38th Meeting of National Working Group on Indonesian Medicinal Plant

ISBN : 978-602-96839-1-2
978-602-96839-3-6

Proceedings

International Conference On Medicinal Plants

The Future of Medicinal Plants:
From Plant to Medicine

Surabaya, 21-22 July 2010

Organized by

POKJANAS

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Universitas Katolik Widya Mandala Surabaya

ISBN : 978-602-96839-1-2
ISBN of Volume 2 : 978-602-96839-3-6

PROCEEDING OF INTERNATIONAL CONFERENCE ON MEDICINAL PLANTS

in occasion of

the 38th Meeting of National Working Group on Indonesian Medicinal Plant

21-21 July 2010
Surabaya, Indonesia

Editor :

Elisabeth C. Widjajakusuma

Organizing Committee

FACULTY OF PHARMACY
WIDYA MANDALA CATHOLIC UNIVERSITY

in collaboration with
National Working Group on Indonesian Medicinal Plants
and German Academic Exchange Service

PREFACE

The International Conference on Medicinal Plants in occasion of the 38th Meeting of National Working Group on Medicinal Plant was held on the campus of Widya Mandala Catholic University in Surabaya during 21-22 July 2010. Over 300 participants had many fruitful discussions and exchanges that contributed to the success of conference. The present volume Proceedings (Volume 2) includes the papers presented at the conference and continues where Volume 1 leaves off.

The 192 abstracts that were presented on two days formed the heart of the conference and provided ample opportunity for discussion. Of the total number of presented abstracts, 63 of these are included in the Volume 1 and 58 in this proceedings volume. Both of the Conference Proceedings cover all aspects on key issues related to medicinal uses of plants, their active ingredients and pharmacological effects, production and cultivation of medicinal plants.

We appreciate the contribution of the participants and on behalf of all the conference participants we would like to express our sincere thanks to plenary speakers, Dr. Mona Tawab, Prof. Henk van Wilgenburg, Prof. Tohru Mitsunaga, Prof. De-An Guo, dr. Arijanto Jonosewojo, SpPD FINASIM, Dr. Bambang Prayogo, Mr. Jimmy Sidharta, Ir. Dwi Mayasari Tjahjono, S.Pd, Dipl. Cidesco,Dipl. Cibtac , and everybody who helped to make conference success and especially to our sponsors

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May you all be richly rewarded by the LORD.

All in all, the Conference was very successful. The plenary lectures and the progress and special reports bridged the gap between the different fields of the development of medicinal plants, making it possible for non-experts in a given area to gain insight into new areas. Also, included among the speakers were several young scientists, namely, students, who brought new perspectives to their fields. I hope this proceedings will promote the interdisciplinary exchange of knowledge and ideas in medicinal plant and related industries.

Dr.phil.nat. Elisabeth Catherina Widjajakusuma
Conference Chairman

ISBN : 978-602-96839-3-6(vol 2)

CONTENT

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DESIGN OF ANIMAL EXPERIMENTS FOR EFFECTIVENESS AND SAFETY STUDIES OF PHYTOMEDICINES: A COMPUTER SIMULATION

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Summary : Experiments for effectiveness and safety studies of phyto-medicines need to be well designed, if they are to give reliable, efficient results. An interactive computer assisted learning program, ExpDesign, is introduced which has been developed for simulating animal experiments, aiming to improve pharmacological and toxicological studies. The program guides students through the steps for designing animal experiments and estimating optimal sample sizes. Principles are introduced for controlling variation, establishing the experimental unit, selecting randomized block and factorial experimental designs and applying the appropriate statistical analysis. In addition PowerCalculation is a supporting tool, which visualize the process of estimating the sample size.

Keywords: Phyto-medicines, experimental design, sample size, computer simulation, CAL.

INTRODUCTION

Pharmacological and experimental studies on botanicals have obtained high level of professional interest since testing has revealed mechanisms of action of some phyto-medicines that yield significant effectiveness. Therefore, there is a pressing need for phyto-pharmacological research showing effectiveness and safety of botanicals based on animal studies. Animal studies, however, should be based on the three R's - Replacement, Refinement and Reduction - meaning that 1) no studies on vertebrates should be done if alternatives are available, 2) suffering of the animals should be minimized and 3) use of laboratory animals should be reduced as much as possible. In the book *The Principles Of Humane Experimental Technique* Russell and Burch (Russell and Burch, 1959) claimed that " . . . reduction remains of great importance, and of all modes of progress it is the one most obviously, immediately and universally advantageous in terms of efficiency". The principles of good experimental design and methods have been well established for over 50 years. Most principles of experimental design were already developed in the 1920s and 1930s by R.A. Fisher (1935). The theory and practical applications of experimental designs have been described in detail in a number of textbooks (Clarke and Kempson, 1997, Cox, 1958, Mead, 1988, Montgomery, 1997). Nowadays mathematical methods exist to determine appropriate sample sizes for different experimental situations. In the literature many recipes for estimating sample size can be found (Chapman *et al.*,1996) and there exist also computer programs such as nQuery Advisor (Prentice and Gloeckler, 1978) that will carry out the calculations. However, such methods have their limitations and there is mounting evidence that many experiments are poorly designed and incorrectly analyzed (Festing, 1995).

The choice of experimental design is important to ethical experiments involving animals. Poor design can result in animal wastage. Any reduction in the use of laboratory animals, which can be achieved without loss of scientific information, therefore, is clearly highly desirable. As Russell and Burch stated, "Every time any particle of statistical method is properly used, fewer animals are employed than would otherwise have been necessary." They also acknowledged that "For reduction purposes, as we have noted, statistical method has a key property – it specifies the minimum number of animals

needed for an experiment." Experiments need to be well designed, if they are to give reliable, efficient results (Festing, 1994). Formal designs, such as completely randomized, randomized block, crossover and sequential designs will be efficient. Factorial designs with two or more independent variables often give more information for a given input of resources. Variability must be controlled in order to minimize experimental error. Uniform, inbred rodents should be chosen, and treatments should be applied with great care. Accurate data must then be collected and appropriately analyzed and their statistical analysis has to be well established. By paying careful attention to all these factors, the quality of the science will be improved, and the information provided by each animal will be increased, leading to a reduction in animal use. The high frequency of statistical errors, however, suggests that many research workers are poorly trained in experimental design and statistics, or do not consult statisticians, or fail to provide the adequate information to the statistician for a proper advice (Festing[a], 2000).

A statistician, consulted in an early phase of planning an experiment, can provide advice about what should be measured in order to reduce animal numbers in an experiment and to obtain as much information per animal as possible. It is the research worker, however, who has to specify the effect size, the power and the significance level. The effect size is the magnitude of the difference between treatment and control means, which the experiment is to be designed to detect. Power analysis calculations require estimates of the standard deviation among experimental units, which must come from previous experiments or the literature, the effect size of biological interest, the required power, the significance level, and the alternative hypothesis whether a one- or two-tailed test is appropriate.

In this paper a computer simulation will be presented aiming to make investigators familiar with some basic principles of experimental design and statistics.

EXPDESIGN: COMPUTER SIMULATION

Realistic experimental conditions, including 'biological variability', can be simulated with computer simulations (van Wilgenburg, 1995). ExpDesign aims to provide a realistic model in which experimental situations can be simulated in order to help students, who will be trained to work with animals, in designing experiments. ExpDesign is a computer aided learning (CAL) program that allows formal designs such as completely randomized, randomized block, crossover and factorial design and their statistical analysis. The effect of unwanted variability, which increases the number of animals used in subsequent experiments, can be simulated in life scenarios. By paying careful attention to the factors that affect variability the student will experience how power can be increased and/or the number of animals can be reduced. With visualized mathematical methods the appropriate sample size can be determined and a final choice of an optimal experimental design can be selected. Accurate data can then be collected for further statistical analysis.

Aims are to enable researchers and students to:

- Explore different experimental designs:
- Design experiments, which will deliver more information, produce more conclusive results improve interpretation and reduce the number of experimental animals required.
- Estimate the number of animals needed to attain the scientific objectives effectively.
- Select a suitable animal model.
- Avoid bias and deal with variability.
- Use appropriate statistical methods.
- Facilitate the discussion with professional statisticians.

The program is highly interactive and consists, beside the simulation of experimental situations, of a tutorial and information in which key issues in experimental design can be explored. These sections are:

Introduction – in which the objectives are summarized and the theoretical background about sample size and improving the precision of an experiment are given. Attention is paid to data types. The data types that occur in animal experiments typically are either continuous (graded) or quantal, i.e. can be count (discrete) or are dichotomous (binary) data. One of the experiments, which can be simulated in the model, 'The analgesic effect of an opiate and some analogues', is introduced and instructions for designing an experiment are given. A tool that is linked to the simulation 'PowerCalculation' for calculating the sample size is also introduced.

Sample size – In this section the student is invited to start an experiment. Since in this phase of the simulation nothing is known about the response and the variability, first a pilot experiment can be set up. To start with, an outbred strain can be chosen. The user is guided through the steps for calculating the sample size. Power analysis requires the researcher to specify an acceptable level of false positives, an acceptable level of false negatives, the smallest effect worth detecting and typical data variation. It will be shown that manipulation of any of these factors will influence the estimated sample size in a predictable way. The tool 'PowerCalculation' is available for visualizing this process.

Choice of animal model and reducing bias– Reduction of the numbers of animals required is possible, if animal variation is decreased. A decrease in variability may be achieved, if measurement error is decreased by using an improved measurement tool. Decrease in animal variation may also result if inbred strains or littermates are used. The use of different strains (inbred or outbred) can be explored in the computer model. Attention is paid to different kinds of phenotypic variation, which can decrease sensitivity, need for larger sample sizes or decreased statistical power so that only large responses can be detected.

Experimental Unit – The nature of the experimental unit will be explored in the following step. In a two-sample t-test the sample size found in the former section can be applied. In the tutorial, feedback is given on the nature of the experimental unit in this design.
Improving Precision – In this section several experimental designs can be selected to improve the precision of an experiment. A "completely randomized" design with a single independent variable is the simplest. This involves assigning experimental units to the different treatments by using an appropriate randomization process. "Blocking" of various types can be used, either as a way of increasing the precision of the experiment, or to take account of any natural structure in the experimental material. Additional independent variables may also be added to give factorial designs. Information from factorial experimental design can be used to design more efficient experiments, either by reducing the numbers of animals used or by increasing the sensitivity so that smaller biological effects can be detected. Factors such as sex, strain, and age of the animals and protocol-specific factors such as timing and methods of administering treatments can have an important influence on the response of animals to experimental treatments. A factorial experimental design approach is more effective and efficient than the approach of varying one factor at a time.

Statistical analysis of experimental data - Student's t-test is suitable for comparing the means of two groups, provided that certain assumptions about normality of the residuals and homogeneity of the variances are met. For analyzing complex factorial or randomized block experimental designs an analysis of variance (ANOVA) is more correct. Student's

t-test in the latter case can lead to too many false positive results and too many false negative results, and to failure to identify interactions among independent variables (Festing[b], 2000). In the model both Student's t-test and ANOVA can be used for the statistical analysis of experimental data and the outcomes can be compared. In cases where, for example, the effect of increasing doses of a drug are studied distributions may be skewed. After logarithmic transformation data may approximately meet again the basic assumptions about normality of the residuals and homogeneity of the variances and ANOVA can be used for the statistical analysis. Other valid statistical methods for non-parametric analysis are reviewed briefly.

Probits program – Experiments with quantal data type cannot be simulated in the ExpDesign program, but the Probits program is linked to the former program and can be used for the analysis of all-or-nothing responses. The most obvious example of an all-or-nothing effect is death, but also other effects can be scored as occurring or not-occurring. Some responses can either be measured on a continuous scale, for example the delay time between the start of a treatment and the time-point at which they occur, or scored as quantal response, for example occurring or not-occurring at a certain time-point. By linking to the Probits program the user can explore the effects, studied before in the ExpDesign program as responses on a continuous scale, now also according to the all-or-nothing principle. This gives the user the opportunity to explore both situations and discover what are the consequences for the sample size if an effect is measured on a continuous scale or measured as occurring or not-occurring.

POWERCALCULATION: TOOL FOR ESTIMATING THE SAMPLE SIZE

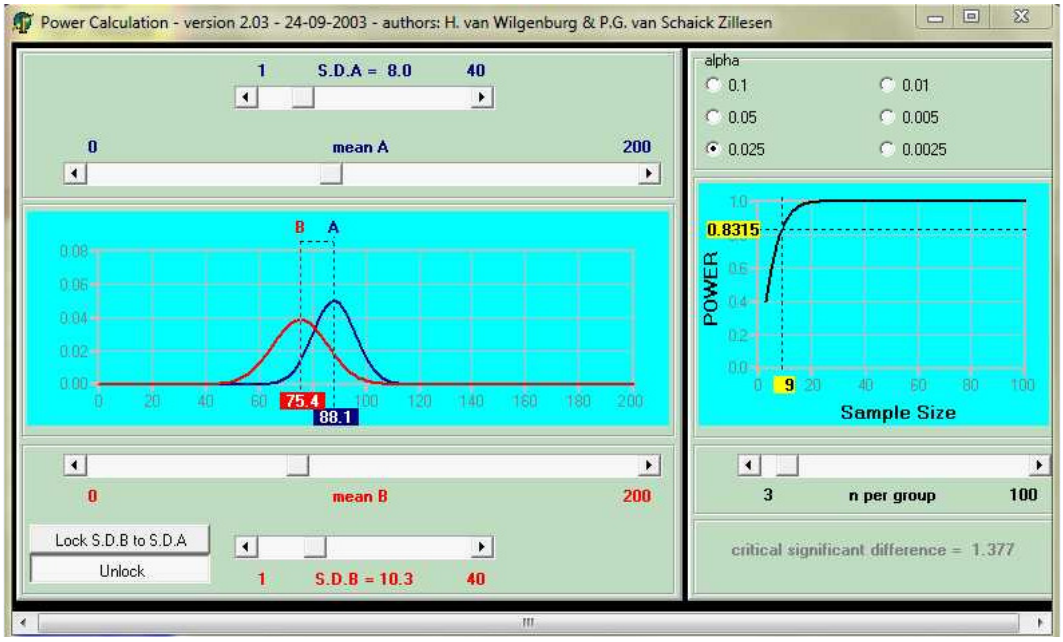

Figure1. PowerCalculation:Tool for estimating sample size of an experiment.

A tool has been developed to visualize the process of power analysis for estimating the appropriate sample size (Fig. 1). Mathematical methods exist for this purpose, but since equations are rather abstract, it is for most investigators less clear how the different parameters affect the sample size. PowerCalculation is an interactive tool. When the mean and sd for the control group is known the distribution curve can be visualized by setting the mean (A) and the sd in the left hand curve. The smallest effect

worth detecting, i.e. the minimum significant difference, is set by shifting the mean (B) of the test group in the desired position. After the significance level, i.e. the level of false positive (alpha for a two-tailed test, or alpha/2 for a one-tailed test) and the power (1 – beta, where beta is the level of false negative) is set, the sample size can be red from the abscissa of the right hand curve. Manipulation of any of these parameters will influence the estimated sample size.

Example: Analgesic effect of a opiate and some analogues

The analgesic effect of opiates can be studied with the hot-plate method. The hot-plate method is introduced with a video clip, showing the paw-licking response of untreated mice, mice treated with NaCl, with the NSAID phenylbutazone and with the opiate morphine. Placed on a hot plate at a temperature of 57 degrees Celsius the animals will start to lick their forepaws after a certain time delay. The response can be measured on a continuous scale, or as a quantal response, i.e. licking or not-licking after 30 seconds or after one minute. The student will be asked to design an experiment to compare the analgesic effect of the opiate pethidine with some analogues. Since the range for the default doses for the analgesic effect of pethidine is known from the literature, it is suggested to start first a pilot experiment with pethidine to find out about the mean delay time and the variability in response. First the experiment can be done with outbred mice. All mice will receive the same dose based on the dose/kg for an adult mouse. After mean response time and sd are found the sample size can be estimated with the PowerCalculation tool, choosing a minimal effect to be detected for the perthidine analogues. The student will be asked for suggestions to reduce the variability in response, in order to reduce the sample size and/or to improve the power. The student can also play with the parameters, such as level of false positive and false negative, one- or two tailed test and minimal significant difference and see what most affects the sample size. The experiment can then be repeated with inbred mice and by applying the appropriate dose/kg/mouse. Next the student can choose for a design with a single independent variable. The student selects one of the three analogues available, apply the same dose to both the pethidine group and the analogue group and performs a Student's t-test on the outcomes. The analogues have been selected from the literature with one being slightly less effective than pethidine, one being about 7% more effective and one being more than 20% effective than pethidine as an analgesic. It will be questioned what has been the experimental unit in this experiment. A better design will be to use a randomized block experiment. The student will discover that the Student's t-test is no longer appropriate in this situation, but that instead ANOVA should be used. More complicated designs can be selected, for example more doses per drug and factorial experimental designs. The sample size should be adjusted using the so-called resources equation (Mead, 1988). This is a rule of thumb, whereby the error term in the analysis of variance relevant to the study is designed to have between 10-20 degrees of freedom. It can be shown that there is little to gain by having more degrees of freedom in the error term (Festing, 1996).

In pharmacological and toxicological studies the ED50 is often given to characterize the dose of a drug that produce 50% of the maximum effect. This value, with 95% limits, can be found with the aid of the statistical procedure called probit analysis for all-or-nothing experiments [15]. By linking to the Probits program the student can design an experiment for establishing the ED50, with 95% limits, for pethidine and its analogues.

DISCUSSION AND CONCLUSIONS

Herbal medicines are widely employed by practitioners of phytotherapy, based on tradition. More recently phyto-pharmacological research has demonstrated the effectiveness of herbal compounds as phytomedicines. For standardization, correct dosage and safety tests, however, reliable animal studies are inevitable. These

experiments can only be ethically justified when experiments are properly designed and translation of research evidence from animals to humans will be possible. The investigator, therefore, should have a basic knowledge of the requirements for good experimental design and statistics. Biological processes, providing that they are intrinsically mathematical and that enough quantitative information is available, can replace animal experiments in practical courses [16]. We have shown that realistic experimental conditions, including 'biological variation' can be simulated with computer simulations. Conditions can be specified for an acceptable level of false positives, an acceptable level of false negatives, the smallest effect worth detecting and typical data variation. With the interactive CAL programs ExpDesign and PowerCalculation animal experiments can be simulated, aiming to improve pharmacological and toxicological studies as required for developing safe and effective phyto-medicines.

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GAS CHROMATOGRAPHY-MASS SPECTROMETRY (GC-MS) BASED METABOLIC FINGERPRINTING OF THREE MALAYSIAN GINGER (*ZINGIBER OFFICINALE ROSC.*)[#]

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[#)]Adapted from: H.J. Mahdi, R. Andayani and Ishak (2010) Metabolic Fingerprinting of Three Malaysian Ginger (Zingiber officinale Roscoe) using Gas Chromatography-Mass Spectrometry, Am.J. App. Sci. 7 (1): 17-23.

Abstract : A comprehensive metabolic fingerprinting of three micro propagated ginger explants, Bukit Tinggi, Tanjung Sepat and Sabah cultivars, was carried out using Gas chromatography coupled with mass spectrometry (GC-MS). The ginger leave tissues were fractionated in a polar (MeOH) and non polar ($CHCl_3$) solvents, subsequently methoximated and silylated prior to GC-MS analysis. By applying this technique, over 300 metabolites (polar and non-polar) in total were detected from each ginger cultivar. However, only about 25% of these compounds can be characterised for the best hit of the molecular ion peaks and the fragmentation patterns by using the Wiley7n.1 and the NIST Mass spectra libraries. Fatty acids and sugars (mono– and disaccharides) as the main constituents of the ginger leaf tissues, a small amount of essential amino acids and some organic acids. A distinct GC-MS metabolic fingerprinting of each ginger cultivar can be further used as "unequivocal pattern recognition" among ginger phenotypes grown in Bukit Tinggi, Tanjung Sepat and Sabah, Malaysia.

***Keywords*:** GC-MS analysis, Metabolic fingerprinting, *Zingiber officinalle* Rosc. (= ginger)

INTRODUCTION

Ginger (*Zingiber officinale* Rosc.) is an important tropical horticultural plant, values all over the world as a spice and its medicinal properties such as anti-emetic, anti microbial, anti platelet activity (Barnes et. al., 2002). A therapeutic benefits of ginger are largely due to its volatile oils – Zingiberene (1%-3%) and a non-volatile compounds – oleoresin (4%-7%) mainly gingerol and shogaol besides other compounds such as starch (50%), fats, waxes, carbohydrates, vitamin and minerals (Robbers et al., 1996; Fnimh, 2001). Several factors such as the geography, age of rhizome at harvest and extraction method determine the relative proportions of chemicals.

Ginger belongs to the family Zingiberaceae which contains about 1300 species and widely distribute in the tropics south and south-eastern Asia specially Indo-Malaysia (Awang, 1992; Simpson, 2006). Ginger plant is completely sterile (poor-seed production) and propagates only by its rhizomes (Berg, 1997). Plant tissue culture technique has commonly been used for micropropagation of ginger.

In present study, we carried out a metabolic fingerprinting analysis of the leaves of micro-propagated ginger cultivars from Bukit Tinggi, Tanjung Sepat and Sabah by using GC-MS in order to to identify the three ginger cultivars. Furthermore, the degree of correlation present among the ginger cultivars will be examined based on direct comparison of the ginger metabolites which is presence either in methanol or chloroform fractions.

MATERIAL AND METHOD

Plant Materials

Ginger plantlets were maintained in Murashige and Skoog (MS) media containing 3 % (w/v) sucrose; 2 mg/l 1-naphtalen acetic acid in combination with 2 mg/l kinetin as growth hormones and agar 0.08% (w/v). Three months old ginger leaf tissues from Sabah, Bukit Tingg and Tanjung Sepat cultivars were used in the experiment.

Sample Preparation

Sample preparation was done according to Jiang et al. (2005) method with a slight modification. The leaves were cut into a small pieces, immediately immersed in to liquid nitroge and were grinded to fine powder while kept frozen under liquid nitrogen, homogenized and were stored at -80 °C freezer until further use.

Extraction and Derivatization of Polar and Non-Polar Metabolites

The extraction and MSTFA-derivatization protocol were carried out according to modified Shepherd's method (Mahdi et al. 2010) Methyl nonadecanoate used as an internal standard for non-polar metabolites and sucrose in case of polar metabolites.

GC-MS Parameters

Methanolic and chloroform fractions were analysed using GC-MS (Agilent Technologies 6890N network GC system) with operating parameters: capillary GC column HP-5MS 5% phenyl methyl siloxane (30 m x 0.25 mm i.d. x 0.25 mm film thickness), a carrier gas Helium (flow rate 1.2 mL/min) and a split-less injection mode. Injector temperature is 250 °C, oven temperature will be set initially at 50 °C, then will be raised to 250°C at a 10 °C/ min rate till the end of analysis. The eluted analytes detected using (5973 network) mass selective detector and electron impact ionization (EID) will be carried out at 70 eV. Data were acquired using Enhanced ChemStation G1701CA version C00.0021, Agilent technology.

Data Analysis of the Ginger Metabolites

We applied some criteria for the selection of suitable ions for identification of a compound. It should has a high peak area (> 0.05 %), unique, and/or be well resolved from other ions with the same mass to charge ratio (m/z) in the defined time window. The identity of compounds were checked using the Wiley7n.1 spectra data base and compared manually with the standard spectrum (NIST Chemistry WebBook) for the best hit ion peaks and the correct fragmentation patterns

RESULTS AND DISCUSSION

In average, about 314 to 385 compounds were detected from the leaf tissues of three micropropagated ginger cultivars – (BT, TS and SB) as shown in Table 1. However, only about. 25% to 30% of these peaks were unequivocally identified from both methanolic– and chloroform fractions of the ginger samples. The ginger BT cv. contained the highest number of detected total metabolites compared to two others ginger cultivars.

Table 1. The number of deteted metabolites of the ginger cultivars

Ginger cultivar	MeOH fraction	$CHCl_3$ fraction	T otal no compound (% identified peak)
	Compounds	Compounds	
BT	207	178	385 (30%)
TS	174	141	315 (24%)
SB	169	145	314 (25.7%)

BT = Bukit Tinggi, TS = Tanjung Sepat and SB = Sabah

Tabel 2. Relative content of ginger metabolites in the MeOH fraction, identified by GC-MS

RT	Name	Qualifier ions (m/z)			Ginger leaf tissues		
					BT	TS	SB
19.7	l-Threonine	147	219	248	0	1	1
20.0	Glycine	147	248	276	0	1	1
21.68	L-Serine	204	218	278	0	1	1
22.38	L-Threonine	147	218	291	0	1	1
24.99	Malic acid	191	233	335	2	2	2
25.53	L-Proline	156	230	258	1	1	2
25.69	Aspartic acid	202	218	232	0	1	1
27..98	Glutamic acid	246	348	363	0	1	1
29.21	L-Asparagine	116	132	231	0	1	0
31.66	Ribonic acid	217	292	333	0	1	1
32.58	Sorbose	147	204	437	1	1	0
33.19	β -D-Galactofuranose	191	217	319	2	2	1
33.78	D-Fructose	217	277	307	2	2	2
34.09	L-Glucose	103	129	231	2	2	2
34.58	Glucose, oxime	189	291	319	1	1	1
35.31	α-D-Glucopyranose	129	147	204	1	1	1
35.92	D-Glucose	103	129	231	2	2	2
37.76	Myo-Inositol	305	320	343	1	2	2
39.63	L-Glucose	147	204	231	1	1	1
41.87	D-Mannopyranose	129	204	219	1	1	1

Note: RT =RetentionTime, BT: Bukit Tinggi, TS: Tanjung Sepat, SB: Sabah
(0) = nil, (1): indicates < 0.5%, (2) indicates 0.5-5% of total integrated peak area of TIC of particular sample

The low percentage of identified peaks (24% - 30%) both in methanolic and chloroform fractions of the three ginger cultivar. It might be due to some reasons such as peak overlappi, a low level of analytes (~ 0.05% - 1.0%) or simply, because no information is available in the spectra data base library so far.

Furthermore, the same type of ginger leaf metabolites were observed in both polar (methanolic) fraction and non-polar (chloroform fraction). As shown in Table 2, sugars (mono and disaccharides) as well as malic acid were presence in all ginger cultivars. Meanwhile, the chloroform fraction of the ginger leaf sample contained

realtively high amount of amino acids namely linoleic acid, linolenic acid and palmitic acid (Table 3).

Table 3. Relative content of ginger metabolites in the $CHCl_3$ fraction, identified by GC-MS

RT	Name	Qualifier ions			Ginger Leaf tissues		
					BT	TS	SB
15.01	Meta-cresol	149	165	180	1	1	1
23.25	α-Neoclovene	91	189	204	1	1	1
25.05	Lauric acid	143	171	214	1	1	1
27.59	Tau. -Cadinol	105	161	204	1	1	1
28.06	α-Gurjunene	175	189	204	1	1	1
28.66	(Z) -3-(4-n-Butyl-3-thienyl) propenal	123	151	194	1	1	1
29.09	m-Xylene	91	282	297	1	1	1
29.60	Myristic acid	87	199	242	1	1	1
30.10	α -Hexylcinnamic aldehyde	91	173	216	1	1	1
31.70	Pentadecanoic acid	143	213	256	1	1	1
31.96	Neophytadiene	109	123	137	1	1	1
32.25	Tetradecanoic acid	117	171	285	1	1	1
32.47	Neophytadiene	123	137	278	1	1	1
33.19	9-Hexadecenoic acid	152	194	236	1	1	1
33.82	Palmitic acid	87	185	270	**3**	**3**	**3**
34.28	Cinnamic acid	219	250	265	1	1	1
35.64	Margaric acid	185	199	284	1	1	1
36.09	Palmitic acid	117	313	328	2	2	2
37.08	Linoleic acid	95	263	294	3	3	3
37.20	Linolenic acid	135	149	236	3	3	3
37.51	Stearic acid	129	199	298	2	2	2
39.00	Linoleic acid	150	164	352	2	1	1
39.10	Oleic acid	145	185	345	2	2	2
39.56	Stearic acid	297	314	356	2	2	2
44.10	Behenic acid	125	311	354	2	1	1
45.64	Tricosanoic acid	97	325	368	1	1	1
47.28	Cholest-7-ene	159	383	399	1	1	1
47.43	Tetracosanoic acid	199	339	382	1	1	1

Note. Rt: retention time, BT: Bukit Tinggi, TS: Tanjung Sepat, SB: Sabah, 1 indicates < 0.5%, 2 indicates 0.5% - 5% and 3 indicates > 5% of total integrated peak area of TIC of a particular sample.

A distinct pattern in the level of metabolites were detected among the three ginger cultivars. Apparently, these chemical variations could due genetic effects since almost all to environmental and intrinsic factors were eliminated. In conclusion, the GC-MS metabolic fingerprinting of derivatized ginger leaves can used as a tool for an identification of ginger cultivars from Bukit Tinggi, Tanjung Sepat and Sabah, Malaysia.

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ANTIBACTERIAL ACTIVITY OF MARINE SPONGES *HALICLONA FASCIGERA* AGAINST *RALSTONIA SOLANACEARUM*

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Abstract : Bacterial wilt caused by *Ralstonia solanacearum* has become a severe problem mainly on ginger of West Sumatera Indonesia, and no effective control measure is available yet. Clearly chemical measures have lost their attractiveness because of development of resistant strains and undesirable effects on our environment. Consequently, biological control of pathogens is gaining great importance worldwide (Lemessa, 2007). To investigate for proper biocontrol agents and to obtain antibacterial antagonists from marine sponges, a vast survey was performed. The marine sponge *Haliclona fascigera* collected from Mandeh Island, West Sumatera was screened in Agar disk diffusion method against *R. solanacearum*. Results showed the isolated compound from the fraction of n-butanol of *Haliclona fascigera* had an ability to inhibit the growth of *R. solanacearum*.

***Keywords*:** marine sponges, *Haliclona fascigera*, antibacterial, *Ralstonia solanacearum*.

INTRODUCTION

Sponges (phylum Porifera) are the multicellular animals (metazoa) that represent an important constitutive group of the coral reef fauna with a widerange of species. Despite being sessile and soft-bodied, sponges appear to be predated only by selected groups of marine animals, such as several fishes, turtles, sea urchins and sea stars, some nudibranches, some kinds of gastropoda and flat worms [Galeano and Martinez, 2007].

Sponges compete for space on the substrate with other sessile organisms such as corals and algae. This mechanism is not well-known but supposed to be chemical [Van Soest , Evelyn, Gomez, Breakman, 2006]. They are known to produce a large number and diversity of bioactive secondary metabolites. Until now, more than 5000 different compounds have been isolated from about 500 species of sponges [Rifai, *et.al.* 2005]. Up to 800 antibiotic have been isolated from marine sponges [Touati, Chaieb, Bakhrouf, Gaddour, 2007]. The first report of antimicrobial activity of sponge extract was by Nigrelli *et al.* [Newbold, Jensen, Fenical, Pawlik, 1999]. Since then, there has been a growing number of antibacterial extracts reported from marine sponges [Edrada, et. al. 2000; Bergquist and Bedford,1978; Burkholder and Rutzler, 1969]. The chemical nature of metabolites isolated from marine sponges has been extensively reviewed by several 2 authors and are studied as interesting target to screen for antimicrobial substances for many reasons [Mc Caffrey and Endean, 1985]. Bacterial wilt caused by *Ralstonia solanacearum* has become a severe problem mainly on ginger of West Sumatera Indonesia, and no effective control measure is available yet. Clearly chemical measures have lost their attractiveness because of development of resistant strains and undesirable effects on our environment. Consequently, biological control of pathogens is gaining great importance worldwide [Lemessa, 2007], since the conventional use of chemical pesticides has been seriously questioned because of environmental and human health hazard [Spurrier, 1990; Mendgen et al. 1992]. To investigate for proper biocontrol agents and to obtain antibacterial antagonists from marine sponges, a vast survey was performed. The methanol extracts of ten marine sponges were screened for their antibacterial

activity. Among them, the extract of *Haliclona fascigera* appeared to be quite promising due to their capacity to inhibit the growth of *R. solanacearum* (Table1). The aim of this study was to investigate the activity spectrum as well as isolation and characterization of the bioactive secondary metabolites from the methanol extracts of *Haliclona fascigera* collected from Mandeh Island, West Sumatera.

EXPERIMENTAL

General method

Melting points were *s* determined on Fisher-John Melting Point Apparatus, UV and IR spectra were obtained using Ultraviolet-Visibel Pharmaspec 1700 (Shimadzu®), and Perkin Elmer Spectrum One spectrofotometers, respectively. Column chromatography was carried out using Merck Si gel 60 and Sephadex LH-20, TLC analysis on Si gel PF254, precoated aluminum sheets. Organic materials were visualized by UV light of wavelength 254 nm.

Animal material

The marine sponge *Haliclona fascigera* was collected from the coral reef at a depth of around 15 m in Mandeh Island of West Sumatera, and the sample was stored in methanol immediately after collection. The specimen was identified by Dr. Nicole J.de Dr. Nicole J.de (Institute of Systematic Population Biology, Amsterdam University, Netherlands). The voucher specimens (MN 07) are deposited at the Natural Product Chemistry Laboratory of Pharmacy Faculty, Andalas University, Indonesia.

Secondary metabolites testing

The presence of secondary metabolites was performed based on modified Simes *et.al*. method for alkaloid, terpenoids/steroids, phenolics and saponins.

Extraction and isolation

The sponge (1 kg, wet wt.) was homogenized and then extracted with MeOH (1 L×3) in 5 L Erlenmeyer at room temperature. The concentrated total extract (44,80 g) was subjected to liquid-liquid partitioning resulting in n-hexane (0,069 g), ethyl acetate (2,40 g) and butanol (6,94 g) soluble fraction. The butanol fraction was subjected to Si gel column chromatography eluting on silica gel, using EtOAc with increasing amounts of MeOH as the mobile phase, to afford eight fractions (A-H). Each fraction was concentrated under reduced pressure and their antibacterial activities against *R. solanacearum* were tested *in vitro* using diffusion agar method. Fraction D shown a strong antibacterial activity compared to other fractions. Recrystallization of fraction D using MeOH and EtOAc yielded compound 1 (22.7 mg).

Antibacterial assays [Acar, 1980; Lay, 2001]

The assay was carried out using plate diffusion agar method. Antibacterial activity was determined against strains of *R. solanacearum*. The bacteria was grown in Nutrient Broth medium and incubated on 35-37°C for 24 hours. Twenty milliliters Mueller Hinton agar inoculated bacteria were introduced into sterile petri dishes. Paper disc (6 mm of diameter) were impregnated 20 µl of extracts/isolated substance deposited on petri dish after solvent evaporation. In the same plate, it was also deposited paper disc containing chloramphenicol as a comparation or positive control and negative control was DMSO. The plate were then incubated for 24 h at 35–37°C before reading the inhibition zone diameter. The inhibition zone diameter around the paper disc correlates with susceptibility of the tested extracts. Further correlations using zone diameter allowed the designation of an organism as “susceptible”, or “resistant” to concentrations of the extract.

RESULTS AND DISCUSSION

The methanol extract of marine sponge *Haliclona fascigera* gave positive test with Liebermann Burchard'reagent indicating that the sponge contained steroid/terpenoid, phenolic and saponin. The result of secondary metabolites testing showed in Table 2. Fractionation of the methanol extract of marine sponge *Haliclona fascigera* with *n*-hexane, ethyl acetate, *n*-butanol, gave *n*-hexane, ethylacetate, and *n*-butanol fractions. Each fraction was concentrated under reduced pressure and their antibacterial activities against *R. solanacearum* were tested *in vitro* using diffusion agar method. *n*-Butanol fraction shown a strong antibacterial activity compared to the *n*-hexane and ethylacetate fractions. (data not shown). The *n*-butanol fraction were fractionated by silica gel chromatography, TLC and recrystallization. The fractions having the activity were collected. Finally, 1 active compounds were isolated as white amorf melted at 143-145 oC. Based on data from chemical reaction with Lieberman Bourchard, ultraviolet and infrared spectra suggest that compound is belong to terpenoid group. The work to elucidate its structure is still in progress. The activity of isolated compound was determined by agar dilution method against four species of bacteria. Its antibacterial activity was evaluated by MIC (Minimum Inhibitory Concentration) and these values are presented in Table 3. This compound showed antibacterial activity with MIC of 0,05% against *R. solanacearum*, and indicates that marine sponges remain an interesting source of new antibacterial metabolites with better activity then some antibiotics. This is not surprising because the sponge *H. fascigera* belonging to this genus possess a wide variety of compound with different biological activities. Thus during the identification of this indicates that marine sponges remain an interesting source of new antibacterial metabolites with better activity then some antibiotics.

Table 1. Antibacterial activity of the marine sponge extracts

No	Sample Code	Identification of marine sponges	Inhibition Zone diameter (mm)		
			5%	3%	1%
1	MN 01	*Cinachyrella* sp	-	-	-
2	MN 02	*Stylissa carteri*	12	11	7
3	MN 03	*Acanthostrongylophora ingens*	8	7	-
4	MN 04	*Petrosia* sp	8	7	-
5	MN 05	*Petrosia* sp	-	-	-
6	MN 06	*Petrosia* sp	8	7	-
7	MN 07	*Haliclona fascigera*	14	13	9
8	MN 08	*Xestospongia testudinaria*	-	-	-
9	MN 09	*Dactylospongia elegans*	-	-	-
10	MN 12	*Hyrtios reticulates*	9	8	-

Table 2. Secondary metabolites testing of marine sponge *Haliclona fascigera*

No	Secondary Metabolite	Result
1	Alkaloid	-
2	Terpen/steroid	+
3	Phenolic	+
4	Saponin	+

Table 3. Antibacterial activity of the compound 1

Concentration (%)	Inhibition Zone diameter (mm)
1	15
0,5	13
0,25	11
0,1	9
0,05	8,25
0,025	-
0,01	-

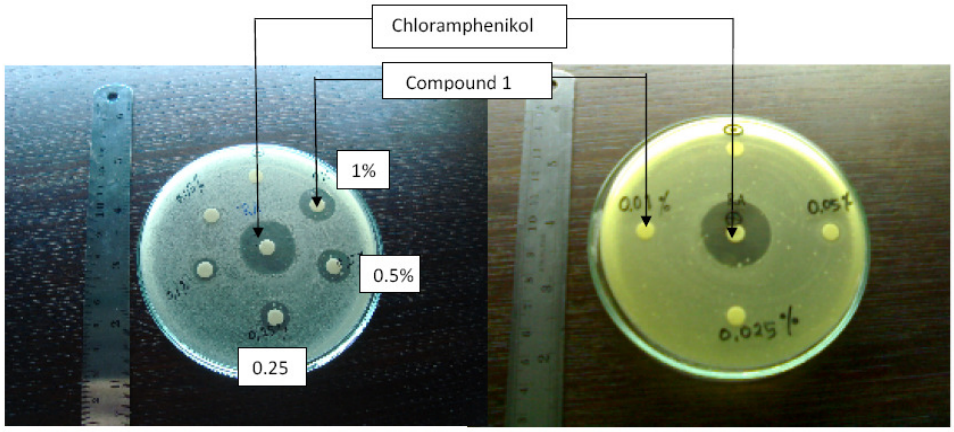

Figure 1. The inhibition zone of the isolated compound 1 to *R. solanacearum*.

CONCLUSION

The marine sponge *Haliclona fascigera* are potential source of antibacterial agents. The isolated compound from this sponge had an ability to inhibit the growth of *R. solanacearum* with MIC value of 0,05% usin plate diffusion agar method.

ACKNOWLEDGMENT

This research was supported by Research Grants from the Indonesian Ministry of Education (Hibah Srategis Nasional Tahun 2009). Authors are especially grateful and appreciate Dr. Nicole. J. de. Voogd, spongologist from Zoological Museum of the University of Amsterdam for sponges identification.

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A BIOACTIVE COMPOUND OF LACTARANE SESQUITERPENE VELLERAL FROM THE STEM BARK OF *DRYMIS BECCARIANA* GIBBS. (WINTERACEAE)

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Abstract : Isolation guided by brine-shrimp (*Artemia salina*) bioassay from the stem bark of *D. beccariana* Gibbs. led to isolate a bioactive compound called Velleral (1) ((3aS,8aS)-2,2,8-trimethyl-3,3a.6.8a-tetrahydro-1H-azulene-5,6-dicarbaldehyde).The structure of this compound was elucidated based on physical and spectroscopic data (UV, IR, , 1H NMR, 13C-NMR and GC-MS). This compound showed significant active in the A. salina bioassay with LC_{50} 2.92 µg/mL and exhibited significant cytotoxicity against maurine P-388 leukemia cells with IC_{50} 3,39 µg /mL. Moreover, the compound exhibited antibacterial and antifungal activity especially against *Staphyloccocus aureus* ATCC 25923 and *Candida albicans* respectively. This result indicates that stem bark of *D. beccariana* is a potential source of bioactive compounds.

Key words : *D. beccariana*, Velleral, *A. salina*, Sel maurine P-388, *S.aureus*, *C. albicans*

INTRODUCTION

D. beccariana (Winteraceae) locally called "Akway" is an upright evergreen shrub with very hot peppery leaves and attractive white flowers in terminal head, new stems are red, grow in full sun and height to around 4 meters. It is found in primary and secondary tropical rain forest especially in high altitude (Heywood, 1993).

The stem barks of *D. beccariana* are used traditionally for the stimulant by indingenous people of Manokwari Papua. The tea prepared from the stem barks of D. beccariana are also used againt bacterial infections as well as inflamations (Lense, 2002). Previous work reported that the stem barks of *D. beccariana* exhibit a wide variety of potent and interesting biological activities (Santoso et al., 2004 and 2005). However, there is no report so far regarding the isolation bioactive compounds of this species.

EXPERIMENTAL

General

HR-FABMS were obtained from GCMS- QP201205 Shimadzu. The UV and IR spectra were recorded on a Variant DMS-100 and Shimadzu 8300 FTIR spectrometers, respectively. ^{1}H dan ^{13}C spectra were determined on a Jeol JNM A-500 at 500 MHZ (^{1}H) and 125 MHZ (^{13}C) respectively. Column Chromatography and analytical TLC utilized Silica Gel Merck 9385, and Merck TLC Silica gel 60 F_{254} (230-400 mesh), respectively. All solvents used were pro analysis (p.a) Merck.

Plant materials

D. beccariana were obtained from district Anggi Manokwari West Papua Province. The voucher specimen was lodged earlier on at the Herbarium of

Manokwarience The State University of Papua with identification code BW 278. It was air dried and ground before extraction.

Extraction and Isolation

The ground dried stem bark of *D. beccariana* (2,2 kg) was extracted with ethanol (EtOH) (3 X), each time by soaking in 5 L of solvent for overnight before it was decanted. The combined extracts were evaporated under reduced pressure to give brown gum (309 g). EtOH extract was portioned with $CHCl_3$/water (1:1). Water fraction was dried by frezee dryer to give residue (25.4 g). $CHCl_3$ solubles was evaporated by vacumm rotary evaporator to give $CHCl_3$ fraction (166 g). $CHCl_3$ fraction then was partitioned between hexane/MeOH (90%) (1:1). MeOH soluble were evaporated giving MeOH fraction (56 g). The recovered hexane solubles geting hexane fraction (86 g). All fractions (5 fractions) were subjected to brine shrimp (*A. salina*) lethality bioassay. The most active fraction (hexane fraction) was fractionated by VLC (Oi 5 cm) using n-heksana:EtOAc solvent system with increasing polarity from ratio 9.5: 0.5 until pure EtOAc, giving seven major fractions A (2.1 g), B (3.2 g), C (3.9 g), D (3.4 g), E (4.3 g), F (3.6 g) dan G (3.9 g). All fractions were subjected to brine shrimp (*A. salina*) lethality bioassay. The most active fraction (F fraction) then was fractionated by VLC (Oi 3 cm) using n-heksana- EtOAc solvent system with increasing polarity from ratio 9 : 1 until pure EtOAc giving six fractions, A1-A6. The fraction A2 was the most active fraction on brine-shrimp bioassay. This fraction had two major TLC bands, B1 and B2. The most active band B1 was separated by preparative TLC using n-hexane:EtOAc (8:2) giving three bands C1, C2 dan C3. Purify the most active band C1 giving compound 1.

Analysis GCGC-MS

The GC Parameter: oven temperature 60^{o}C, oven balance time 1 minute, injection temperature 300^{o}C, interface temperature 300^{o}C, length of column 30 m, column diameter 0.25 mm, column pressure 22 kPa, flow velocity in the column 0.5 mL/minute, linier velocity 26.3 cm./sec, split ratio 113, flow total 60 mL/minute, 30 minutes; MS parameter : initial m/z 33.00, final m/z 550.00, scan interval 0.5 sec., scan speed 1250 amu/sec.

Compound 1

Solid yellowish powder, melting point 114-118^{o}C, UV (MeOH) λmax, nm: 232 dan 202. IR (KBr) ν_{max}, cm^{-1}: 2740, 2840, 2972,1710, 1597; EIMS m/z 232 [M+]; ^{1}H NMR (500 MHz, CDCl3) δ_H ppm: 9.40 (^{1}H, s. H-5), 9.37 (^{1}H, s, H-13), 6.58 (^{1}H, d, 8,4 Hz, H-4), 6.48 (^{1}H, d, 11 Hz, H-8), 2.70 (^{1}H, m, H- 9), 2.55 (^{1}H, m, H-3), 2,09 (^{1}H, m, H-2), 1.87 (^{1}H, *ddd*, 1.9 Hz, 6.5 Hz, 12 Hz, Ha-10), 1.78 (^{1}H, *ddd*, 1,9 Hz, 8 Hz, 12 Hz, Ha-1), 1.41 (^{1}H, *dd*, 12 Hz, 12Hz, Hb-10), 1.36 (^{1}H, *dd*, 12 Hz, 7.2 Hz, Hb-1), 1.16 (^{3}H, s, H-12), 1.11 (^{3}H, s, H-14) dan 0.98 (^{3}H, s, H-15); ^{13}C (125 MHz, $CDCl_3$) δ_C 196,1 (C-5), 194.2 (C-13) 164.1 (C-4), 163.0 (C-8), 140.1 (C-6), 142.2 (C-7), 48.9 dan 46.0 (C-1 dan C-10), 44.4 dan 42.9 (C-2 dan C-9), 36.8 (C-11), 36.1 (C-3), 30.2 dan 28.0 (C-14 dan C-15), 22.2 (C-12).

Brine Shrimp (*Artemia salina*) Lethality Bioassay

Egg hatching

Nineteen grams of commercially available Bio-Mix sea water were dissolved in 500 mL distilled water. A clean dry petri dish containing 60-70 ml of artificial sea water served as a brine shrimp mini-pond. Approximately 50 mg of brine shrimp eggs was placed on the pond. Larvae for subsequent assays were collected 48 hours after introduction of the eggs into the mini-pond.

Preparation of test solution for testing

Clean vials were calibrated to 5 mL. The crude extracts of EtOH, hexane, water, MeOH and chloroform were tested at concentration of 500, 100 and 50 μg/mL. Fraction was tested at 40, 25 and 10 μg/mL. The pure fractions were tested at 20, 10 and 5 μg/mL. Untreated sea water and 2% DMSO in sea water served as control for the assay. Two or three replicates were prepared for each tes solution and control. The assays were performed at room temperature, without any mechanical agitation, for 24 hours.

Determination of LC_{50}

The LC_{50} values were determined using a Finney computer program (probit analysis) on personal computer at 95% confidence intervals (McLaughlin et al., 1991).

Antimicrobial Assay

Preparation of test organisms

The organisms, *S. aereus* (ATCC 25923) and *E. coli* (ATCC 25922) were streaked on nutrient agar then incubated at 37^{o}C for 24 hours. *C. albicans* was streaked on Emerson agar then incubated at room temperature for 24 hours.

Preparation of assay plates

Fifteen mL of melted sterile nutrient agar (for bacteria) and sterile Emerson agar (for fungi) was poured and distributed evenly in sterile Petri dishes the allowed to harden. The test organisms, from the nutrient agar and Emerson agar cultures were streaked into the solidified agar plates.

Preparation of sample for assay

Sterile Whatman antibiotic assay disc (5 mm diameter) was impregnated with 0.5 mL 50 μg/mL $CHCl_3$ of compound 1 and put in Petri dish. The Petri dish was dried in a vacuum oven at 40^{o}C for 15 minutes to remove residual solvent. The same procedure was done for negative control (chloroform) and positive control erythromycin and ampicilin.

Testing for antibiotic activity

The back of the seeded plates was marked to indicate the starting point. The whatman antibiotic discs impregnated with sample (two replicates) were placed in clockwise order on seeded agar plates (Emerson agar for fungi and Nutrient agar for bacteria) and left for 24 hours at room temperature (for fungi) and at 37^{o}C (for bacteria). Chloroform was used as a negative control, whereas erythromycin and ampicilin were used as a positive control. The diameter of clear zone of inhibition was measured (Sukari and Takahashi, 1988).

Cytotoxicity assay (Alley *et al.*, 1988)

P-388 cells were seeded into 96-well plates at an initial cell density of approximately 3 x 104 cells cm-3. After 24 h of incubation for cell attachment and growth, varying concentration of samples were added. The compounds added were first dissolved in DMSO at the required concentration. Subsequent six desirable concentrations of samples were prepared using phosphoric buffer solution, pH 7.30-7.65. Control was recieved only DMSO. The assay was terminated after an 48 h incubation period by adding MTT reagent (thiazol blue) and the incubation was continued for another 4 h, in which the MTT-Stock solution containing SDS was added and another 24 h of incubation was conducted. Optical density was read by using a microplate reader at 550 nm. IC_{50} value were taken from the plotted live cells compared to control (%), recieving only PBS and DMSO, versus the tested concentration of compounds (FM). The IC_{50} value is the concentration required for 50% growth inhibition. Each assay and analysis was run in triplicate and averaged.

RESULTS AND DISCUSSION

Compound 1 was obtained as yellowish powder, m.p 114-118°C. The UV Spectrum exhibited absorptions at 232 nm and 202 nm, these absorptions indicated the presence of chromoporic carbonyl function (C=O) conjugated with unsaturated α,β as a result of n π* electronic transition. The IR spectrum showed the C-H stretching of aldehyde function at 2750 cm^{-1} and 2820 cm^{-1}, this was supported with the presence of the moderate band in 1709 cm^{-1} which is typical absorption of conjugated carbonyl function (C=O).

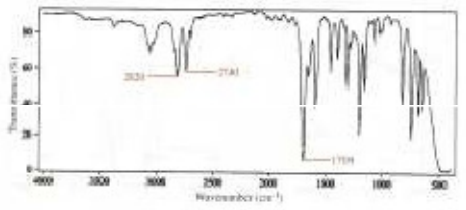

Figure 1. IR Spectrum of Compound 1

Whereas the absorption at 2920 cm^{-1} indicated the C-H stretchings of $–CH_2$ and $–CH_3$ alifatic. pair signal carbonyl function at δ_H 9.40 and 9.37 which suggested the presence of two aldehyde functions of compound 1, which is also supported by the ^{13}C NMR spectrum at δ_C 196.1 and 194.2. The presence doublet signals at δ_H 6.48 and 6.58 with coupling constants of 11 Hz and 8.4 Hz respectively were assigned to vinyl proton of H-4 and H-8. The three multiplet signals at δ_H 2.70 , 2.55 and 2.09 were assigned to H-9, H-3 and H-2 respectively. The two triplet signals at δ_H 1,87 (^1H, *ddd*, J= 1,9 Hz, 6,5 Hz and 12 Hz,) and δ_H 1,78 (^1H, *ddd*, 1,9 Hz, 8 Hz, and 12 Hz) were assigned to Ha-10 and Ha-1. Whereas the two doublet signals at δ_H 1.41 (^1H, *dd*, 12 Hz, 12Hz) and δ_H 1.36 (^1H, *dd*, 12 Hz, 7.2 Hz,) were assigned to Hb-10 and Hb-1 respectively. The three singlet signals at δ_H 1.16, 1.11 and 0.98 were assigned to H-12, H-14 dan H-15 respectively. The ^{13}C-NMR spectrum showed 15 signals for 15 carbon atoms. Based on their chemical shift values, 9 carbon atoms were assigned to C-sp^3, 4 carbon atoms were assigned to C-sp^2 and another 2 carbon atoms were assigned to carbonyl carbons. The molecular formula was determined to be $C_{15}H_{20}O_2$ by EIMS which showed a molecular ion peak at m/z 232 [M+]. Comparison mass spectrum fragmentation with Wiley7. Library data base, compound 1 had high similarity with velleral compound. On the basis of physical and spectroscopic data presented above, compound 1 was found to be sesquiterpen lactarane which is velleral, ((3aS,8aS)-2,2,8-trimethyl-3,3a.6.8atetrahydro-1H-azulene-5,6-dicarbaldehyde.

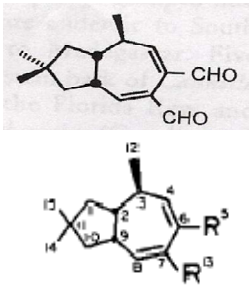

Figure 2. Compound 1

Compound 1 exhibited significant active in the *A. salina* bioassay with LC_{50} = 2.92 g/mL. Compound 1 also showed cytotoxicity against maurine P-388 leukemia cells with LC_{50} = 3.39 g/mL. Furthermore, compound 1 showed activity against gram positive

(+) *S. aureus*, as well as fungus (*C.albicans*) with 16.6 mm and 9 mm diameter inhibition zone at the dose of 25 μg respectively. No inhibition was observed with gram negative (*E. coli*). Even, compound 1 exhibited higher inhibition zone than that of erythromycin against gram positive *S. aureus*.

Table 1. Antimicrobial activity of compound 1.

Compound	Dose (μg)	Diameter of inhibition zone (mm)		
		C. albican	*S. aureus*	*E. coli*
1	25	9	16,6	-
Erytromycin	25	15	14	16
Ampisilin	25	18	22,5	7

The activities of compound 1 mentioned above agree with the previous report by Jonanssonh et al (1995) which proposed that the unsaturated compound of α,β-1,4-dialdehyde has broad activities such as citotoxicity, mutagenic, bactericidal, fungicidal, antifeedant, algaecidal, anti-hipercolesterol, antiinflamantory, pestiside, antitumor (Jonanssonh et al, 1995). In fact, there are many unsaturated compounds of α,β-1,4-dialdehyde already patented and commercially sold as modern medicines such as aframodial for reducing cholesterol in the blood and warburganal for genome virus inactivator (Roel, 2000). This is the first report so far regarding the present of lactarane sesquiterpene velleral in D. beccariana Gibbs. This compound have been isolated and identified in genus Lactarius such as Lactarius vellerius and genus Russula, Marasmius and Lentinius.

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PRELIMINARY STUDY ON *PHAEOMERIA SP* ECONOMIC POTENCY

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Abstract: Experiment to show influence of Phaeomeria sp rhizome extract treatment to germantion of soybean and green peanut had been done at Laboratory of plant ecology, Biology Department, Faculty of Science, University of Sriwijaya, Indralaya, South Sumatera, Indonesia during February 2008. Data analysis shows that Phaeomeria sp had potency on herbicide, ornamental and medicinal plant.

INTRODUCTION

Zingiberaceae family members have been known by South East Asian peoples as spices and medicinal plants. The famous species of them were *Curcuma domestica, Alpinia galanga, Zingiber officinale,* and *Costus speciosus. Phaeomeria* or *Nicolaia* or *Etlingia* , is a genus of Zingiberaceae that have not known much yet, because of their living in the forest. Some of them known because of the form,colour and size of flower; *Phaeomeria speciosa* or *Etlingia elatior* or *Nicolaia speciosa*.

In South Sumatera forest, there is a member of *Phaeomeria* living with specific character; the leaves is two colours, green on upper side and red underneath. Form of rhizome, stem, leaves and flower just like *Phaeomeria speciosa*. Peoples near of forest sometime use the rhizome for medicinal needs, liniment. They said that the rhizome has specific smell and make skin become hot after use as liniment. According to ARS Systematic Botanist, Etlingera/Phaeomeria is a member of Zingiberaceae family, Alpinioideae subfamily, and Alpinieae tribe (Anonymous,2008a)

To show the potency of these plant, in this research we use the extract of rhizome as treatment to germination of soybean and green peanut (*Glycine max & Phaseolus radiatus*). Usage of plants extract as germination test have been conducted by lot of botanist; extract of pine nidles has known inhibit the radicle growth of soybean (Marisa, 1990).

MATERIAL AND METHODS

Rhizome cut and grind and extracted by filtering and made solution with concentration 0 %, 2 %, 4 %, 6 % , by adding aquadest. Ten of soybean seeds placed in a plastic dish with a piece of filter paper. Each treatment was replicated 6 times. Let the

germination process during 4 days under dark condition (in the laboratory bench), and the percentage of germination and length of radicle measured then. Data processed by analysis of variance (F test) to find wether treatments influence the germination percentage and length of radicle. The same test also done to greenpeanut seeds with concentration of rhizome extract; 0 %, 7%, 12,5 %, and 18 %.

RESULT & DISCUSSION

Result of germination test of rhizome extract treatment to percentage of germination and length of radicles of soybean should be seen at below tables.

Table 1. Percentage of germination after rhizome extract treatment (%)

No	Treatment	Mean of Germination percentage
1	Control	88.3
2	2 %	91.6
3	4 %	80.0
4	6 %	68.3

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Table 2. Length of radicle after 4 days germination (cm)

No	Treatment	Length of radical (cm)
1	CONTROL (0%)	4.08
2	2 %	3.25
3	4 %	2.66
4	6 %	1.46

Germination test to green peanut should be seen at below tables.

Table 3. Percentage of germination after rhizome extract treatment (%)

No	Treatment	Mean of germination percentage
1	control	91.6
2	7 %	91.6
3	12.5 %	88.3
4	18 %	88.3

Table 4. length of radicle after 4 days germination (cm)

No	Treatment	Length of radicle (cm)
1	Control	2.485
2	7 %	1.42
3	12.5 %	1.285
4	18 %	1.02

Statistical analysis for above data through analysis of variance at degree of error 5 % should be seen at last page. It is shown that treatments influenced the percentage of germination.

The above data show that extract of Phaeomeria rhizome could inhibite germination of soybean and green peanut seeds. Marisa (1990) has reported that extract of pine nidles also inhibited the germination process of soybean. It is mean that rhizome of Phaemorea has a potency for bioactive like herbicide.

Ornamental; search of cybernet had found that a company from New Zealand, Russell Fransham (exotic and unusual plants landscaping commercial and retail, Clements Road, Matapouri Bay RD 3, Wangarei) sell lot of exotic flower plants, included

Phaeomeria/Nicolaia. They sold it at $ 20 USD/pot (Russsell Fransham, 2008). So, because our plant has two colour of leaves, might be it should more expensive than Russell Fransham plant. It is mean, beside it had chemical bioactive potency, but ornamental potency too.

Medicinalplant; furthermore, because of poeples near of forest use it as liniment, mean Phaeomeria sp with two color of leaves had the potency on medicinal thing. Deeper research could be made by pharmacologs about it. For a while, Anonymous (2008b) reported that Phaeomeria speciosa has some chemical content like saponin, flavonoid, and polyphenol. Culture of Phaeomeria should be done by planting the rhizome, because if we plant their seeds, it needs 2 years duration for germination (Fazlisyam, 2008).

SUMMARY

Phaeomeria sp that living in south sumatera, with two color leaves (green and red) had some potency as economic plant, herbicidal, ornamental and medicinal plant. Culture of these plants and further study should be made.

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APPENDIX:

Analysis of variance on rhizome extract treatment to germination percentage of soyabean (1), soybean length of radicle (2), , and green peanut length of radicle (3). germination percentage of green peanut(4)

1.

Varians resource	df	SS	MS	Analysed F	Tabled F (ά .05)
Treatment	3	19.46	6.49	7.02*	3.10
Error	20	18.5	0.925		
Total	23	37.96			

2.

Varians resource	df	SS	MS	Analysed F	Tabled F (ά .05)
Treatment	3	21.87	7.29	3.82*	3.10
Error	20	38.29	1.91		
Total	23	60.16			

3.

Varians resource	df	SS	MS	Analysed F	Tabled F (ά .05)
Treatment	3	7.453	2.484	6.387*	3.10
Error	20	7.605	0.380		
Total	23	15.058			

4

Varians resource	df	SS	MS	Analysed F	Tabled F (ά .05)
Treatment	3	1.79	0.6	0.37	3.10
Error	20	32.17	1.61		
Total	23	33.96			

VASOACTIVE EFFECTS OF *CONNARUS GRANDIS* LEAVES EXTRACTS ON DIFFERENT STRAINS OF RATS

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Abstract : Vasoactive effect of two fractions (water and CHCl3) of *Connarus grandis* Jack leaves on 7 Sprague Dawley (SD) and 6 Spontaneously Hypertensive Rat (SHR) has been studied. Rats were anaesthetized (pentobarbitone 60 mg/kg intraperitoneal), carotid artery was cannulated to monitor blood pressure and heart rate, and an insition was made on the med-line of abdomen to expose the left kidney. A flow meter probe laser Doppler was placed on the kidney to measure its blood flow (Flowmeter, Power Lab® ADI Instrument). After animal set up is completed, 2 ml of saline was given as commencement and then stabilized for 1 hour. In the first part, the doses-vasoactive responses curve to noradrenaline at doses of 200, 400, and 800 ng/kg BW was made. After 30 minutes resting time, the second dose-response curve was made to water fraction of *C. grandis* at doses of 20, 40, 80, and 100 mg/kg BW. In the last part, the response curve was made to chloroform fraction at doses of 5, 10, and 20 mg/kg BW. All of the drugs were given twice i.v. ly and the vasoactive response was made as kidney perfusion (blood flow). Results showed that the two fractions of *C. grandis* increased kidney perfusion significantly (P>0,05) without significant changed in the heart rate (p>0.1). There was no significant different between responses of SHR and SD rats (p>0.1). These indicated that both water and $CHCl_3$ fractions of *C. grandis* leaves posses vasodilatation effect on both SHR and SD rats. The vasodilatation effect of the water fraction is better as compare to that of CHCl3 fraction.

Key words: *Connarus grandis Jack leaves, water fraction, $CHCl_3$ fraction, renal perfusion, vasodilatation.*

INTRODUCTION

It takes long time or even long-life to therapy hypertension since the disease is diagnosed. Most of anti-hypertensive drug is expensive or unsaved if used for such long time. *Conarus grandis* Jack. Connaraceae is one of medicinal plant originated from Indonesia and has been documented at the Kebun Raya, Bogor. This woody climbing tree that found in Sumatra, Jawa and Bangka (Backer & Backhuizen, 1965) has been used traditionally to treat ashma (Heyne,1950, Anonim, 1986; Goh &Tan, 2000), while Lewis (1977) described that, different genus of Connaraceae family used for oral infection, and according to Burkil (1966), some Connarus genus are used to treat worm infection, fever, asthma and itching. Armenia (1990) found that the ethanolic extract of *Connarus grandis* leaves active as central nervous system depressant, sympatholytic/ parasympathomymetic, muscle relaxant and or vasodilator. These facts were approved by a prolong sleeping time of mice induced by pentobarbitone (Armenia, 1990; Sulastri, 1993; and Yuniar,1997), retracted chicken head (muscle relaxation)(Armenia dan Arifin, 1991), and antagonizes convulsion induced by strychnine nitrate (Armenia, 1992). and has anti-bacterial property (Armenia dan Akmal, 1996). The most important is that the leaves extract decreased BP of normotention rats (Armenia, 1990, Noveri *et al.*, 1994, Permana-Sari *et al*., 1997), and for most of all, the crude ethanolic extract posses a better activity as compared to its fractions or isolated compound.

Resent study reported that the ethanolic extract of *C. grandis* leaves decrease blood pressure of different models hypertensive rats {(spontaneously hyper-tensive rats (SHR) and renal hypertensive (2K1C Goldblat hypertensive) rats)} (Armenia *et al*.,

2008), and NaCl+ prednison induced hypertensive rats (Yuliandra *et al*., 2008) where SHRs were more responsive. Such hypotensive effect may ralated to the sympatholytic and or parasympathomymetic activities of the extract (Armenia, 1990). This fact was approved by Erita *et al*. (2008) whom reported that the ethanolic extract shifted the dose-responses curve of dobutamine induced frog's heart contraction to the right while Sadikin *et al*. (2008) reported that the same extract relaxed the rat illeum smooth muscle.

This most resent study is focused on the vasoactive effects of water and $CHCl_3$ fractions of *C. grandis* leaves on rat. This was done by measuring the rat renal perfution using noradrenaline as comparison.

EXPERIMENTAL

Materials and Methods

An amount of 2 groups of rats were used in this study. The first group was the Spraque dawleys (SD) rats (7 rats) and the second group was SHR (6 rats). The following teatment was done to each group of rats: the rats were anesthethized (pentobar-bital Na 60 mg/kgBW i.p.) and the hair of neck and abdomen were shaved. The insition was made on the neck, and the trachea was cannulated to facilitate spontaneous breathing, and the carotid artery was cannulated to monitor blood pressure (BP) and heart rate (HR) (PowerLab® ADInstrument, Australia). The jugular vein was cannulated infuse normal saline (Perfusor secura FT 50ml, B.Braun). To maintain anesthethise, pentobarbitone Na 2 mg / kgBB/hour was added into saline infusion. The infusion rate was made 6ml/hour (Armenia, 2000; Rathore, 2008). To facilitate spontaneously urination, a cannulae was placed on the bladder. A midline insition was made on the abdomen (Cautery®) and the left kidney was exposed. A laser dopler flow probe was places on the dorsal posterior end of the kidney and then connected to data aquisition (Power Lab®)ADInstruments, Australia). Dose-respons curves of renal perfusion to noradrenalin at doses of 200, 400, dan 800 ng/KgBW, water fraction of *C grandis* at doses of 20, 40, 80, dan 100 mg/KgBB and its CHCl3 fraction at doses of 5, 10 dan 20 mg/KgBB were measured, each were done twice. All drugs were given ivly through jugular vein and 30 resting time (the perfution back to normal) was performed between each dose-respons (Rathore, 2008).

The blood pressure, heart rate were measured, and the percentage increase or decrease of RBP was calculated. The tested drug was said to be vasodilator if it increase the RBP of the rat, and vice versa for the vasoconstriction.

Data Analysis

Renal vasoactive response to tested drug was taken as an average ± standard error (SE). Data was analyzed statistically using two way ANOVA follwed by Duncan Post Hoc Test, and significant was taken at $p<0.05$ (SPSS® for Windows Release 17.0.0).

RESULTS AND DISCUSSION

From 500 g of dried leaves of *C. grandis*, an amount of 44.72 g of ethanolic extract was obtained, and after fractionated and evaporated an amount of 4.5 g and 0.9 g of water and $CHCl_3$ fractions were gained respectively.

The normal renal blood perfusion (RBP) of Spraque dawleys (SD) rats and SHR were not significantly different ($P > 0.1$) with the averages of 258 and 267 bpu (blood perfusion units) respectively. On the other hands, the heart rate of SD rat was lower as compared to that in SHR with the averages for SD and SHR 310 and 388 bpm respectively, and so as the blood pressure (BP) ($p<0.01$), with the average BP of SD and SHR 124 and 160 respectively (Tabel 1).

Table 1. Average un-treated RBP, BP and HR of SD and SHR

Rats	RBP (bpu)	BP (mmHg)	HR (bpm)
SD	258 ± 8	124 ± 11	310 ± 12
SHR	267 ± 20	160 ± 16*	388 ± 10*

HR to NA

Administration of noradrenaline to rats tend to increase RBP significantly ($p<0.1$) with the average RBP at doses of 200, 400, dan 800 ng/KgBW of 7 ± 2%, 11 ± 2%, dan 14 ± 2% respectively. RBP to NA of SD rat was lower as compared to that in SHR ($P < 0,05$) with the averages of 14 ± 2 % and 6 ± 2 % respectively. There was no interaction influence between rat strain and NA doses to BP ($P > 0,1$) (Table 2).

Table 2. Change in HR to NA in SD rats and SHR

NA Doses (ng/KgBB)	% Decrease of HR in		Averages ± SE
	SD	SHR	
200	9 ± 2	4 ±2	$7±2_a$
400	15 ± 2	7± 2	11±2 ab
800	20 ± 2	7± 2	14± 2b
Average ± SE3	14 ± 2 a	6 ± 2 b	

HR to Water Fraction of *C. grandis*

Adminstration of water fraction of *C. grandis* did not influence the HR of the rat ($p>0.1$), and there was no different in HR between SD rat and SHR ($p>0.1$). The average decrease of rat HR treated with the water fraction of *C. grandis* at doses of 20, 40, 80 dan 100 mg/KgBW were 8 ± 3%, 13 ± 3%, 19± 3% a and 22± 3% (Tabel 6). The average HR of SD and SHR treated with water fraction of *C. grandis* were 16 ± 2% and 11± 2% respectively (Tabel VI). There was no significant interaction influence between water fraction doses and rat strain to rat HR ($P > 0,1$) (Table 3).

Table 3. Change in HR to WF in SD rats and SHR

Dosis FA (mg/KgBB)	& Change in HR ± SE1		Rata-rata ± SE2
	SD	SHR	
20	-9.3617	-11.7767	-0.569a
40	-12.6267	-7.7283	-0.178a
80	-16.2067	-10.9683	-3.588ab
100	-26.4683	-14.2	-0.334b
Averages ± SE3	-16.166a	-11.168a	

SE1, SE2 and SE3 were 4, 3 and 2 respectively.

HR to Chloroform Fraction

The influence of $CHCl_3$ fraction of *C. grandis* to the rat HR was similar to that given by water fraction HR ($P>0,1$). The average of rat HR to $CHCl_3$ fraction of C. grandis at doses of 20, 40, 80 dan 100 mg/Kg BW were 13 ± 5%, 13 ± 5% and 12± 5% respectively (Tabel 6). The average HR of SD rat and SHR were -8 ± 3 and $-17 ± 5_a$ respectively (Table 4).

Table 4. Change in HR to CF in SD rats and SHR

Dosis FK (mg/KgBB)	% Change in HR		Averages ± SE
	SD1	SHR	
5	-11	-15	-13 ± 5
10	-15	-11	-13 ± 5
20	2	-26	-12 ± 5
Average ± SE	-8 ± 3	-17 ± 5	

Renal Blood Perfusion (RBP)

Renal Blood Perfusion (RBP) to Noradre-naline (NA)

Noradrenaline at doses of 200, 400, dan 800 ng/KgBW decreased renal blood perfusion significantly (P < 0,05) with the average decrease of blood perfusion unit (BPU) of 13 ± 2 %, 17 ± 2%, dan 32 ± 2% respectively. The RBP to NA of SD and SHR were not significantly different (P > 0.1) with the average decrease of BPU of 22% ± 2 and 21 ± 2% respectively. However, there was an interaction influence between rat strain and NA doses to RBP (p < 0.05). In this situation in SD rat, the decrease of RBP at 200 mg of NA was lower as compared to that showed in SHR. In SD rat the response were then increased with NA doses, while in SHR, the RBP at 400 ng of NA was decreased more but then increased with dose of NA (Figure 1). In addition, the maximum decrease of RBP to the highest dose of NA in SHR was lower as compared to that in SD rat at the same NA dose (Table 5 and Fig. 1).

Table 5. RBP responses to NA in SD rats and SHR

Dosis NA (ng/KgBB)	%Change of RBP in		Average
	SD	SHR	
200	-8	-18	-13 ± 2b
400	-21	-14	-17 ± 2b
800	-37	-32	-35 ± 2a
	22 ± 2a	-21 ± 2a	

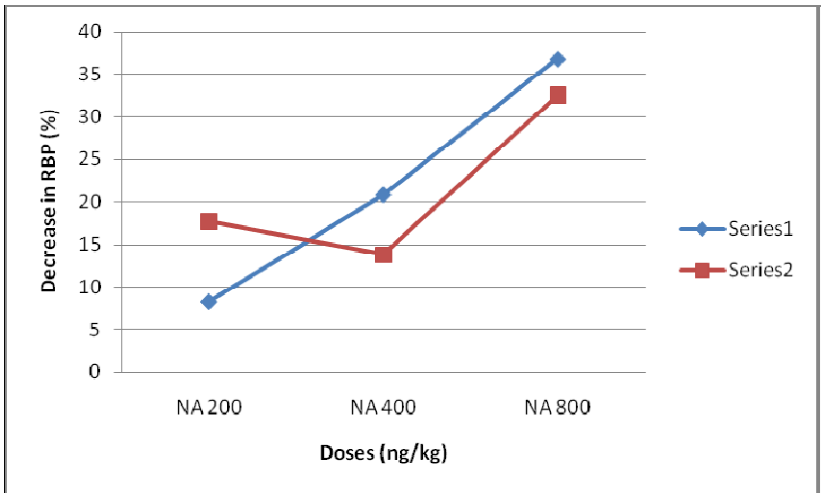

Fig 1. RBP to NA in SD (blue) rat and SHR (red)

Renal Blood Perfusion to Water Fraction of *C. grandis* leaves (WF))

Administration of the water fraction of *C. grandis* leaves increased RBP significantly (P < 0,05) with the averages blood perfusion unit (BPU) at doses of 20, 40, 80 of 7.81 ± 2.29%, 12.88 ± 2.29%, 19.59 ± 2.29% dan 22.31 ± 2.292% respectively

(Figre 1). RBP of SD rat and SHR under the influence of WF were not significantly different (P > 0.1) with the averages of 13.95 ± 1.56% and 17.34 ± 1.68 % respectively (Tabel 3). There is no interaction influence between rat strain and WF doses to renal perfusion (p >0.1) (Fig 2, Table 6)

Table 6. RBP responses to WF in SD rats and SHR

Doses (mg/KgBW)	**% Change of RBP in**		**Averages ± SE**
	SD	SHR	
20	6	9	8 ± 2a
40	13	12	13 ± 2a
80	18	21	19 ± 2b
100	18	27	22 ± 2b
Rata-rata ± SE	14±2a	17± 2a	

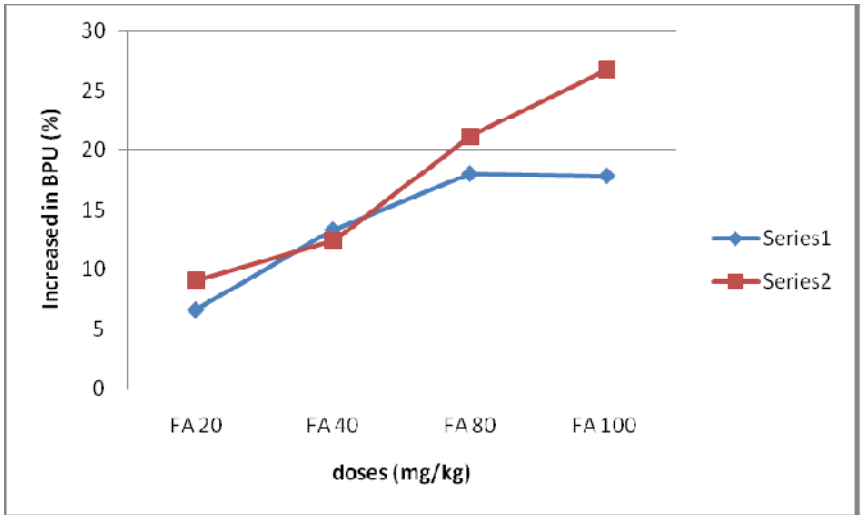

Fig 2. RBP to WF in SD (blue) and SHR (red)

Renal Perfusion to Chloroform Fraction of *C. grandis*

Administration of $CHCl_3$ fraction of *C. grandis* at dose of 5 m10 mg/kg BW increased the RP but when the dose was increased to 20 mg/KgBW, the RBP decreased significantly (P < 0,05). The average increase and decrease of RBP at doses of 5, 10, dan 20 mg/KgBB of chloroform fraction of *C. grandis* were 10.34 ± 3.41%, 10,63 ± 3.41% dan -0.33 ± 3.76% respectively (Figure 1). There was no different RBP between SD rat and SHR and interaction of FC doses and rat strain to RBP (P > 0,1). The average change of BPU in SD rat and SHR were 5.00 ± 2.38 % and 8.96 ± 3.31% respectively (Fig. 3 and Table 7)

Table 7. RBP responses to CF in SD rats and SHR

Doses (mg/KgBB)	**% Change of RBP in**		**Averages ± SE**
	SD	SHR	
5	11	9	10 ± 3a
10	10	11	11 ± 3a
20	-6	6	-0.3 ± 4b
Averages ± SE	5 ± 2a	9 ± 3a	

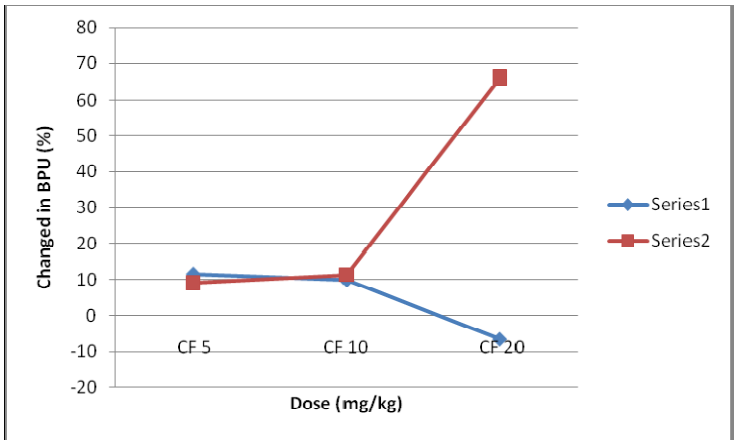

Fig. 3. RBP to CF in SD rat (blue) and SHR (red)

Figure 4 below showed the comparison between averages vasoactive response of water fraction and $CHCl_3$ fraction.

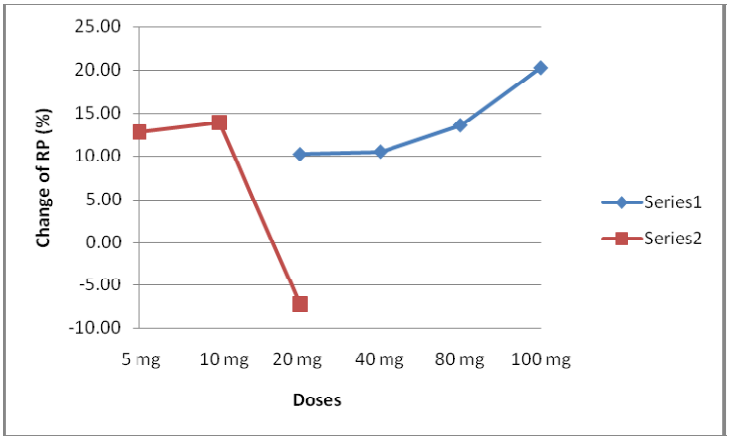

Fig 4. RBP to water (blue) and CHCl3 (red) fractions

DISCUSSION

SD rat and SHR are different in their blood pressure values, where for SD rat of ± 120 mmHg (Armenia, 2008; Porter *et al*., 2007; Yuliandra, 2008), while in SHR of of ± 160 mmHg (Collison *et al*., 2000; Armenia, 2001; and Nugroho, 2006). In this experiment, mean arterial pressure of SD rat and SHR were 124 ± 11,04 and 160,5 ± 16,04 respectively, about similar to those reported before.

Water and $CHCl_3$ fractions of *Connarus grandis* were used in this experiment based on their effectiveness as blood pressure lowering effect as reported previously (Noveri, 1994). Armenia (1990) described that the ethanolic extract of this plant leaves lowered blood pressure of normotensive rats at dose of 10 mg/KgBW and in hypertensive at at doses of 40 mg/KgBB (Armenia, *et. al*, 2008), while Ratih (1994) reported that $CHCl_3$ fraction effective at 5 mg/kg BW. In this study we modified the doses as 20, 40, 80, dan 100 mg/KgBW of water fractionand of 5, 10 dan 20 mg/KgBW for $CHCl_3$ fraction.

Noradrenaline act via adrenergic receptor and constrict blood vessels and was used as comparison. In this study its effect showed as a reduction of renal perfusion (Katzung, 2000; Bellomo & Giantomasso, 2001).

By measuring renal blood perfusion, at the same time we evaluate the blood flow in microcyrculation in a certain periode (Myers, *et.al,* 1994). So far this methode is

seldomly used, but beside it is easy to perform, it is also more sensitif and fulfill the clinical requirement for microvascular blood flow measurement (Leahy, 2001). In addition, with this method the blood flow can be monitored continously (Leahy, 2001; Morales, 2005). At the same time, the rats heart rate and blood pressure were also monitored to control their condition during experiment. All of these parameters were measured using PowerLab® ADInstrument, Australia.

Renal blood flow and heart rate of normotension SD rat and SHR were not significantly different even though the BP of SHR was higher than that in SD. This condition is due to local auto-regulation mechanism (Doohan, 2000., Guyton, 2006) and the kidney itself (Nasr, *et al*., 2005) to maintain the blood flow at the normal level. When the blood pressure is too high, there will be an increase in blood flow and oxygen (Doohan, 2000). As the consequent, the kidney will release some vasocontrictor (Nasr, *et al*., 2005) that constrict the blood vessels and return the blood flow back to normal even though the blood pressure is higher.

In this study, water and $CHCl_3$ fractions of *C. grandis* leaves increased the renal blood perfusion (vasodilatation) on SD rat and SHR. An interesting fenomena found here that the increase in blood flow under the influence of water fraction is doses related, while the effect of $CHCl_3$ fraction was unlikely. In the last condition, $CHCl_3$ at higher dose decreased renal blood flow, even though not significant. This may due to the different chemical composition content of these two fractions. As describe by Cramer and coworkers (1992), Jacobson and coworkers (1993) and some others, that chemical structure is related to its affinity to certain receptor and their activity, that will affect their potency and pharmacology. On the other hands, more than one chemical content in these fraction may act synergic or vice-verse (Warren, 2004). The change of renal blood flow was not followed by a change in heart rate. This result is similar with that reported by previous researcher (Yuliandra, 2007; Armenia, 2008), who found the lowering blood pressure effect of ethaolic extract of *C. grandis* without affecting heart rate on rat.

Armenia (1990), reported that the ethanolic extract of C. grandis leaves possest musscle relaxation effect, parasympa-thomymetic and or sympatholytic activities. These activities are in agreement of the later studies by Armenia and Arifin (1991) with the chicken head retraction effect ; and Sadikin (2008) with the intestinal smooth muscle relaxation effect which can be inhibited by SO4-atropine. These may bring the vasodilatation effect of the fractions that shown in this present study.

From these results, it can be concluded that the water fractions of *C. grandis* leaves dilates blood vessels, while $CHCl_3$ fraction dilates it at lower doses but constricts it when the dose is increased . Further study is needed to eveluate which chemical and receptor(s) are involved in mediating vasoactive effect of these fwo fractions.

ACKNOWLEDGEMENT

This research is sponsored by Fundamental Reseach Grant, DP2M Dikti, 2008.

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SEED GERMINATION AND MEDICINAL PROPERTIES OF *ARECA CATECHU* L.

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Abstract : Palm (Arecaceae family) is a high diversity species, especially in Indonesia. Palm species known are useful as building materials, oil, energy, foods, handicrafts, ornamental plants and medicinal plants. Purwodadi Botanical Garden as an institution for ex-situ plants conservation, its have 12 species of Arecaceae potential as a medicinal plant, one of them is *Areca catechu* L that discuss in this paper. Palm propagation generally depends on seeds. Therefore we need information about seed germination as well as its potential. This research was conducted at the Purwodadi Botanical Gardens with observations in the garden and information about their potential based on literature. The expected results are used as guidelines in conservation and cultivation of palm.

***Keywords*:** Areca catechu, Medicinal Plants, Germination.

INTRODUCTION

Palm in botany classification in Arecaceae family. Palm in the world have estimated 200-300 genera and around 2000-3000 species spread throughout the tropics and sub-tropical (LBN-LIPI, 1978; Witono, 1998; Sharma, 2002; Chin, 2003). Indonesia is the world's center of palm diversity, the palm in the world among 46 genera (576 species) found in Indonesia and 29 genera are endemic (Witono, 1998). Palm widely known to have various uses in life as building materials, household, handicrafts, food resources, oil resources, energy resources, medicinal plants, ornamental plants and environmental conservation.

Purwodadi Botanical Garden as plants conservation *ex-situ* agency, has the duty to conservation, exploration, collection and maintenance of Indonesia plants, especially of lowland dry plants, that have value and potential (Asikin, 2006). One of the most interesting the Purwodadi Botanic Garden collection is the family Arecaceae. Purwodadi Botanical Garden has a collection 358 specimens from 93 species and 54 genera. 12 species of Arecaceae have potential as medicinal plants such as *Actinorhytis calapparia, Areca catechu, Areca triandra, Arenga pinnata, Borassus flabellifera, Calyptrocalyx spicatus, Caryota mitis, Cocos nucifera, Corypha utan, Licuala rumphii, Oncosperma horridum,* and *Phoenix dactylifera.* Potentials medicine derived from the part of palms from roots, stems, bark, sap, fiber, leaf, flower, fruit water, fruit, seed coat and seed (Irawanto, 2008).

In the other hands the effort to management and maintenance of the Purwodadi Botanical Gardens collection have several technical problem, the attack of pests and diseases, the propagation generally depend on seeds, the seed germination requires a relatively long time on a particular type of palm. Therefore, it is interesting to do research, because the potentially as a medicinal plant. This study aimed to observe the seed germination of medicinal palms, i.e. *Areca catechu* L. from the Purwodadi Botanic Garden collection. The information expected to become the basis for conservation research and cultivation of palm species.

METHODOLOGY

Research conducted at the Purwodadi Botanical Gardens that consist of seed germination observation and medicine potential from literature. The secondary data, such

as information of seeds and fruiting rhythm from the Seed Collection Unit, plants database from the Registration Unit. The germination did at greenhouse on the Nursery Unit, since March to July 2010. Seed material collected from the garden and soaked in water for easy germination. Seed sowing on the sand media contain 30 seeds. Observations were made every week, until the germination phase is complete. Result analysis with tables and figures that description of seed germination.

RESULTS AND DISCUSSION

Description of *Areca catechu* L.

Local name: Betel nut palm (En); Pinang (In); Pinang, Pineung (Ac); Batang mayang, Pinang, Pining (Bt); Batang pinang (Mk); Bawah, Ugai, Urai (Lp); Pinang, Gehat, Kahat (Dy); Jambe (Sd); Jambe, Wohan (Jw); Penang (Md); Winu (Sb); Wenyi (Su) Keu, Ehu, Glok (Fl); Wua (Sl); Tilade (Si); Maman, Poposo, Mamaan, Tenga of wua, Nyangan (Sw Ut); Luhuto, Luguto (Gr); Poko rapo (Mk); Alosi (Bg); Pua m Bua, Mpua (Rt); Pua, Bua (To); Bua, Hua, Soi, Pua, Hualo, Kanai, Hua yain (Sr), Soin, Yeli, Fua, Flut, Fifin miha, Bua (Bu); Palin, Mila, Mela (Hm).

Distribution: the exact origin of *Areca catechu* is not known, but most probably it originated from central Malesia where it is known to be of very ancient cultivation and where it is variability of the genus Areca is greatest. Malesia to the Indians cultivated; commonly found in South and Southeast Asia; in Java in 1500 above sea level cultivated.

An erect, slender, unarmed, unbranched, solitary, pleonanthic, monoecious palm, up to 30 m tall with terminal crown of 8-12 leaves. Root system dense but superficial, most roots within a 1 m radius from the trunk in the top 60 cm of soil. Trunk cylindrical, 15-30 m tall, 10-15 cm in diameter, grey-brown, densely and regularly ringed with leaf scars. Leaves arranged spirally, crowded at the trunk top, 1-1.5 m long, paripinnate, sheathing, sheath completely encircling the stem like a tube, 0.5-1 m long, pinnae 30-50, sub opposite, linear to lanceolate, longest in the center of the blade, longitudinally plait d, apex dentate or irregularly incised, dark green. Inflorescence erect, appearing on the trunk below the crown leaves, 30-60 cm long, branched broomlike to 3 orders basally, tertiary branches filiform, spicate, 15-25 cm long, very fragrant. Fruit an ovoid drupe, 3-6 cm x 2-5 cm, orange to reddish, usually 1-seeded, pericarp fibrous, about 6 mm thick. Seed (so-call nut) ovoid, globose or ellipsoidal, 3-4 cm x 2-4 cm, endosperm ruminate with hard reddish tissue from inner integument running horizontally for some distance into pale brown endosperm (Vossen, 2000). The morphological features can be seen in Figure 1.

Figure 1. Morphological characteristics of *Areca catechu* L.

Medicinal Potential of *Areca catechu* L.

In Vossen (2000) uses as medicine: Diuretic, laxative (seed coat); diarrhea, stomach pain, ulcers, Antiseptic (leaf); scabies, diphtheria, menorrhagia, epistaxis, antelmintik, diarrhea, dysentery, dental disease, tonic, increase sexual vitality, lumbago, dysuria (seed), worms, kidney stone, nose bleeds, wounds, skin diseases, tonic (fruit).

According Purwadaksi (2007), parts of plants used for medicine is seed that contain several active substances such as alkoloid, arekolina, arekolidina, arekaina, guvakolina, guvasina, choline, and tannin. Alkaloids are toxic to several species of parasitic worms, his usefulness to treat abdominal pain, diarrhea, intestinal worms, measles, scabies, and ulcers. However, when taken in high doses can kill sperm.

According to Sihombing (2000), betel leaves contain essential oils that can cure sore throat disorders, base and vessels broncial. Young leaves can be bitter medicine muscle aches. Seed is useful for food, industrial raw materials such as fabric dyes and medicines. Areca as potions compiler has entered in the priority list of WHO (World Health Organization) United Nations. Areca nuts have been used as medicine since thousands back century, especially in Egypt. Up to now there are 23 countries that use nuts as a traditional medicine. Areca seed properties such as: drug worm, eczema, toothache, colds, wounds, scabies, diphtheria, menstrual pain, nosebleeds, ulcers, diarrhea, ulceration, and libido enhancer.

According Wijajakusuma (2008) nut as drug worms, digestive disturbances, swelling, whitish, diarrhea, delayed menstruation, cough with phlegm, diphtheria, cleaning and strengthening teeth. dried seed with side effects that may occur is nausea and vomiting, abdominal pain, dizziness and anxiety, to reduce the toxic effects, recommend using seed that has been peeled, boiled, used after a cold.

According Dalimantha (2009) can be eaten as fresh vegetable or pickled, while the fruit is one of the ingredients for betel nut. Leaf used to wrap food, ingredients, and increases the appetite. Seeds bitter taste, spicy, warm and astringent. Nutritious raise qi, antelmitik, carmintive, menstrual shed, diuretic, shed phlegm, improve digestion, laxative, and stop the attacks of malaria. Nut stimulates the release of water saliva and gastric fluids that can improve digestive functions. Fruit taste bitter, warm, astringent, circulation of blood, urine shed (duretik) and laxatives.

Chemical content of 0.3 to 0.7% seed contains alkaloids that work cholinergic, such as arecoline ($C_8H_{13}NO_{2)}$, arecolidine, arecain, guvacoline, guvacine, homoarecoline, and isoguvacine. Also contains 15% condensed tannins, areca red, 14% fat (palmitic, oleic, linoleic, palmitoleic, stearic, caproic, caprylic, lauric, myristic acid), saponins (diosgenin), steroids (kryptogenin, ß-sitosterol), amino acids, choline, and catechin. fresh seed contain about 50% more alkaloids than the palm seed is processed. The skin fruit contains condensed tannins areca nut, arecoline, worked as a drug to paralyze Taenia worms, especially Taenia solium. Arecoline also efficacious, sedative, antiviral, and antifungal. Cholinergic work will increase the secretion and intestinal peristalsis, slow the heart rhyme and reducing blood pressure.

Seeds are used for treatment: intestinal worms (taeniasis, fasciolopsiasis, ascariasis), flatulence caused by digestive disorders (dyspepsia), swelling due to fluid retention (edema), berries, weak legs, feeling full in the chest, wounds, cough with phlegm, diarrhea, stomach pain and heartburn caused by buildup of food is not digested, delayed menstruation, leucorrhoea, malaria, and minimize the pupils (miosis) in glaucoma. Leaves used to treat: no appetite, back ache (lumbago). Coir is used to overcome: digestive disorders such as abdominal bloating (dyspepsia), difficult defecation (constipation), edema and berries for a little urine.

Alkaloid contained in the fruit quite dangerous to the nervous system, commonly occur are nausea, and vomiting (20-30%), abdominal pain, dizziness, and nervous. Drinking cold medicine to reduce the incidence of vomiting. A rare side effect is stomach ulcers are accompanied by vomiting blood. The signs of overdose: a lot of salivation, vomiting, drowsiness, and seizures. Medicine act: do washing the stomach with a solution of permanganate and give potassium atropine injections. To reduce the toxic effects, recommend the use of areca seed that has been dried or even better if the seeds dry areca boiled before drinking and drunk after a cold. Habit of betel nut seeds can increase the incidence of cancer fibroisi submucosal cheek mucosa and buccal cancer.

Purwodadi Botanical Garden Collection of *Areca catechu* L.

Purwodadi Botanical Garden have 37 specimens / individual plants of *Areca catechu* L. as a palm collection, as can be seen in Figure 2. Collections are located on garden / planting location that call vak, such as III.A.I. 42, 79-79a-79b; III.B. 31-31a-31b-31c; III.F. 31-31a-31b-31c; III.F. 43-43a; III.G. 7, 23-23a-23b-23c; III.H.I. 20-20a-20b -20c, 37-37a, 39-39a; XIV.G.II. 11. and I.A. 25-25a; III.D. 56 for *Areca catechu* L. var. *alba* Blume. The plant of *Areca catechu* L. came from seedling Bogor Botanical Gardens, propagation of seeds Purwodadi Botanical Garden, and from Indonesia flora exploration to Nasional Park of Rawa Aopa Watamohai Kendari in Southeast Sulawesi; Buton Island in Southeast Sulawesi; Nasional Park of Morowali Poso, Central Sulawesi; Nasional Park of Bone Dumago, North Sulawesi; and Supriori Mountain Biak Papua. Collection of *Areca catechu* L. in Purwodadi Botanic Garden can be seen in Figure 2.

Figure 2. Purwodadi Botanic Garden Collection of *Areca catechu* L.

Seeds Germination of *Areca catechu* L.

In general characterization of seeds can be divided into three types: (1) orthodox (2) recalcitrant and (3) intermediate (Hong, 1998). The character is used to determine appropriate storage conditions and storage time. Orthodox is the term used to describe seeds that can be dried until low moisture content (5%) and successfully stored at low temperature or below freezing temperatures in the long term. While recalcitrant is the term used to describe seeds that can not survive in the dry condition at a relatively high humidity (30-40%) and are not tolerant of low temperatures, where the seeds quickly lose viability (ability to germinate) and can not be stored in long time (Schmidt, 2000).

Palm seeds are generally recalcitrant (Baskin, 1998) while according to Smith (2003) is not recalcitrant or may be intermediate. However the characteristic of palm seed is very diversity. Because geography and ecology is very wide and most are still not clearly known characteristics of the seeds (Hong, 1998) and has not been much research on palm seed fields.

Seeds of *Areca catechu* L. collected from the fruit that fell on the ground, based on seed collecting data in September, December (2005), January, February, August, September, November (2006), January, March (2007), January, February, August (2008). *Areca catechu* L. is palm species that fruit rhythm disaffected by the climate, because almost all year fruiting with a large quantity (Irawanto, 2009). This is an advantage of *Areca catechu* L. can fruit without knowing the season, its produce regularly and continuously (Sihombing, 2000).

The fruit productivity of *Areca catechu* L. from one tree at least 350 each year. Where a young tree can fruit after 4-6 years age, each year can be harvested 4-6 times and typically consists of 20-60 units. The productivity period at 10-15 years and even up to 20 years, if more than 20 years generally will decrease (Lutony, 1993).

Seed sowing direct on sand media in a greenhouse, during germination keep media in warm and humid condition. Before sowing, seeds are soaked in water for easy germination. Seed do not need to be removed from fruit or disposed coat seed, seed covered with soil as thick as 10 cm, and to prevent rain water affected or sunlight direct

exposed, give shade and watering every day (Lutony, 1993). Figure of fruits or seeds and seedlings of *Areca catechu* L. can be seen in Figure 3.

Figure 3. Fruits, Seeds and Seedling *Areca catechu* L.

Seeds of *Areca catechu* L. is large. Size of seed is play a importance in germination, which large seeds will have stored food reserves more and can be used for germination at the early growth phase (Baskin, 1998).

Germination of palm marked by the emergence of new shoots that generally hypogeal. Palm germination types can be classified into three types: (1) non-ligular remote, (2) remote ligular, and (3) adjacent ligular (Tomlinson, 1990) can be seen in Figure 4. Type the remote if it is marked by the expansion of the cotyledon axis that are developing positively geotropis. Where is the expansion of cotyledons develop into plumule at some distance from the seed. Ligule appearance of instability, called non-ligular remote germination, if there is a remote ligular ligule. While adjacent ligular is not marked with the expansion of the layer of cotyledon. The cotyledons grown with ligule. Where germination type *Areca catechu* L. is the adjacent ligular.

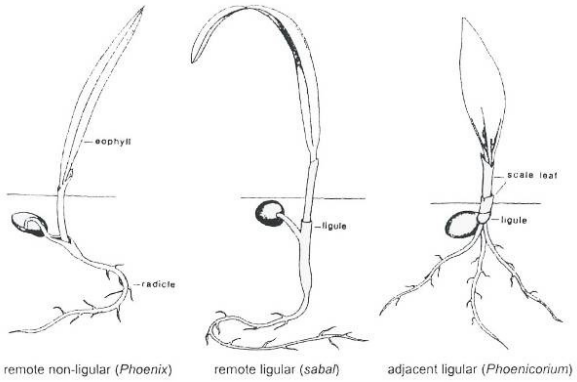

Figure 4. Germination type Palem

Seeds of *Areca catechu* L. has viablitas 80-100%, which germinated only at 75 days after planting, it is possible to occur during dormancy, both dormasi morphology, physiology and mechanics. Generally MPD (Morpho-physiological dormancy) can be solved, for dormancy and morphology of temperature 28 ^{o} C, for physiological dormancy temperatures 39 ^{o} C and mechanical dormancy of hard seed coat which can be broken down with water. In some palms in tropical regions of the embryo will grow slowly after spreading seeds will germinate quickly, because the optimal temperature and the availability of adequate water (Baskin, 1998). Therefore, germination of *Areca catechu* L. before sowing is soaked in water for 14 days. The soaking process can provide the stimulus to accelerate seed germination and ready for planting (Robinson, 2002). The results for seed germination of *Areca catechu* L. 20 days faster than without soaking.

In palms there are six phases of germination: (1) operculum appear, (2) button, plumule, cotyledon appeared, the emergence of radicle or first leaf scale, (3) the

emergence of second leaf scale, (4) eophyll growth, (5) development eophyll single or bifid and (6) growth of first leaves (Latifah, 2004). Where the phases of germination of *Areca catechu* L. can be seen in Figure 5. with a description of the first phase is marked by the emergence of operculum, around 5-10 days; the second phase is marked by a clear plumule emergence of radicle followed until the first leaf emerged scale, about 20-25 days; third phase of the first scale leaf scale leaf growth followed by a second elongated, about 5-10 days; phase The fourth is marked by the growth of eophyll appeared to elongate, approximately 5-10 days; fifth phase is the development of eophyll shaped bifid, about 5-10 days and the sixth phase is marked by the first leaves appear, the first leaf growth until the second leaf appeared, approximately 20 days.

The seed germination of *Areca catechu* L. from starting until last phase time needed about 75 days.

Figure 5. Germination Phase of *Areca catechu* L.

CONCLUSION

Areca catechu L. is a palm species that have potential as a medicinal plants. Seeds of *Areca catechu* L. are large and high viability (80-100%). Type germination is adjecent ligular with germination phase during 75 days that the germination begin 55 days.

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THE BIOACTIVITY TEST OF MANGOSTEEN (*GARCINIA MANGOSTANA* L. GUTTIFERAE) PERICARP AGAINST *STAPHYLOCOCCUS AUREUS* AND *ESCHERICHIA COLI* BACTERIA

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Abstract The extracts of mangosteen (*Garcinia mangostana* L. Guttiferae) pericarp obtained by water, ethanol and methanol distillation and the fractination extract using *n*-hexane and ethylacetate. The simplisia was phytochemically screen, and the antibacteria activity of the extracts were tested against various bacteria caused diarhea and dysentery diseases such as *Staphylococcus aureus* and *Escherichia coli.* Method used for the test is agar disc diffusion. The result showed that mangosteen pericarp contains of chemical compounds such as flavonoids, saponin, tannin, quinone and steroid/triterpenoid. The antibacteria activity test showed the inhibition zones. It indicated that the extracts has a good potential to inhibite growth of those bacteria.

Keywords : Mangosteen pericarp, Bioactivity test*, Staphylococcus aureus , Eschericha coli*

INTRODUCTION

Mangosteen (*Garcinia mangostana* L.) is one of the plants in Guttiferae family. The *Garcinia mangostana* fruit pericarp with dark red colour content of chemical component xanthone has been potently as antioxidant, antifungi, antivirus, do as natural antibiotic and increase the immunity sistem (Suksamrarn *et al.*, 2003).

Indigenous medical practitionist use these fruit pericarp for treatment of stomach bacterial infections such as diarrhea and dysentery diseases, and used to treat painful menstruation. Now these fruit pericarp also used as treat of cervix cancer.

This research used dry pericarp which extracted with water, ethanol and methanol. The present study is aimed to determine the bioactivities of all extracts of this fruit pericarp possessed antibacterial activity against *Staphylococcus aureus (*ATCC 25923) and *Escherichia coli (*ATCC 25922) used agar disc diffusion method

MATERIALS AND METHODS

Plant material

The pericarp of mangosteen fruits were collected from Bogor, then washed and cleaned of dirt, removed the stems and thinly sliced dried with 45ºC temperature drying oven for 3 days until dried with 3% moisture content. The dry mangosteen pericarp were powdered and extracted by aquadest, ethanol and metanol, and fractinated with *n*-hexane and etil acetate solvent. All of the extract were then collected and stored in incubator at 4ºC.

Determination of phytochemistry content

The chemical components of mangosteen pericarp was determined by a Hewlett-Packard 5890 series II plus gas chromatography Hewlett-Packard 5972 series mass selective detector. The operating parameters were as follows: Column: HP 5 MS 30 m. x 0.25 mm x 0.25 mm; inlet temperature 250ºC; detector temperature 280ºC; inject volume

1µl; column temperature 80ºC-280ºC; rate 15ºC/min. The spectra were recorded and compared with the library.

Bioactivity tests

Brine shrimp lethality assay

The extracts of different concentrations of 0.1, 0.05, 0.025, 0.01, and 0.005 mg/ml of mangosteen pericarp extract were prepared by dilution all of the extract with DMSO (dimethylsulfoxide) and sea water. Three replications of each concentration of sample were tested for brine shrimp lethality bioassay as described by Meyer *et al* (1982). LD_{50} values were determined using the probit analysis method (Finney, 1971).

Antibacterial activity

Antibacterial activity of all of the extracts were assayed by the agar disc diffusion method (Jawetz, *et al.* 1986) against Gram-positive bacteria (*Staphylococcus aureus* ATCC 25923) and Gram-negative bacteria (*Escherichia coli* ATCC 25922) .These bacteria was kindly provided by the laboratory for Medical and Pharmaceutical Technology, Agency of Assesment and Application of Technology (BPPT).

Briefly, a sterile paper disc (8 mm diameter) impregnated with 10 µl of an extract was placed on the surface of each plate previously inoculated with bacterial inoculum and incubated for 24 h at 37ºC. Disc of Chloramphenicol 10 µg used as positive controle. The results of agar disc diffusion assay were evaluated by measuring the inhibition zone diameters (in mm).

Antioxidant activity

The mangosteen pericarp extracts were studied for the ability to scavenge the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical by comparison with a well known synthetic antioxidant, butylated hydroxytoluene (BHT).

Briefly, a portion of sample solution was mixed with the same volume of 6 x 10-5 M DPPH in methanol and allowed to stand at room temperature for 30 minutes. The absorbance were then measured at 520 nm (Hatano *et al*., 1989).

RESULTS AND DISCUSSION

The mangosteen pericarp collected from the field screened to content alkaloids, flavonoids, saponins, quinons, tannin, steroid/triterpenoids and volatile oil. The chemical compounds of the mangosteen pericarp exhibited dard red colour. As shown in Table 1, the chemical components of mangosteen pericarp were found to be flavonoids, saponins, tannins, quinones and steroid/triterpenoids. Among them, the compound that exhibited the highest content was flavonids such as xanthone (123,97 mg per 100 ml of mangiosteen pericarp fresh water). This component has been reported to show many biological activities, such as antioxidant, antifungi, antivirus, natural antibiotic, increase the immunity sistem and anticancer (Suksamram *et al*., 2003).

Another report indicated that the main components of mangosteen pericarp were containt Xanthone (3-Isomangostin, Alfa mangostin, Gamma-mangostin), Garcinone A, Garcinone B, C, D dan garcinone E, Maclurin and Mangostenol (http://mzeden-blog.blogspot.com/). These results are somewhat different from our result which might imply that the climatic, habitats and geographic conditions in different areas may affect the production of chemical components.

Table 1. The chemical components of mangosteen pericarp

No.	Components	Notes (+/-)
1	Alkaloid	-
2	Flavonoid	+
3	Saponin	+
4	Tannin	+
5	Quinone	+
6	Steroid / Triterpenoid	+
7	Volatile Oil	-
8	Coumarin	-

For brine shrimp lethality assay (BSLT), the chemical compound of mangosteen pericarp give appreciable activity against brine shrimp lethality test as shown in Table 2 below.

Table 2. The LD_{50} value of BSLT of mangosteen pericarp extract

No.	Extract	LD_{50} value of BSLT (mg/ml)
1	Extract I	10
2	Extract II	22
3	Extract III	23
4	Extract VI	5
5	Extract V	8
6	Extract VI	8
7	Extract VII	21

Notes :

Extract I	: Aquadest extract
Extract II	: Ethanol extract
Extract III	: Methanol extract
Extract IV	: *n*-hexane fraction from ethanol extract
Extract V	: Ethylacetate fraction from ethanol extract
Extract VI	: *n*-hexane fraction from methanol extract
Extract VII	: Ethylacetate fraction from methanol extract

The result indicated that chemical compound of mangosteen pericarp might possess some physiological activities since this chemical compounds were toxic to brine shrimp.
Regarding antimicrobial activity, the extracts of mangosteen pericarp exhibited marked activity against Gram-positive and Gram-negative bacteria, by using agar disc diffusion method (Table 3). The result revealed that the extracts possessed marked antimicrobial activity against Gram-positive bacteria and against Gram-negative bacteria with the maximal inhibition zones 14 mm for *Staphylococcus aureus* bacteria and 16 mm for *Escherichia coli* bacteria six hours after incubation. This inhibition zones was smaller

than that of standard antifungal Chloramphenicol (diameter = 20 mm.). It is because that the extracts contain many components which enhanced and reduced the growth effect of the bacteria.

Tabel 3. Diameter of inhibition zone (mm) antimicrobial activity of mangosteen pericarp extracts

Extract of mangosteen pericarp (1 g/ml/, 10 µl/disc)	*S. aureus* ATCC 25923 (Gram-positive bacteria	*E. coli* ATCC 25922 (Gram-negative bacteria)
Extract I	14	16
Extract II	11	13
Extract III	8	11
Extract IV	10	9
Extract V	12	13
Extract VI	11	12
Extract VII	12	13
Chloramphenicol (10 µg/disc)	20	20

The antioxidant activity (DPPH assay), the chemicals components of mangosteen pericarp was active at concentration 100 µg/ml. The hRF value of these oil was 52.5%. It shown this plant may be useful for other disease not only caused by bacteria.

In conclusion, the main components, could be used as a biomarker for standardization of this plant and the results of bioactivities suggest that the chemical compounds could to be used for treatment of some microbial infections, which also agrees with the traditional use of this plant in treatment of those bacterial-derived stomach diseases, such as diarrhea and dysentery diseases. Moreover, mangosteen pericarp should also be subjected to more elaborated bioassay for specific pharmacological activities.

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PHYTOCHEMICAL STUDY OF KETAPANG BARK (*TERMINALIA CATAPPA* L.)

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Abstract: *Terminalia catappa* L. is a tree which is known in Indonesia as ketapang or in other countries like England as tropical/indian almond. All part of ketapang used as traditional medicine and its pharmacological activity had been reported by many researchers. In this present work, the phytochemical study was covered material preparation, extraction, fractionation, characteristic determination of crude drug and extract, separation, purification and identification of isolated compound. The bark was extracted by reflux with 95% ethanol. An amount of extract was dissolved in hot water, then fractionated by liquid-liquid extraction (LLE) using n-hexane, chloroform, and ethyl acetate respectively. The extract and fractions analyzed by thin layer chromatography (TLC) and showed that n-hexane fraction had an interested compound. Further separation and purification of n-hexane fraction by combination of classical column chromatography and preparative TLC, have got isolated compound BK. Characterization of BK by ultraviolet-visible spectrophotometry, infrared spectrophotometry, liquid chromatography-mass spectrometry (LC-MS/ESI), and nuclear magnetic resonance spectroscopy (^{1}H, ^{13}C, HMQC and HMBC) identified that BK was steroidal compound with 390.20 (m/z) of molecular weight and $C_{27}H_{34}O_2$ of molecular formula, and predicted have aromatic ring in A and B position and acetoxy group in C-3 position.

***Keywords*:** Ketapang, *Terminalia catappa* L., phytochemical study, steroids

INTRODUCTION

Terminalia catappa L. are naturally widespread in subtropical and tropical zones of Indian and Pacific Oceans and planted extensively throughout the tropics. In Indonesian archipelago, ketapang grown wild at low altitude, seashore or near by littoral area of Java Island until 800 m above the sea level and cultivated usually to take the fruits (Heyne, 1950; Thomson and Evans, 2006).

The leaves of this Combretaceous plant were widely used as a folk medicine in Southeast Asia for dermatosis and hepatitis. A lot of pharmacological studies have reported that the extract of leaves and fruits have anticancer, antioxidant, anti-HIV reverse transcriptase, anti-inflammatory, antidiabetic and hepatoprotective activities. This plant was popularly known as 'deshibadam' in Ayurvedic medicine. Juice of young leaves are employed externally in ointment for leprosy and scabies and internally for colic and headache. In addition, the seed also have aphrodisiac activity (Jing *et.al,* 2004; Ratnasooriya and Dharmasiri, 2000; Tenpe *et al.,* 2007).

In Indonesia, ketapang bark was empirically used on medication of dysentery. The kernel seed used for laxantia, and the leaves for treatment of rheumatism, headache, colic, tanning and dye black agent on fabrican cloth (Heyne, 1950; Kasahara and Hemmi, 1995).

All ethanol extract of 12 species of Terminalia genera (Combretaceae) leaves have shown an antimicrobial activity minimal against three of *Escherichia coli, Staphylococcus aureus, Candida albicans* and *Microsporum gipseum*. Further research showed that ethanol and water extract of fresh fallen leaves gave the largest inhibition diameter against the same microbe and *Pseudomonas aeroginosa, Trichophyton*

mentagrophytes, Pityrosporum ovale and *Epidermophyton flocosum*. Furthermore, antifungal testing showed that ointment with 10% of this extract healing up on rabbit's skin infected by *Epidermophyton flocosum* and *Candida albicans* (Suganda, A.G. *et al.*, 2004, 2006, Sukandar, E. Y. *et al.*, 2007).

Ketapang contains several chemicals that distributed on every parts of this plant. The kernel seed consist of fatty acids and tannins like punicalin, punicalagin, terflavins A ,B, and tercatein. The dried fallen leaves was present of flavone glycosides like apigenin 6-*C*-(2²-*O*-galloyl)-b-*D*-glucopyranoside,apigenin-8-*C*-(2²-*O*-galloyl)-b-*D*glucopyranoside, isovitexin, vitexin, isoorientin, and rutin. (Lin Hsu and Ta-Chen, 1999; Heyne, 1950; Lin *et al.*, 2000). Phenolic compounds were found in root, fruit shell and bark. The fruit had also cyanidin-3-glucoside and corilagin. The bark possessed of gallic acid, ellagic acid, 2,3-(*S*)-HHDP-D-glucose, casuarinin, castalagin, grandinin, castalin; 3-methoxy-4-hydroxy phenol-1-*O*-b-D-(6-*O*-galloyl)-gluco side; 3,5-dimethoxy-4-hydroxyphenol-1-*O*-b-D-(6-*O*-galloyl)glucosede;(-)-epicatechin-3-*O*-gallate;(-)-epigallocate chin-3-*O*-gallate, procyanidin B-1; 3-*O*-galloyl procyanidin B-2; acutissimin A and eugenigrandin A (Lin et. al., 2000; Thomson and Evans, 2006; Nagappa *et al.*, 2003).

Phytochemical study of ketapang bark original species from Indonesia has not been reported. Previous screening indicated only the presence of flavonoid, tannin, saponin, kuinon, and mono/sesquiterpene (Sumintir, 2008). These research was done to provide basic chemicals reference of ketapang and hopefully an isolated compound would intended to be direction for further elaboration.

METHODS AND RESULTS

Materials and tools

Materials: ketapang bark, 95% ethanol, aquadest, n-hexane, chloroform, ethyl acetate, toluene, ether, acetone, methanol, amilalcohol, hydrochloric acid, sulfuric acid, nitric acid, boric acid, citric acid, acetic acid glacial, formic acid, formaldehyde, ammonia, bismuth-subnitric, mercury(II)chloride, alumunium (III) chloride, chloral hydrate, kalium hydroxide, sodium acetic, magnesium powder, alumunium foil, precoated silica gel GF254, and silica gel 60 (0.063-0.200 mm) for column.

Tools: grinder, reflux, rotavapor (BUCHI), electric dryer (MITSEDA HD 350), separatory funnel, freeze dryer (EYELA FD 81), microscope (OLYMPUS CX31), crucible porcelain, furnace (CARBOLITE), toluene-distillator, classical column chromatography, common glassware in laboratory, high performance liquid chromatoghraphy (HAWLETT PACKARD 1100), ultraviolet-visible spektrophotometer (DESAGA), infrared spectrophotometer (FT/IR JASCO 4200), Liquid Chromatography-Mass Spectrometry /Electron Spray Ionization (LC-MS/ESI), Mariner Biospectrometry), and nuclear magnetic resonantion spectrometry (JEOL JNM ECA 500).

Sample preparation

The bark from main wooden rod of ketapang, was collected on September 2008 from local area of Indonesian Institute of Science (LIPI) Bandung. The Material was sorted, cleaned, cuted in small quadrangle (2.5-3.0 cm), air-dried then heated in oven (40°C), and finally grinded.

The plant was identified in Herbarium Bandungense, School of Science and Biological Technology (SITH) ITB, that belonged to Combretaceae, species *Terminalia catappa* L, with sinonim name *Terminalia moluccana* Lamk.*, Terminalia procera* Roxb.*,* and *Terminalia mauritiana* Blanco.

Extractions

Powdered crude drug was Extracted by reflux using 95% ethanol (6 hour). Filtrat was condensed first using vacuum rotavaporator then electric dryer. A 973.04 g of extract

was obtained from 4 kg of crude drug, equivalent with 24.32% (w/w) of extractive matters (rendemen).

Fractionation

Fractionation conducted by liquid-liquid extraction (LLE) using n-hexane, chloroform, and ethyl acetate respectively, where the extract was dissolved in aquadest previously. About 200g extract fractionated resulted in 2.81 g n-hexane fraction (1.4%); 1.04 g chloroform fraction (0.52%); 1.50 g ethyl acetate fraction (0.75 %) and 102.85 g of water fraction (51.42%).

Determination of paramater quality

Crude drug and extract were determined by strandard procedure (Ditjen POM, 1987, 1979), parameters was covered water-soluble extractive matters, ethanol-soluble extractive matters, loss on drying, total ash content, water-soluble ash content, acid-insoluble ash content, and water content. Specifically to crude drug followed by macroscopical and microscopical analysis.

Macroscopical analysis of katapang bark showed the appearance of cuted bark in long fibrous layer, hard, thick (2-5 mm), visible like soft path in inner surface and outer surface was rugged, wrinkled, fractured, brown color and specific odor. Powdered crude drug have cream-pale brown color. By microscopical analysis fragments identified were shclerenchime, parenchime, fibrous bark, rocked cells, amylum, oil cells, and chrystal of rossette oxalate. The result of determinations are available in Figure 1 and Table 1.

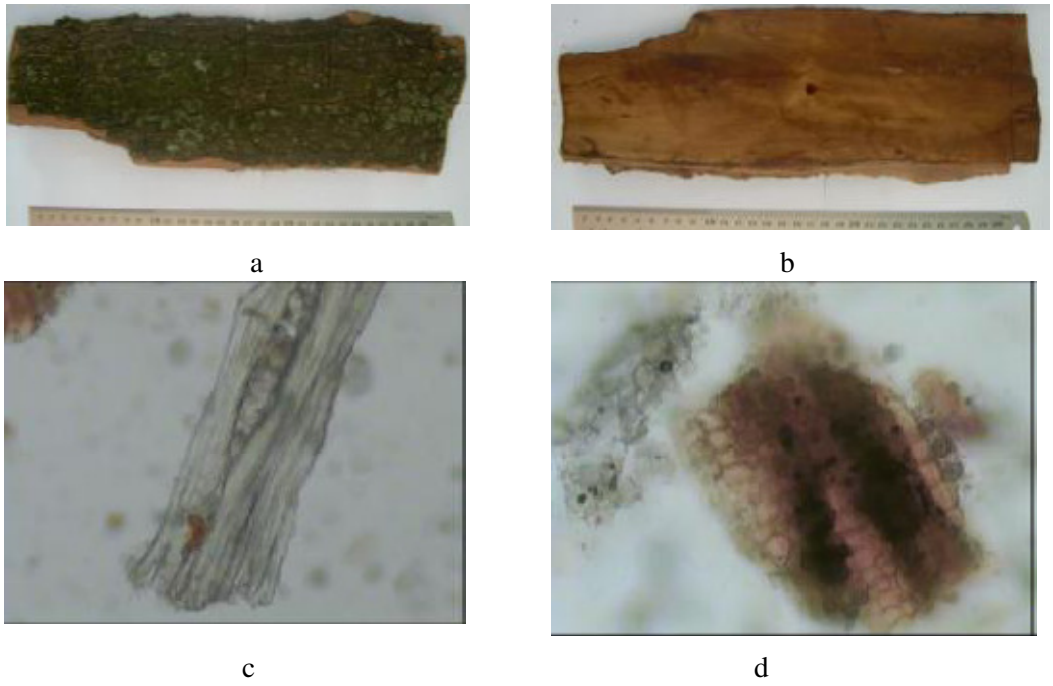


Figure 1 Characteristic of macroscopic and microscopic

Note :

(a) Outer-(b)inner-surface (c)fibrous bark(d)oil cells and chrystal of rossette oxalate in parenchime

Table 1 Characteristic of Quality

Parameter	Content % (w/v)	
	Crude Drug	**Extract**
Water-soluble extractive matters	14.50	49.45
Ethanol-soluble extractive matters	19.00	64.55
Loss on drying	10.54	21.63
Total ash content	15.07	1.67
Water-soluble ash content	4.50	0.38
Acid-insoluble ash content	11.09	1.27
Water content (% v/w)	8.88	17.85

Chemical content investigation

Chemical content investigation was done by phytochemical screening and thin layer chromatography (TLC).

Phytochemical screening

Phytochemical screening were determined from crude drug, extract and all fractions of LLE, that covered alkaloid, flavonoid, kuinon, saponin, tannin, steroid dan triterpenoid using standard procedures (Ditjen POM, 1979). The results are available in Table 2.

Table 2 Result of Phytochemical Screening

Compounds	I	II	III	IV	V	VI
Alkaloid	-	-	-	-	-	-
Flavonoid	+	+	+	-	+	+
Kuinone	+	+	+	+	+	+
Saponin	+	+	+	-	-	-
Catechin	+	+	+	+	-	-
Gallotanin	+	+	+	+	-	+
Steroid/Triterpenoid	+	+	-	+	+	+

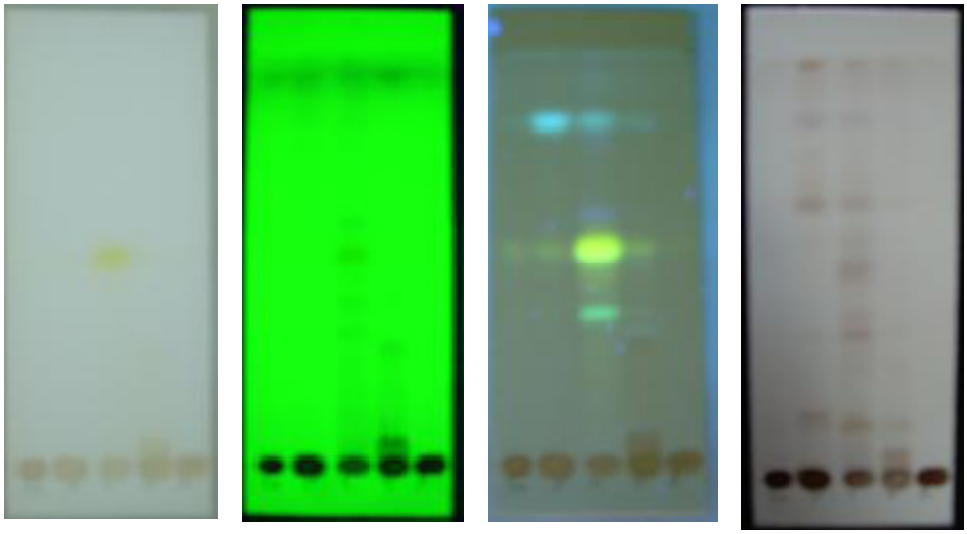
Note:

I=crude drug; II= ethanol extract ; III=water fraction ; IV = n-hexane fraction; V = chloroform fraction; VI= ethyl acetate fraction; + = present; - = absent

Thin layer chromatography (TLC)

Ethanol extract and LLE fractions were investigated by TLC using precoated silica gel GF254 and toluene:ether (1:1) solvent mixture. Chromatogram was observed under 254 nm, 366 nm and after 10% of sulfuric acid as spray reagent. Revealed the profile or numerous chemical contents of ketapang bark marked by every spots.

In Figure 2 showed that are two spots which have dominan in intensity. They're observed under UV 366 nm, as greenish-blue flourescence spot ('biru kehijauan'=BK) in n-hexane fraction (Rf 0.78-0.85) and yellow flourescence spot (Rf 0.45-0.54) in chloroform fraction. At this research, for further process initially focused on n-hexane fraction to isolate the greenish-blue flourescence spot ('biru kehijauan' = BK).



a b c d

e

Figure 1 Chromatogram of extraction and fractionation result

Note:

Sample: (1) extract, (2) n-hexane fraction, (3) chloroform fraction, (4) ethyl acetate fraction, (5) water fraction. **condition**: precoated silica gel GF254, eluen: toluene:eter (1:1) solvent mixture, **observed** under (a) visible (b) UV 254 nm (c) UV 366 nm (d) visible with 10% of sulfuric acid (e) UV 366 nm with 10% of sulfuric acid

Separation and purification of fractions

Classical column of chromatography (CC) and preparative TLC was chosen for further separation and purification of BK in n-hexane fraction. n-hexane:ethyl acetate (11:1) solvent mixture used as mobile phase, where BK identified in Rf 0.35. Classical column of chromatography was conventionally prepared as follow: about 450 ml of eluen; 30.14 g silica gel for column; size 2 cm of column diameter; 17.5 cm of column heigh after stable. Sample 1.03 g of n-hexane fraction putted down carefully on surface column to formed a flat layer. Elution process began slowly until interested compound fully separated, this process monitored by TLC.

From CC obtained 43 of sub fractions where BK was concentrated in 17-26 sub frations. Base on similarity pattern of chromatogram TLC then clasified as grouped 17-18 of sub

fraction (4.2 mg), 19-22 sub fraction (8.2 mg) and 23-26 sub fraction (7.3 mg). Each groups then purified by preparative TLC under the same condition. BK on band shape then removed or scraped from the plate, redissolved with eter, liquid phase decanted, air-dried or placed in acid room to remove the solvent, residue was BK.

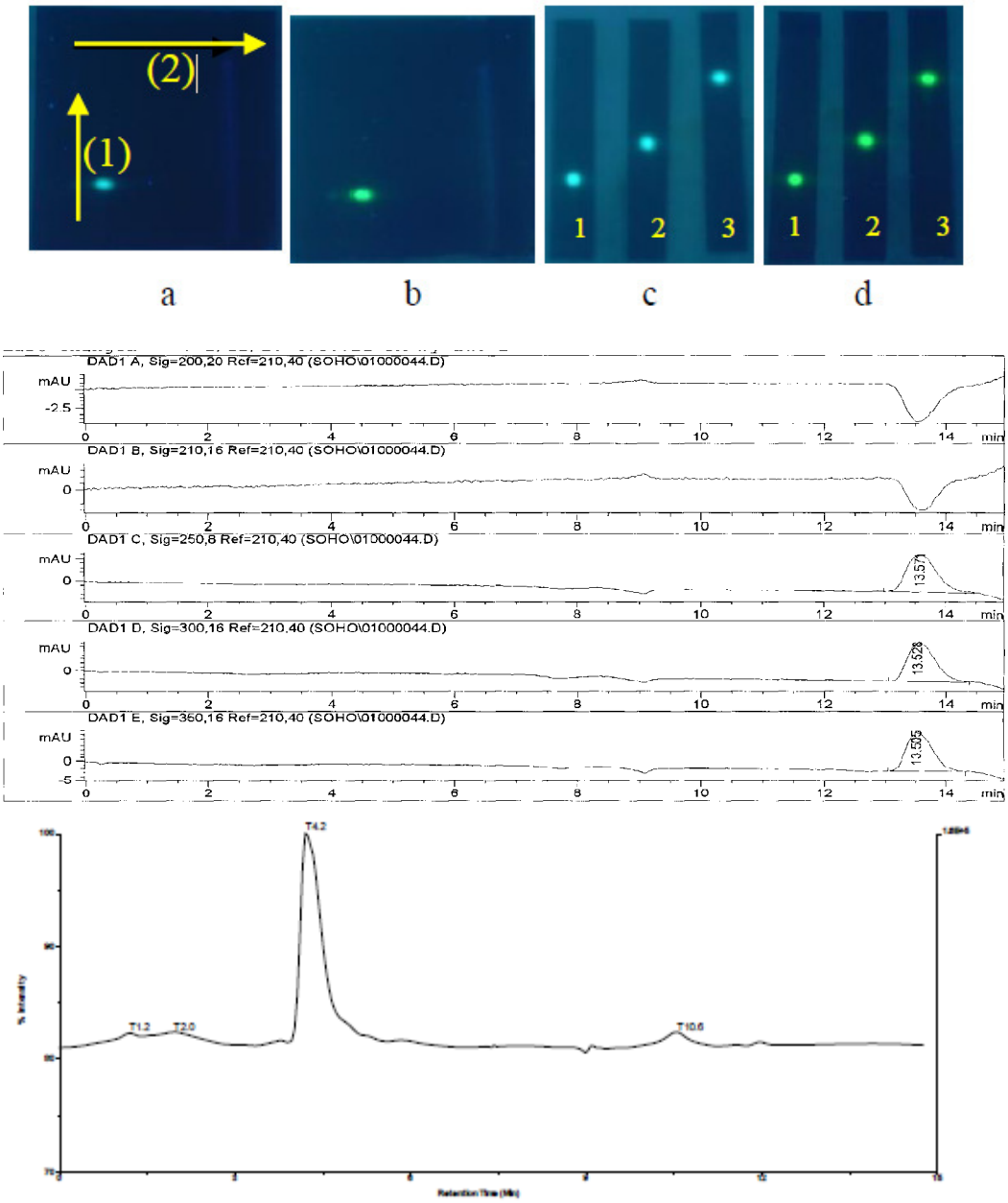


Figure 2 Chromatogram of purity tests

Note: **TLC:** stationary phase: precoated silica gel GF 254, mobile phase (1) n-hexane:ethyl acetate (11:1) solvent mixture and (2) n-hexane:chloroform (3:7) solvent mixture (3) chloroform:ethyl acetate (9:1) solvent mixture, obseved under UV 366 nm (a and c), UV 366 nm with 10% sulfuric acid (b and d). **HPLC :** ODS hypersil column, mobile phase methanol (100%), flow rate 1 ml/minute, DAD detector, temperature 25□C, stop T 15 minute. **LC(LC-MS/ESI):** mobile phase: methanol:water (80:20) solvent mixture; Injection Volume 20 □l; flow rate 1 ml/minute; C18 (RP 18) Supelco column, size 150mm of lenght, size 5 µm of particle. System used: ESI (*electrospray ionization*), positive ion mode.

Purity of isolate BK was checked by two dimensional TLC, TLC with three diffrerent mobile phase, HPLC and LC (connected with MS in LC-MS/ESI). On each chromatogram appeared only one spot with different value of Rf or Rt during observation under their specific condition. These may conclude that isolate BK was pure. In chromatogram resulted by LC-MS/ESI, showed that isolate BK was dominan of peak although small peak of impurities stil exist. After direct calculation on data, area under curve was 77.45% of precentage. It's also figure out the purity level of isolate BK. Chromatogram are available in Figure 3.

Characterization of Isolated compound

Isolate BK visually like a stiky mass, fair white and almost transparent when dissolved. It's soluble in ethanol, methanol and ethyl acetate but more soluble in chloroform, n-hexane, diethyl ether. Monitored by TLC, BK appeared as greenish-blue flourescence spot ('biru kehijauan'=BK) under UV 366 nm, but under UV 254 nm this invisible. After sprayed with Liebermann-Burchard (LB) reagent, BK turned into green flourescence spot under 366nm, this also appearance BK after sprayed with 10% sulfuric acid, beside as light green spot visually when heated. Reaction BK with anisaldehyde reagent was appeared as yellow spot in orange/pink backgrounds under UV 366 nm and visible. Isolat BK it's not reacted with 5% aluminium (III) chloride solution and 5% sitroboric solution when used as spray reagent. Chromatogram are available in Figure 3

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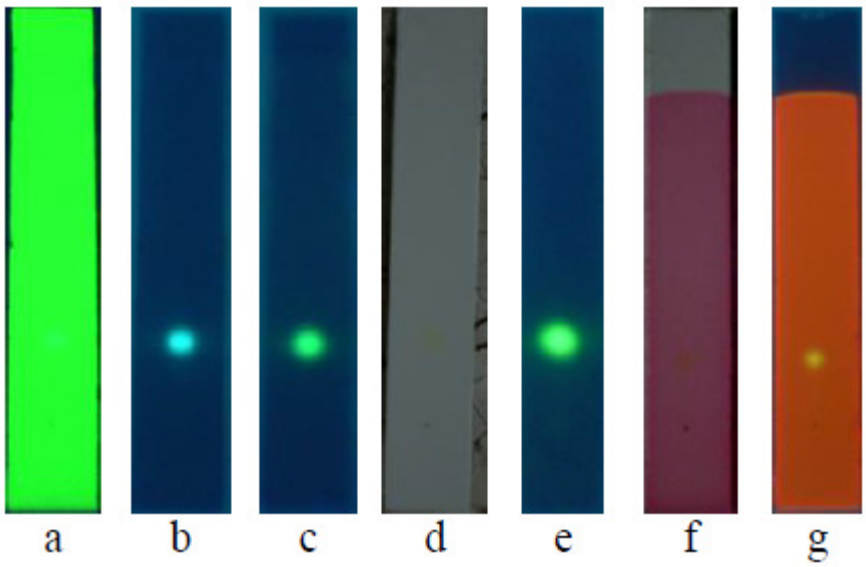

Figure 3 Chromatogram of BK with some spray reagent

Note: stationary phase: precoated silica gel GF 254, mobile phase n-hexane:ethyl acetate (11:1) solvent mixture; obseved under (a) UV 254 nm (b) UV 366 nm (c) UV 366 nm with 10% sulfuric acid, (d) visible with LB reagent, (e) UV 366 nm with LB reagent, (f) visible with anisaldehyde, (g) UV 366 nm with anisaldehyde

Application of TLC also verified that BK was not an arthefak. It's done by spotting an amount of isolate BK and extract under the same condition. This co-chromatogram pattern showed that isolate BK contained in the extract in small portion, so that BK must not formed as arthefak. Profile of BK by utraviolet-visible spectrum, described the presence of conjugative bond within it's structural compound. As the absorbance was detected at wavelenght of 208 nm dan 355 nm. Previously, 95% ethanol was measured as blank solvent to proof that spectrum only derived from sample. Infrared spectrophotometry was measured with potasium bromida (KBr) pellet as sample handling. Base on spectrum, structure of BK possessed some fungsional groups as

follow: hidroxy (O-H) at 3378,67 cm^{-1}, stretched C-H at 2931,27 cm^{-1} and 2857,99 cm^{-1}, group C=O at 1735,62 cm^{-1}, and bended C-H at 1461,78 cm^{-1} and 1376,93 cm^{-1}. Beside that, mass spectrum of LC-MS/ESI showed the ion peak at $[M+1]_+$ 391,20 (m/z), this implied that the molecular weight of BK was 390,20 (m/z). The H-NMR spectrum of BK used $CDCl_3$ as solvent, have shown a strong and sharp signal at δ 0,8-1,0 ppm that indicated of methyl groups ($-CH_3$). signal also appear at existence area of group-CH_2 (δ 1.2-1.4 ppm), group C-H (δ 1.4-1.7), group CH_3-COO- (δ 2.0-2.2 ppm), group CH- (bound to esther group) (δ 3.3-3.9 ppm), group $R_2C=CH_2$ (δ 4.6-5.0 ppm), $R_2C=CHR$ (δ 5.2-5.7 ppm), and proton aromatic (ArH) (δ 6.0-9.0 ppm). Whereas spectrum C-NMR showed appearance of signals that indicated of alkyl groups (5-60 ppm), group C–O (δ 45-90 ppm), carbon in aromatic ring (δ 90-60 ppm), carbon of alkena (δ 100-170 ppm), group –COO- (δ 150-185 ppm). Spectrum are available in Figure 5. Measurenment by two dimensional NMR comprise of *Heteronuclear Multiple Quantum Coherence* (HMQC) and *Heteronuclear Multiple Bond Correlation* (HMBC). Data summaries in Table 3 and Table 4.

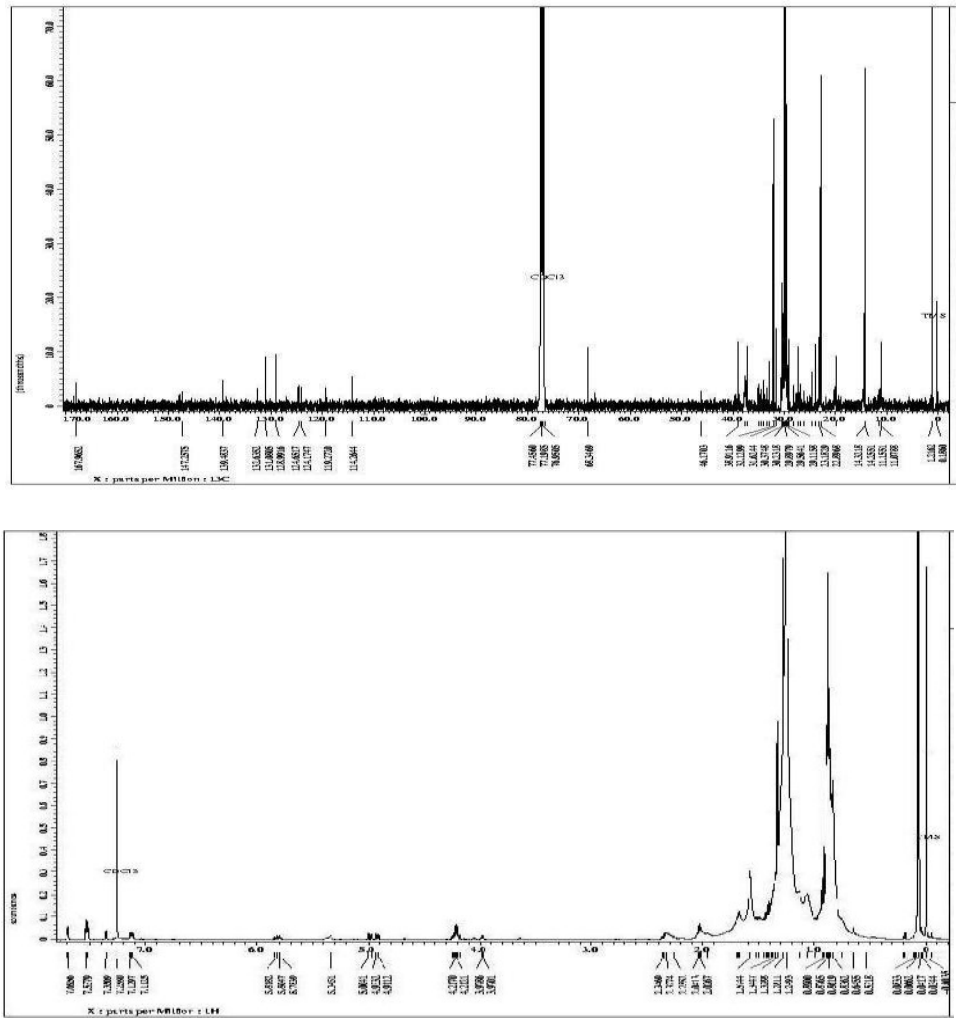

Figure 5. H- and C- NMR spectrum of BK

Table 3. Data summary from HMQC spectrum

δ_C (ppm)	δ_H (ppm)	δ_C (ppm)	δ_H (ppm)
11.1551		46.1705	
14.3218	0.880	68.3469	4.21
19.9208	0.838	114.2644	4.9-5.0
22.8908	1.331	119.2720	7.53
23.1829	1.26	124.2747	7.12
23.9269		124.6517	7.35
27.2844		128.9916	7.7
29.5641	1.25	131.0805	7.52
29.8979		132.6352	
30.3748		139.4837	
31.6244		147.2575	
32.1299		147.8012	
37.2901		167.9652	
38.9116			

Table 4 Data summary from HMBC spectrum

δ_C (ppm)	δ_H (ppm)	δ_C (ppm)	δ_H (ppm)
0.838	37.2901	2.02	114.2644, 139.4837
0.877	22.8908	4.21	23.9269, 30.5465
0.880	38.9116	7.12	124.6517, 147.80
0.918	38.9116	7.35	124.1747, 147.80
1.253	29.8979	7.52	139,4837, 147.2575
1.281	14.3218, 147.2575	7.70	131.0805
1.331	30.3748		

DISCUSSION

Ketapang that used in this research was determined belonged to Combretaceae, species *Terminalia catappa* L, with sinonim name *Terminalia moluccana* Lamk., *Terminalia procera* Roxb., and *Terminalia mauritiana* Blanco. The bark was taken from main wooden rod of ketapang, then cleaned, air-dried then powdered in order to be extracted, fractionated, and characterization.

The result of macroscopical and microscopical analysis showed a similarly profile of ketapang with the bark of other medicinal plants. Interested fragments appeared was the presence or abundance of oil cells with specific odor and chrystal of rossette oxalate.

By determination of quality parameters and phytochemical screening, was figured out the quality and chemical component katapang bark. Hopefully this will provided basic reference to explore ketapang in herbal medicine.

Qualitative information from TLC after extraction and fractionation process, directed focused on the objective of further separation and purification i.e isolation of greenish-blue flourescence spot ('biru kehijauan'=BK) in n-hexane fraction. The result of previous condition on chromatogram TLC, BK sited at Rf 0.78-0.85. After re-optimize the condition resulted an appropriate mobile phase for further separation and purification,that was n-hexane:ethyl acetate (11:1) solvent mixture, where BK sited in Rf 0.35. By the combination methods of CC and preparative TLC, BK was fully isolated. Purity level checked by two dimensional TLC, TLC with three diffrerent mobile phase, HPLC, and LC-MS/ESI, under specific condition concluded that isolate BK was dominan with 77.45% of purity level.

In chromatogram by TLC, after sprayed by Liebermann-Burchard reagent, BK appear as green flourescence spot under 366 nm, as light green color visually, supposed that steroidal compound. According to Goad and Akihisa (1997), steroidal compound reacted positively with LB if produced green or blue or pink color. From that reference also stated that in UV-Vis spectrum, double bond of steroidal compound will indicated at range wavelength of 190-220 nm. As result that the absorbance of BK indicated at wavelength of 208 nm dan 355 nm implied that BK owned conjugative double bond, had also clarified of it's green flouresence under UV 366 nm. In addition from infrared spectrum showed that BK possessed fungtional group of O-H, C-H dan C=O within structural compound.

The H-NMR spectrum of isolat BK showed a similarly with specific pattern of stearyl acetate, that marked as strong and sharp signal at δ 0.8-1.0 ppm that indicated of methyl groups (-CH_3), signal at δ 4.2 ppm and δ 2.02 ppm indicated of acetoxy group, and signal at δ 4.9-5.0 ppm indicated of vinilic double bond. From C-NMR spectrum peak was appeared signals at δ 5-60 ppm indicated of alkyl groups, signal at δ 68,34 ppm indicated of carbon bound to esther group, and signal at δ 167.96 indicated of carbonyl group. Further analyze of compiled data from H- and C-NMR ensure the previously prediction that BK was steroidal compound. In general steroidal skeletone of cholestane, subtitution of acetoxy group possible in C-3 position (3β-hydroxyl).

Two dimensional NMR spectrum of HMQC and HMBC showed some kind position of carbon such as the presence of carbon connected with proton aromatic, carbon connected with proton vinilic, one esther carbon connected with their proton, carbon connected with proton of methyl, and carbon disconnected with any protons (carbon without proton). These conclude that isolate BK in their structure possibly have aromatic bonding, vinilic bonding, and or binding with methyl groups in side chain.

Mass spectra of LC-MS/ESI given clued that the molecular weight of BK was 390.20 (m/z). In general, steroidal compound have 388- 426 (m/z) of molecular weight which depend on parent skeletone and side chain of their structure. These information was supported the previously prediction that BK was steroid compound. And then, molecular formula possibly predicted as $C_{27}H_{34}O_2$. The value of Hydrogen Deficiency Index calculated based formula, that was 11. It's mean that within structural molecul, BK had 11 of double bonding. It can breaking down by decreased 4 from the value allocated for the skeleton (siklo-pentano-perhidro-fenantren ring system), and decreased 1 the value for double bond of one carbonil group, so that there still remain 6 double bond within structure of BK. In such a way that formerly prediction of the presence oromatic ring within structure of BK more supported.

Steroidal compounds that have been reported had aromatic ring in their structure, are very rare derive from plants. As a mean to identify and elucidate molecular structure of BK, so dealing with alternative approached. And then the resulted data of characterization were compared with reference resources. Herein some reference that reported of steroid with aromatic ring in their structural skeletone. Aromatic ring in A position can be found in steroidal hormone like estradiol, estrone, estriol and geodisterol in marine sponge (Gui- Yang-Sheng Wang and Crews, 1999, Ikan, 1991). Aromatic ring in B position can be found in fungi of species *Phycomyces blakesleeanus*, such as *Phycomysterol A, Phycomysterol B* and *Neoergosterol* (Barrero *et al.*, 1998). And Aromatic rings both in A and B position like equilenin and its derivative (Pouchert and Behnko, 1993, Hill *et al.*, 2008). Structure of each compound are available in Figure 6.

Table 5. H-NMR spectrum of BK, equilenin and it's derivative

Isolate BK	Equilenin (AL)	Equilenin (CDU)		17α-dihydro-equilin sulfate		Equilin sulfate (6-OH)	
δ (ppm)	δ (ppm)	δ (ppm)	H	δ (ppm)	H	δ (ppm)	H
0.838	1.0	1.30	18	0.52	18	0.70	18
0.877	1.3	1.68	12b	1.17	20	1.17	20
0.880	1.7-1.9	1.93	12a	1.52	15a	1.78	12a
0.918	1.9-2.02	2.01	16b	1.60	6a	1.88	15a
1.253	2.2-2.7	2.11	16a	1.73	12a	2.00	12b
1.281	3.0-3.2	2.19	15b	2.04	12b	2.32	16a
1.331	3.4	2.44	15a	2.16	15b	2.39	15b
2.02	7.1	2.80	11b	2.27	16b	2.62	16b
4.21	7.2	2.90	11a	2.99	11a	3.08	11a
4.9-5.0	7.5-7.6	3.05	14	3.09	19	3.10	19
7.12	7.8	6.93	7	3.10	14	3.12	11b
7.35	9.6	6.96	2	3.13	11b	3.14	14
7.52		7.18	4	3.76	17	6.69	7
7.53		7.72	6	4.50	17(OH)	7.33	2
7.70		8.10	1	6.62	7	7.77	1
		9.43	3(OH)	7.30	2	7.89	4
				7.75	1	9.83	6(OH)
				7.85	4		
				9.64	6(OH)		

Note: Showed a similarly of chemical shift value
AL = chemical shift value of equilenin from the Aldrich Library; CDU= chemical shift value of equilenin in NMR prediction using software (Chemdraw ultra); 17α-dihidro Equilin sulfate and Equilin sulfate (6-OH) used as standard on measured condition in natrium salt form

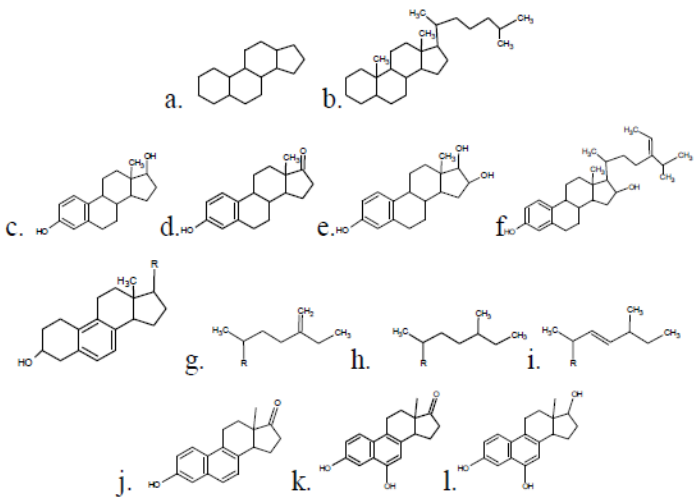

Figure 6 Structure of selected steroid

Note: (a).Siclopentanoperhydrophenantren ring system; (b).Skeletone of cholestane;(c). Estradiol;(d). Estrone; (e).Estriol; (f).Geodysterol; (g). Phycomysterol A; (h). Phycomysterol B; (i). Neoergosterol; (j). Equilenin; (k).6-hidroksi Equilin; (l). 17α-dihidroequilin

Chemical shift value of proton aromatic in NMR spectrum at δ 6.0-9.5 ppm. Data on Table 5 showed a similarly of chemical shift value at proton aromatic at isolat BK with equilenin and its derivative. Its meant that skeletone of isolat BK have similarly in aromatic rings position with Equilenin and its derivative, that was in both A dan B rings. One of the difference was disappearance on chemical shift value proton of hydroxyl group from BK unlike in C-3, C-6 and C-17 position from equilenin and its derivative. Its may conclude that hydroxyl group of BK binding with another fungtional group. As predicted previously that BK might subtituted by acetoxy group in C-3 position. It also explained that chemical shift value of hydroxyl group from BK in C-3 position was disappeared.

Chemical shift value of carbon aromatic in NMR spectrum at δ 90-160 ppm. Data on Table 6 showed a similarly of chemical shift value at carbon aromatic in isolat BK with equilenin and its derivative . Its meant that skeletone of isolat BK have similarly in aromatic rings position with Equilenin and its derivative, that was in both A dan B rings. One of the difference was appearance on chemical shift value at δ 160-220 ppm indicated of carbonyl group. In this case, represented to acetoxy in C-3 position of isolate BK and represented to ketone group in C-17 position of Equilenin and its derivative.

Table 6. C-NMR Spectrum of BK, Equilenin and It's derivative

Isolate BK	**Equilenin (AL)**	**Equilenin (CDU)**		**17α-dihydro-equilin sulfate**		**Equilin sulfate (6-OH)**	
δ (ppm)	**δ (ppm)**	**δ (ppm)**	H	**δ (ppm)**	H	**δ (ppm)**	H
11.1551	11.00	18.0	18	8.6	20	8.6	20
14.3218	21.00	21.8	12	15.6	18	12.7	18
19.9208	23.00	26.4	11	23.4	11	21.4	15
22.8909	28.50	28.9	15	24.6	15	22.9	11
23.1829	36.00	36.8	16	29.2	12	28.8	12
23.9269	38.00-40.00	46.7	13	32.9	16	36.1	16
27.2844	45.00	47.5	14	44.4	13	45.7	19
29.5641	46.00	106.5	7	44.5	14	45.9	14
29.8979	110.00	118.0	6	45.7	19	46.9	13
30.3748	118.00	125.2	4	77.3	17(OH)	106.0	7
31.6244	124.00	125.7	2	107.5	7	111.7	4
32.1299	124.00	127.8	10	111.7	4	120.1	9
37.2901	126.00	128.4	1	119.9	9	121.8	2
38.9116	130.00	129.1	9	121.5	2	123.7	1
46.1705	133.00	129.6	8	123.6	1	124.2	5
68.3469	139.00	133.0	5	129.4	5	129.4	10
114.2644	154.00	153.4	3	136.0	10	133.3	8
119.2720	178.00	203.0	17	149.4	8	149.7	3
124.2747				150.7	3	151.2	6(OH)
124.6517					6(OH)	218.9	17
128.9916							
131.0805							
132.6352							
139.4837							
147.2575							
147.8012							
167.9652							

Note: Showed a similarly of chemical shift value

AL = chemical shift value of equilenin from the Aldrich Library; CDU= chemical shift value of equilenin in NMR prediction using software (Chemdraw ultra); 17α-dihidro Equilin sulfate and Equilin sulfate (6-OH) used as standard on measured condition in natrium salt form

The similarity on chemical shift value of H-and C-NMR spectrum at aromatic ring position from BK with Equilenin and it's derivative, implied that each compound have similar parent steroidal skeleton and location of aromatic ring, that sited in A and B position. However, the configuration or structural complete of BK are still unclear.

CONCLUSION

Phytochemical study of katapang bark was conducted by analyze of specific and non specific parameter of plant, provided information of characteristic of its chemical compounds. By the process of extraction, fractionation, separation and purification, with monitored by TLC, have been fully isolated compound BK from n-hexane fraction. Characterization of BK by ultraviolet-visible spectrophotometry,Infrared spectrophotometry, liquid chromatography-mass spectrometry (LC-MS/ESI), and nuclear agnetic resonance spectroscopy ($_1$H, $_{13}$C, HMQC and HMBC) identified that BK was steroidal compound with 390,20 (m/z) of molecular weight and $C_{27}H_{34}O_2$ of molecular formula, and predicted have aromatic ring in A and B position and acetoxy group in C-3 position.

Advanced research may directed to optimize condition of purification in order to isolation BK with higher of purity level, besides the other chemicals of ketapang.

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IDENTIFICATION OF FLAVONOIDS IN *CABOMBA FURCATA* FROM TASIK CHINI, PAHANG MALAYSIA

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Abstract : Early studies of *Cabomba furcata* from Chini Lake, Pahang, Malaysia has shown the presence of bioactive phytochemicals such as flavonoids which have not been identified yet. Flavonoids are believed to have good contributions to the humankind and they have the potential to be commercialized in pharmaceutical field. This study was done to identify the flavonoid components that are present in *C. furcata* and then to quantify the flavonoids. The analysis and identification of flavonoids were carried out using 3 methods, spectrophotometry uv-visible (uv-vis), high performance liquid chromatography (HPLC) and liquid chromatography-mass spectrometry (LC-MS). Extraction was performed by using five (5) different solvents namely water, methanol, chloroform, methanol-water (1:1) and methanol-chloroform (2:1) and it was found that methanol-water gives the highest extraction yield (8.7%). Preliminary test with spectrophotometry uv-vis had shown the presence of gallic acid, catechin, syringic acid and ellagic acid. Chemical analysis performed with HPLC and LC-MS had confirmed the presence of catechin, gallic acid, syringic acid and ellagic acid in the aquatic plant. Quantity of catechin in the mixture of methanol-water extract is the highest, that is 596.5 mg, followed by methanol extract (445.3 mg), water extract (360 mg), mixture of methanol-chloroform (297.8 mg) and the lowest is from chloroform extract (133.6 mg).

Keywords: Soxhlet extraction, flavonoids, catechin, *Cabomba furcata*, HPLC

INTRODUCTION

Tasik Chini is one of the famous fresh water lakes in peninsular Malaysia. It is located near the Pahang River in central Pahang. Tasik Chini is the second largest fresh water lake in Malaysia and is made up of a series of 12 lakes that is endowed with a rich bio-diversified lush tropical wilderness that is home to 138 species of flora, 300 species of non-aquatic live and 144 species of fresh water fish.

Located just about 100 kilometers from the capital of Pahang, Kuantan, has turned Tasik Chini into a place that has great affinity among tourists locally and abroad. Tasik Chini is also the meeting place for people who enjoy fishing and bird watchers as there are about two hundred (200) species of birds being recorded to populate the area around October until Mac every year. These numbers will further increase due to migration activity that occurs because of cold weather in the northern part of Asia.

However, the beautiful ecosystem of the lake which is estimated to be about 12565 acres is gradually declining because of substantial increase in environmentally harmful activities including agriculture, mining and illegal logging (M.B Gasim et. Al 2006). This situation is further aggravated by the present of certain aquatic plants such as *Cabomba furcata* which are believe to be harmful to the natural ecosystem of the lake. This type of aquatic plant has destroyed the natural habitat for waterlily or commonly known as *Nymphaea.* Problems that occur in Tasik Chini have attracted a lot of researchers to conduct detail analysis on how to maintain the ecological system of the lake for the future generations.

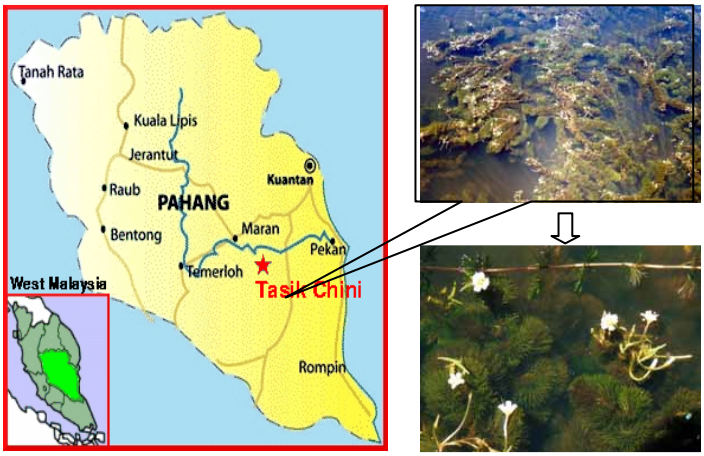


Figure 1. Chini Lake in Pahang dan *Cabomba furcata* which dominates the aquatic plants

Most of the research focus on the declining water quality of the lake, the significant reduction in the number of fish and the increase in the number of bacterial coliform and *E. coli* (M.S Othman *et al.* 2006, 2007). *C. furcata* plant has a certain characteristics that will enable it to become dominant as compared to other aquatic species such as water lily or *Nymphaea*. Jia 2008 has conducted phytochemical testing on *C. furcata* and the results showed that alkaloid components, flavanoid and saponin exist in the plant. From the research, only one type of flavanoid from flavonol group, myrisetin has been successfully identified together with gallic acid. Further testing has been done on the alkaloid components by Syairah 2009 and from the research we knew that the alkaloids are of nicotine and tomatine type.

In this research, flavonoid was chosen as the subject for further analysis in order to identify other flavonoid groups that might be present by using a type of solvent known as chloroform. The existence of other flavonoid that might exist will be studied theoretically so that we can make a clarification whether or not it gives a negative feedback towards other aquatic plants in the lake and contributed to the dominance growth of *C. furcata*. The purpose of this study is to determine the percentage of extract that can be obtained by using chloroform together with methanol as the solvent. This study is also conducted in order to identify the types of flavonoid in the extract and also to determine the concentration of chemical components in selected flavonoid groups. *Cabomba* is a water plant genus, one of two belonging to the family Cabombaceae. This plant can be easily identified because it has divided submerged leaves in the shape of a fan and it submerges completely under water.

This plant is much favored by aquarists as an ornamental and oxygenating plant for fish tanks. Its use in the aquarium trade has led to some species being introduced to parts of the world, such as Australia, where they have become pestilential weeds. Even though Cabomba is very famous for its traditional use in the aquarium, its growth in other places can cause serious problems as mentioned before. This is due to the highly accelerated growth rate which enables the plant to become dominant over other types of natural aquatic plants in the habitat. This will definitely affect the natural balance of the ecological system.

METHODOLOGY

Raw materials

The raw material that has been used in this study was ekor kucing plants (*Cabomba furcata*) which was found in Chini Lake, Pahang. Chemicals used for the early detection of flavonoids were concentrated HCl and zinc powder (Zn). The chemicals used for extractions were methanol, chloroform, mixture of methanol-chloroform (2:1) and

mixture of methanol-water (1:1). Standards used were catechin, ellagic acid, gallic acid, myrisetin and syringic acid. For fractionation, the medium used were Sephadex G-10 and silica gel.

Apparatus

The apparatus used in this work were blender, Soxhlet extractor (Hamilton. Amerika Serikat), vacuum evaporator (Heidolph, Germany) and weighing machine (Sortorius, Germany). Apparatus used for chemical analysis were spectrophotometer (UV-*Visible* 1601 PC, Shimadzu), HPLC (Model C1313A, Agilent 1100 Series)., LCMS microTOF$_Q$ (Bruker) and colom ODS hypersil (Agilent, Germany).

Preparation of raw materials

Samples were washed and dried in a 45^{o}C oven until the weight was constant. It was then kept in an air-sealed bags.

Solvents

Grind *Cabomba furcata* was weighed and 10 gr of the plant was then placed in the soxhlet apparatus. Using 150 mL of solvents (methanol, water, methanol-water, chloroform and methanol-chloroform), extractions were performed for 4 hours. The extracts were then dried using vacuum evaporator with the speed of 80 rpm dan at temperature 65^o C (methanol,chloroform and methanol-chloroform), 100^{o}C (water) and 90^{o}C (methanol-water).

Detection of flavonoids

Shinoda Test

A few drops of concentrated hydrochloric acid (HCl) dan 0.5 g zinc powder (Zn) were added to 5 ml of cabomba extracts. Appearance of pale pink or magenta color shows the presence of flavonoids (Mojab *et al.,* 2003).

Spectrophotometer uv-visible

Serial dilutions were done to the dried extracts and then the samples were read with spectrophotometer uv-vis. The wavelength of highest peaks obtained from the reading of extracts by spectrophotometer were compared with the wavelength of highest peak obtained from every standard used.

HPLC

20 µL of filtered extracts were injected to HPLC. The temperature was set to be 30oC while the flowrate was 1mL/min. Static phase was C18 column while mobile phase A was a mixture of water and phosphoric acid with 999:1 ratio and acetonitrile was used as mobile phase B. The gradient used was 8%-22% of B for 35 minutes and for the next 10 minutes,the gradient was changed to be from 22% to 8% of B.

LCMS

20 µL of filtered extracts were injected to HPLC. The temperature was set to be 30^{o}C while the flowrate was 0.8mL/min. Static phase was C18 column while mobile phase A was a mixture of water and formic acid with 999:1 ratio and acetonitrile was used as mobile phase B. The gradient used was 8%-22% of B for 35 minutes and for the next 10 minutes,the gradient was changed to be from 22% to 8% of B.

RESULTS AND DISCUSSION

Shinoda Test

When zinc is mixed slowly with concentrated hydrochloric acid in a test tube that contains methanol, water and mixture of methanol-water extracts, the color of the mixture

changed initially to red before forming a brownish solution. However, for the chloroform extract, the addition of concentrated hydrochloric acid and zinc will not cause any changes to the color of the solution. The latter might occur due to the original dark color of chloroform extract that will limit the observation.

The color change in the mixture of methanol-chloroform extract can be clearly seen when zinc and 3 drops of hydrochloric acids were added into the extract as the formation of dark-red solution can be clearly observed in the middle of the test tube containing the extract. However, when concentrated hydrochloric acid was continually added into the mixture, the color changed immediately into brown. This observation demonstrates the presence of flavonoid in the methanol-chloroform extract. Figure 2 below shows the change in color that occurs in of the extract being tested.

Figure 2. The color change before and after the addition of concentrated hydrochloric acid and zinc

Table 1. Shinoda's Test

Extract	Results
Water	Positive
Methanol-Water	Positive
Methanol	Positive
Methanol-Chloroform	Positive
Chloroform	No change

According to the observations (Table 1.), all extracts except for chloroform extract showed the presence of flavonoid. Flavonoid components from flavanonol group might be available in the extract as the addition of concentrated hydrochloric acid and zinc had caused the color of the mixture to change to magenta. The presence of flavonoids from flavanone and flavonol groups may also give the same color changes (color may vary from weak pink to magenta) or no color at all when concentrated hydrochloric acid and zinc are added into the tested samples (Jones and Kinghorn).

Spectrophotometer UV-Visible

The figure below shows the preliminary testing that has been done in order to determine the optimum solvent in the tested extracts. The results showed that, extraction method using mixture of methanol-water will give a more concentrated extract compared to the methods using methanol, water, chloroform and a mixture of methanol-chloroform. Therefore, it can be concluded that the mixture of methanol and water with 1:1 ratio is the optimum solvent in this experiment.

The profile displayed below was obtained when all five extracts were dried by using vacuum evaporator in order to remove the solvents before serial dilutions were done on all dried extracts. The chromatographs of all extracts had shown the same profiles that have one peak at wavelength 250nm until 300nm and many different peaks at wavelength less than 250 nm. This might be due to the presence of other photochemical components in the extracts.

At wavelength more than 300nm, all extracts show the same profile. However this is not the case for extract containing chloroform because of the presence of peaks when the wavelength increased. However, the peaks displayed in this situation will not be discussed as the wavelength used in the experiment is only limited within the range of 200 nm until 400 nm (Figure 3).

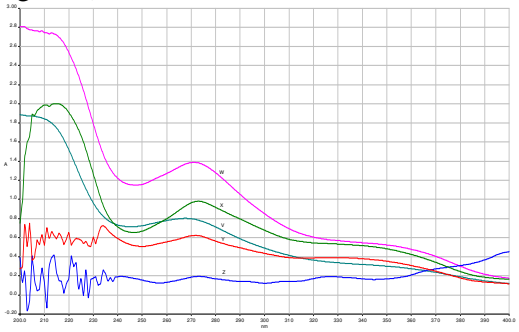


Figure 3. Profile of all 5 extracts at 200 nm – 400 nm. W=methanol-water, X=methanol, V=water, Y=methanol-chloroform, Z=chloroform

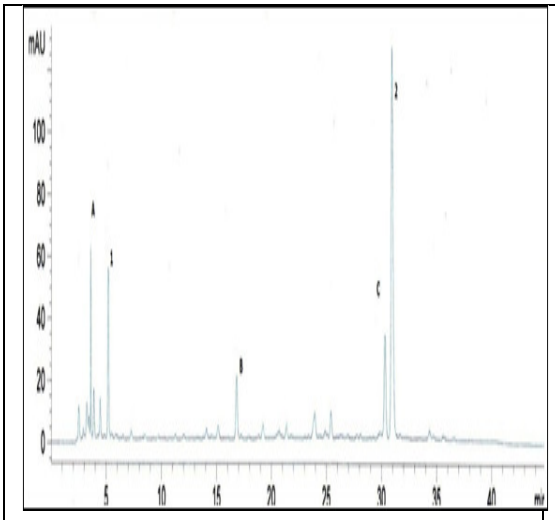
Table 2 listed all the results that show the presence of standard materials used by using the profile of all five extracts with standard materials. From the observation, all standard materials used give positive results.

Table 2. The presence of standard materials by using spectrophotometer *uv-vis*

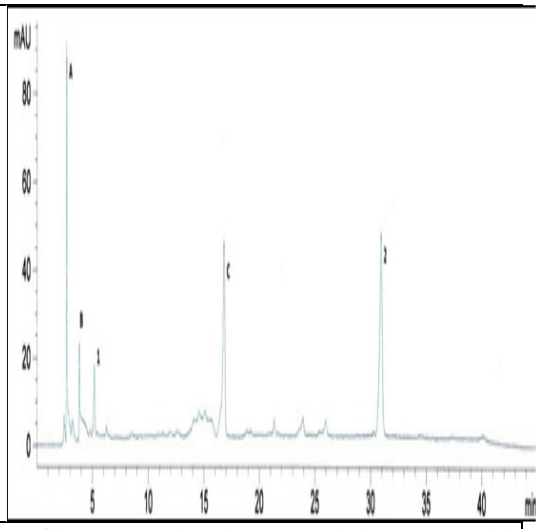
Extract	Gallic acid	cathecin	Siringic acid	Ellagic acid
Water	+	+	+	+
Methanol	+	+	+	+
Methanol-Water	+	+	+	+
Methanol-Chloroform	+	+	+	+
Chloroform	+	+	+	+

HPLC ANALYSIS

Table 3 below listed the retention time for standards used in this study at wavelength 270 nm. The retention time for gallic acid and ellagic acid obtained in this study were a bit different when compared to the time retention for both acids in the study done by Masturah Markom et. al (5.4 minutes for gallic acid and 31.2 minutes for ellagic acid). However, the difference is not that significant. This could be due to the percentage of phosphoric acid being used in this study is much lower (85%). Figure 4 below displayed the profile for standard materials obtained at wavelength 270nm.

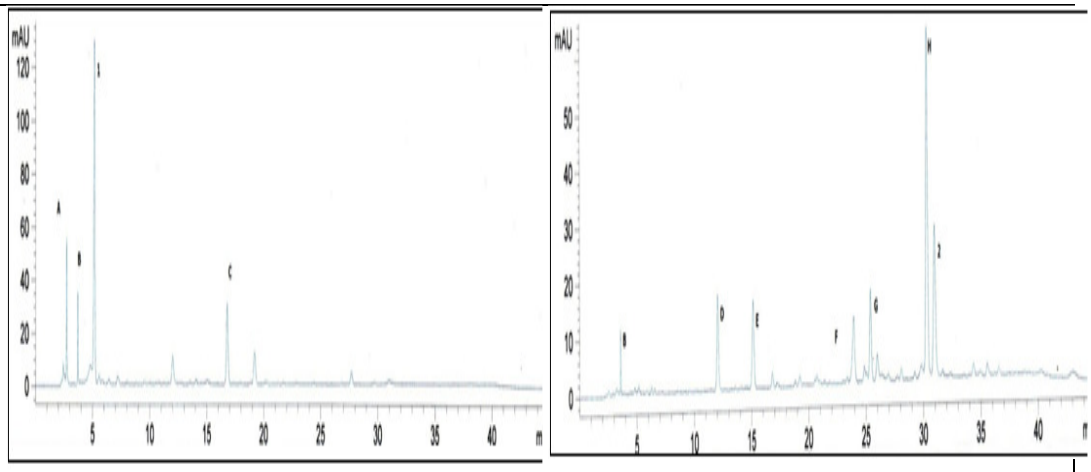


Profil HPLC ekstrak air D. 1= asid gallik, 2=asid ellagik, komponen A,B,C= tidak dikenalpasti



Profil HPLC ekstrak metanol-air D. 1=asid gallik, 2= asid ellagik, komponen A,B,C = tidak dikenalpasti

In Figure 8 and 9, the peaks for gallic acid and ellagic acid in the water extract (fraction D) and mixture of methanol-water extract (fraction D), can be clearly observed at two separate retention times, 5.16 minutes and 30.9 minutes. For figure 4.8, the calculated relative area for gallic acid is 9.78% whereas the relative area for ellagic acid is 40.74%. This data indicates that ellagic acid shows a significantly higher relative area compared to gallic acid. The percentage of relative area for gallic acid in Figure 9 is 4.35 while relative area for ellagic acid is 25%. Peak B in figure 4.8 and peak C in Figure 9 were recorded at the same retention time, 16.8 minutes, and this peak refers to the same component that has managed to be extracted. However, the specific name for the component was unable to be identified.

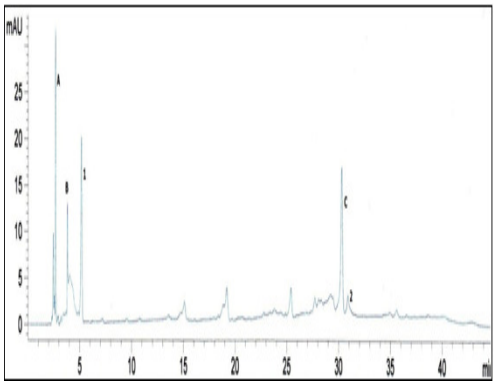


Profil HPLC ekstrak metanol a) B, b) F. 1 = asid gallik, 2 = asid ellagik, komponen A-H = tidak dikenalpasti

Figure 10 (a) shows the presence of gallic acid in fraction B of the methanol extract with relative area 40.8% whereas ellagic acid is present in fraction F of the same extract (Figure 10b). The chromatograf also indicates the presence of high concentration

of unknown component,H, at retention time 30.3 minute with relative area of 30.98% as compared to ellagic acid (15.68%).

Chromatograf for the mixture of methanol-chloroform extract in figure 4.11 below shows the presence of gallic acid and ellagic acid with relative area of 15.2% and 3.26%. At retention time 30.3 minutes, an unidentified componet C with relative area 19.34%, has been detected.



Standard Materials		Retention time (λ=270nm)	Molecular weight
Gallic acid $C_7H_6O_5$		2.9	170
Catechin $C_{15}H_6O_8$		18.2	290
Stringic acid $C_9H_{10}O_5$		23.3	198
Ellagic acid $C_{14}H_6O_8$		35.6	302

Figure 4.11: HPLC's profile for methanol-chloroform extract C. 1= gallic acid, 2= ellagic acid, components A, B, C = unknown

From the HPLC's results, solvents such as methanol, water and the mixture of methanol-water are capable of extracting significant component of gallic acid and ellagic acid as compared to mixture of methanol-chloroform and chloroform based on the height of the peaks obtained in the result. This mightbe caused by the nature of water and methanol which are widely known as polar solvents, therefore they have the ability to extract more gallic acid and ellagic acid as both of them are polarised components. Catechin and siringic acid cannot be detected in the chromatographs as they might be left in the column when fractionation was done.

LCMS ANALYSIS

Table 5 listed all the molecular formula and weight of the standard material used in LCMS study. For Table 6, retention time for standard materials in LCMS study is different from retention time in HPLC test. This is due to the lower flow rate used in LCMS study, 0.8 mL/min.

According to Figure 12, methanol extract B, the second peak shows a very high intensity, 37839, with retention time 2.9 minutes. In Figure 6, acid gallic with molecular weight of 170 has been recorded at time retention 2.9 minutes, but Figure 13 shows the fraction of components present in that particular peak to have high ratio of weight to charge (m/z) at 274.87. this might be due to the present of other components that can be detected at the same retention time with concentration higher than gallic acid. Gallic acid can also combine and react with other components available in the sample which might cause the difference in molecular weight obtained.

Figue 14 shows the LCMS's profile for methanol extract F with the highest peak at 38th minute and intensity of 81043. However, the main component present at that particular peak with m/z 449.1 could not be identified. Peak 9 in the profile has the same retention time with ellagic acid at 35.8 minutes. Despite that, according to Figure 15, the highest m/z value for the peak is 611.16 whereas the molecular weight for ellagic acid is 302. There are possibilities that individula ellagic acid units might be combining or reacting with other components available in the sample because of the significant different in the molecular weight recorded. From the LCMS result, it can be concluded that the extract should be put on several separation and purification processes so that a more accurate result can be obtained. There are very few studies done to identify

flavonoid by using LCMS. This might be due the bioactive properties of flavonoid which is not suitable for the use of LCMS. However, if the extract is put on stringent purification processes, the use of LCMS could be viable.

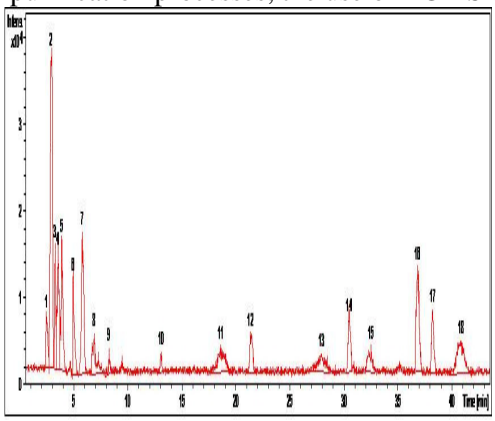


Figure 12. Profil LCMS ekstrak metanol B

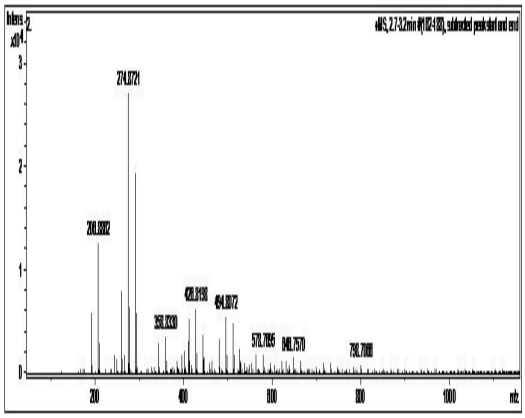


Figure13. Pecahan komponen-komponen yang terdapat di dalam puncak 2 ekstrak metanol B

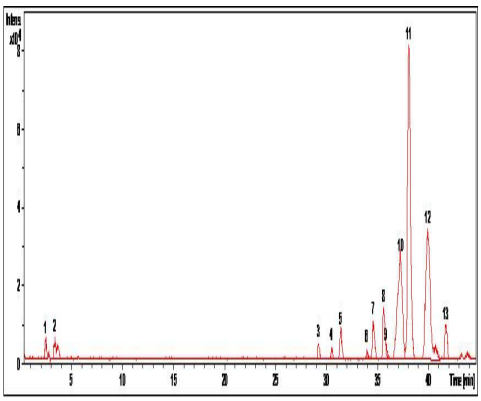


Fig4.14 : Profil LCMS ekstrak metanol F

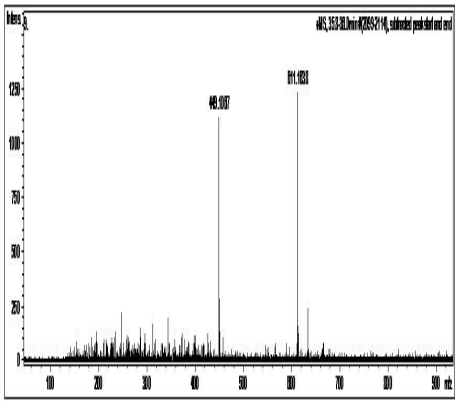


Fig4.15 : Pecahan komponen-komponen yang terdapat di dalam puncak 8 ekstrak metanol F

CONCLUSION

1. Catechin, gallic acid, ellagic acid and syringic acid are present in *C. furcata*.
2. Methanol-water (1:1) solvent is the best solvent in this study.
3. Quantity of catechin extracted by methanol-water solvent gives the highest yield i.e 596.5 mg whereas chloroform gives the lowest concentration of catechin (133.6 mg)

RECOMMENDATION

1. Before fractionation process, test on the extracts by using HPLC and LCMS should also be done so that comparison can be made between the samples.
2. The reading using spectrophotometry *uv-vis* for quantification should be made thrice to get more accurate readings.

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ANTI-OBESITY OF MOUSE BY SNIFFING CYPRESS ESSENTIAL OIL

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Abstract : White cypress (*Callitris glaucophylla*) is belonged to Cupressaceae and distributed mainly in New South Wales state of Australia. Extractive of the heartwood of cypress is known to antitermite (Watanabe[a] *et al.*, 2005; Watanabe[b] *et al.*, 2005) and antifungal activities. Previous work suggested that inhalation or intake of cypress oil affected anti-obesity in mice and rat (Mitsunaga and Tamaru, 2008). The aim of the present study is to identify the active component causing anti-obesity effects by sniffing of cypress essential oil. Three cypress oils were obtained according to the different distillation time from hot toluene extracts of cypress heart wood meal. Here we examined the effects of cypress oil flavor against mice lipopysis, as the result olfactory stimulation with cypress oil reduced body weight, liver weight, and epididymal adipose tissues weight. Moreover, triglyceride (TG) concentration in the liver and plasma and total cholesterol (TC) concentration in the liver of mice inhaled cypress oil was significantly reduced. Especially, a fraction distilled in an early distillation time markedly decreased TG and weights of epididymal adipose tissues. Finally, (-)-citronellic acid, guaiol, and α-, β-, γ-eudesmol, main components of the fraction, induced a response similar to those caused by cypress oil. Thus, the scent of cypress oil, and particularly its comparatively high volatile components, affects enhances lipolysis to reduce body weight of mice.

INTRODUCTION

Obesity is not only a phenomenon detested in beauty but also one of the life-style related diseases. Recently the utilization of natural products is promoted at significant speed in all over the world. Among then we interested in the anti-obesity using a scent of plant materials. For instance, it was reported scent of grapefruit oil excited the sympathetic nerves innervating the brown adipose tissue and adrenal gland and inhibited the parasympathetic gastric nerve (Jiao *et al.*, 2005). Therefore olfactory stimulation with scent of grapefruit oil is known to affect autonomic nerves, increases lipolysis, and reduces appetite and body weight. However, these studies were only herbaceous plants or fruits and wood which have rich biomass and can obtained massive essential oil were not study so far. White cypress (*Callitris glaucophylla*) is belonged to Cupressaceae and olfactory stimulation with scent of the heartwood of cypress is known to antitermite. Previous work suggested that inhalation or intake of cypress oil affected anti-obesity in mice and rat. In the current study, we examined whether the scent of cypress oil obtained by steam distillation of hot toluene extracts from cypress wood can affect anti-obesity in mice.

MATERIALS AND METHODS

Steam distillation

Hot toluene extracts from cypress wood and water were got into round flask, and heated with mantle heater. Three fractions (H, M, and L) were obtained by steam distillation time (0-76 h, 76-137 h, and 137-144 h, respectively). These fractions were analyzed by GC-MS respectively.

Preparation of H distillate

Main components of H distillate was fractionated by preparative HPLC and partition. Three fractions (Fr. 1-3) obtained by preparative HPLC and Acidic fraction obtained by fractionation with alkaline were analyzed by GC-MS respectively.

Animal care

Male Slc:ddY mice (5 weeks of age) were housed in a room maintained at 25□ and illuminated for 12 h (7:00-19:00). Experiments were conducted after animals had been allowed to adapt to their housing condition for 1 week. Each oil of H, M, and L distillate (Culture Experiment 1) and main components of H distillate (Culture Experiment 2) were dissolved ethanol, and suspended in 1,000 volumes of water. Control solution was not included essencial oil. Each cypress oil suspended in 1,000 volumes of water were bubbled with pump, its headspace gas were exposed to mice. For the Culture Experiment 1, twenty four mice were divided into each group of four (HFD, H, M, and L group); HFD group was exposed control solution, and H, M, and L groups were exposed each cypress oil (H, M, and L) suspended in 1,000 volumes of water. After acclimation for 1 week by feeding the MF(Oriental Yeast Co., Tokyo) and bubbling control solution, mice were kept 38 days by feeding the HFD (contained 20% lard, 1% cholesterol, 0.25% sodium cholate) and bubbling cypress oil suspended in 1,000 volumes of water. Similarly, for the Culture Experiment 2, twenty four mice were divided into each group of four (HFD, (-)-citronellic acid (CA), guaiol and α-, β-, γ-eudesmol (GE), and guaiol (G)); HFD group was exposed control solution, and CA, GE, and G groups were exposed Acidic fraction, Fr. 3, guaiol suspended in 1,000 volumes of water, respectively. After acclimation for 1 week, mice were kept 52 days by feeding the HFD and bubbling cypress oil suspended in 1,000 volumes of water. Food and water were freely available. Food intake and body weight were determined thrice a week. Cypress oil suspended in 1,000 volumes of water was changed once a week. At the end of the experimental period, plasma was obtained mice under the ether anesthesia. To assess effects of the olfactory stimulation on the fat accumulations in tissues, tissue weights of the liver and epididymal adipose tissues were measured when the animals were sacrificed. Liver lipid were isolated by the method of Bligh & Dyer, total cholesterol (TC) and triglyceride (TG) of plasma and liver were quantitated by using test kit respectively.

RESULTS

Three fractions (H, M, and L) were obtained by steam distillation time. H distillate contained (-)-citronellic acid, guaiol, α-, β-, γ-eudesmol, and M distillate contained H distillate compounds and ilicic acid methyl ester, costic acid, dihydrocolumellarin, columellarin. L distillate didn't contained little guaiol, α-, β-, γ-eudesmol, and composed mostly of sesquiterpene like dihydrocolumellarin.

When the effects of scent of three distillate fractions were examined in mice, food intake of mice treated with cypress oil was similar to that of mice treated with control solution. The body weight of the mice exposed with cypress oil was reduced remarkably compared with HFD (Fig. 1). Moreover, tissue weights of the liver, TG concentration in the liver and plasma, and TC in the liver of mice treated with distillate fractions were also reduced. Especially, H distillate which contained many highly-volatile compounds was shown significant reduction of epididymal adipose tissues weights (Fig. 2) and TG concentration in the liver.

Next, we examined the effects of main components of H distillate, because the aim was to identify active component of antiobesity effects caused by scent of cypress oil. Three fractions (Fr.1-3) were obtained by preparative HPLC. Fr. 1 contained (-)-citronellic acid, Fr. 2 contained dihydrocolumellarin, Fr. 3 contained guaiol, α-, β-, γ-eudesmol, respectively. Thus, Acidic fraction obtained by partition contained mainly (-)-

citronellic acid. When we examined effects of scent of H distillate main components, (-)-citronellic acid, guaiol, and α-, β-, γ-eudesmol, there induced responses similar to those caused by H distillate. Especially, mice inhaled Fr. 3, guaiol and α-, β-, γ-eudesmol, had significantly low liver weights (Fig. 3) and TG concentration.

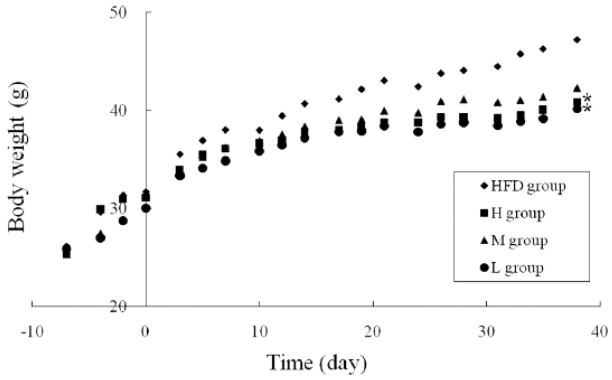

Fig. 1 Body weight change of mice inhaled distillate of hot toluene extracts from cypress wood *p<0.05 vs. HFD group

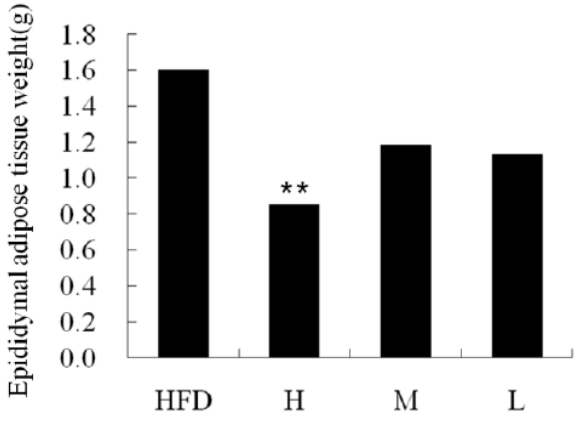

Fig. 2 Epididymal adipose tissue weights of mice inhaled distillate of hot toluene extracts from cypress wood **p<0.01 vs. HFD group

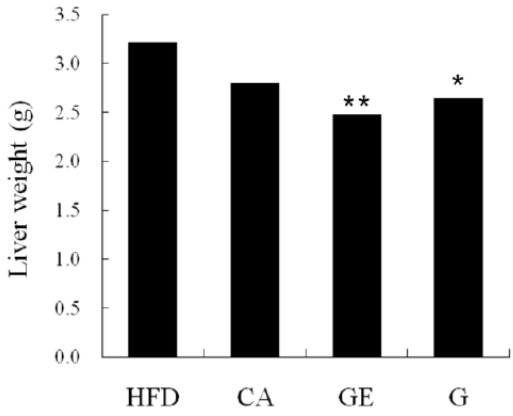

Fig. 3 Liver weights of mice inhaled H distillate **p<0.01 vs. HFD group *p<0.05 vs. HFD group

To determine mechanism of antiobesity caused by scent of cypress oil, measurement of sympathetic nerves activities are now in progress. We performed experiment with the sniffing of H distillate. In the result, we observed that olfactory stimulation with scent of H distillate fractions excited the sympathetic nerves innervating the brown adipose tissues.

From the above findings we conclude that the smell of cypress oil, especially guaiol and α-, β-, γ-eudesmol, affects autonomic nerves, increases lipolysis and heat production, and reduces body weight and adipose tissues weight.

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EFFECT OF *ARTOCARPUS ALTILIS* DECOCTION UNRIPE ON ADVANCED GLYCATION END PRODUCTS IN HYPERGLICEMIA INDUCED RATS *(RATTUS NORVEGIUS)*

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Abstract: Hyperglycemia is a condition in which an excess of sugar (glucose) circulates in the blood. Hyperglycemia promotes oxidative stress. Oxidative stress to the continuance to end the formation Advanced Glycation End Products (AGEs) that may accelerate the formation of advanced glycation end products (AGEs). The increase in AGEs formation may damage organs and tissues such as kidney, liver, brain, lung and nerve. It is also known to be an antioxidant capable of protecting cell membrane and may help prevent AGEs accumulation. This research is aimed to study the effect of decoction of unripe *A. altilis* on AGEs inhibitor liver of hyperglycemic male rats (*Rattus norvegicus*). It was a experimental study with posttest-only with control design, consisted of five groups of treatment. Group 1: control rats given only buffer; group 2: diabetic control (STZ 55mg/kg body weight of rats);group 3,4,5: diabetic rats treated with decoction of unripe *A.altilis* to dose of 0.266; 0.533; 1.066 mg/g body weight of rats/day/100ml) in aqueous solution orally for 30 days. The average of AGEs level groups 1, 2, 3, 4, 5 were 0.724 (mol l^{-1}), 1.128 (mol l^{-1}), 0.937 (mol l^{-1}), 0.805 (mol l^{-1}), 1.262 (mol l^{-1}), respectively. In conclusion, *A. altilis* decoction of unripe was capable of inhibiting AGEs formation in hyperglycemia induced rats in dose of 0.266 mg/g, and 0.533 mg/g body weight of rats in 100 ml.

***Keywords** : AGEs, Artocarpus altilis, streptozotocin*

INTRODUCTION

Hyperglycemia is a condition in which an excess of sugar (glucose) circulates in the blood, therefore it plays a role in promoting complication of diabetes mellitus. Chronic hyperglycemia in diabetes contributes to long-term disorders, disfunction or failure of some body organs especially eyes, kidney, neurons, heart and blood vessels (Peppa, 2003). Diabetes can stimulates the increase of Oxygen Free Radicals (OFRs) (Lawrence, 2004) such as superoxide anion (O_2), hydroxyl radical (OH), and Hydrogen peroxide (H_2O_2) (Halliwell, 1999; Ceriello and Motz, 2004). Formation of OFRs in diabetes are caused by the involvement auto-oxidation of glucose, non-enzymatic protein glycosylation (AGEs), and activation of poliol line (Setiawan and Suhartono, 2005). OFRs cause the increase of oxidative stress of various tissues which indicated by the damage of protein, lipid and DNA (Evans *et al.*, 2002). Some researches have revealed the occurrence of oxidative stress caused by diabetes. According to Lonn, diabetes is a condition which correlates with the increase of oxidative stress as a consequence of hyperglycemia (Atamer *et al.*, 1996). Singh et al reported that there is a strong positive correlation among the metabolism control, the length of diabetes mellitus and the seriousness of oxidative stress induction (Singh *et al.*, 1997). Various studies have shown deficiency of total antioxidant defense status of diabetes patients caused by hyperglycemia, one of which is the glutathione compound which plays a role in the activity of glutathione peroxidase (GPx) (Setiawan and Suhartono, 2005; Lightfoot, 2006). GPx is in peroxide group which can be found in erythrocyte, plasma, liver and other tissues in human body (Winarsi, 2007). GPx acts as an endogenous antioxidant

compound which plays a role of protecting cells, not only from H_2O_2 but also from other organic peroxides (Chen and Schopfer, 1999). Besides GPx, other antioxidant enzyme which takes part in catalyzing H_2O_2 is catalase. To reduce the negative impact of oxidant, as well as to inhibit and terminate the oxidative damage, antioxidants intake is required. Antioxidants can be found in various nature plants, one of which is *A. altilis*. There is an empirical proof related with the use of unripe *A. altilis* in reducing blood-sugar levels of diabetes mellitus patients.

In Indonesia, *A. altilis* has been used as a traditional medicine for diabetes mellitus, high blood pressure, and chronic kidney failure, as well as a cure for swollen or itchy skin. Many beneficial substances such as vitamins and minerals are found in *A. altilis* plant. The leaves and fruits contain flavonoid and vitamin C (Hakim, 2007; Ersam *et al.*, 2003). Flavonoid and vitamin C are known as exogenous antioxidants which act to reduce free radicals (Kirsh *et al.*, 2006). The flavonoid group consists of flavon, isoflavon, flavonol, flavonon, and antocyanin. Two isoprenilflavon compounds from santon, i.e. artonol B and from furanohidrobenzosanton i.e. cycloartobilosanton are new compounds discovered in *A. altilis* with the highest oxidation level (Hakim, 2007; Ersam *et al.*, 2003). Antioxidants in *A. altilis* fruit are suspected to be beneficial in reducing oxidative stress in diabetic condition. However there is no scientific research that has proven this expectation hence this research is conducted. This research aimed to prove the influence of decoction of unripe *A. artilis* to the levels of AGEs of male rats (*R. norvegicus*) with diabetes. It is expected that this research can provide some information on the effects of decoction of unripe *A. altilis* to oxidative stress in hyperglycemic condition and as an antioxidant supplement to prevent further complication of diabetic sufferer.

METHODS

Streptozosin was purchased from CV. Kristalindo Biolab Surabaya. All the other chemicals used were of analytical grade and purchased from commercial sources.

Fruit of *A. altilis* were collected from Banjarbaru,South Kalimantan. Identification of samples was done by using standard botanical monographs. They were further confirmed with the Department of Biology, MIPA Faculty of Lambung Mangkurat University. *A. artilis* started with a certain weight of unripe material which is boiled with aquades until the volume of the water becomes 1/3 of its initial volume. The concentration of decoction was set to be 0.266 , 0.533 and 1.066 mg/g body weight/day/100 ml. Male *Rattus novergicus* rats, weighing 300-330 g obtained from Research Unit of animal Yogyakarta. The animal were maintained on standard rat feed and ad libitum water. The animals fasted overnight and diabetes was induced by single intraperitoneal injection of freshly-prepared STZ (55 mg/Kg body weight of rats) in 0.1 M citrate buffer (pH 4.5).The animals were allowed to drink 5% glucose solution overnight to overcome the drug-induced hypoglycaemia. Control rats were injected with citrate buffer alone. The animals were considered as diabetic, if their blood glucose values were above 200 mg/dL on the third day after the STZ injection. The treatment was started on the fourth day after the STZ injection and this was considered the first day of treatment. The Treatment was continued for 30 days (Kaleem *et al.*, 2006). The rats were divided into five groups comprising six animals in each group as follows: Group I: control rats given only buffer; group II: diabetic control (STZ 55mg/kg body weight of rats);group III: diabetic rats treated with *A.altilis* (0.266 mg/g body weight of rats/day/100ml) in aqueous solution orally for 30 days; group IV: diabetic rats treated with *A.altilis* (0.533mg/g body weight of rats/day/100ml) in aqueous solution orally for 30 days; group V: diabetic rats treated with *A.altilis* (1.066 mg/g body weight of rats/day/100ml) in aqueous solution orally for 30 days. After completion of treatment, the animals were sacrificed. Blood was collected in tubes containing potassium oxalate and sodium fluoride. Plasma was used for estimation of glucose using the easy touch.

The liver tissue were excited and rinsed in ice-cold saline. Tissue were cut into small pieces and homogenized in Tris-HCL buffer (pH 7.4). The homogenate was centrifuged and supernatant was used for AGEs measurement. AGEs was estimated using the method of Voziyan and coworkers (2003). AGEs spectrophotometric were carried out in UV-visible at λ=340 nm (genesys 20) spectrophotometer. All data were statistically evaluated using computer. Hypothesis testing methods included one way analysis of variance (ANOVA) followed by least significant differences test Tukey HSD. P-values of less than 0.05 were considered to indicate statistical significances. All the result were expressed as mean±standard deviation (SD) for six animals in each group.

RESULTS

Based on the research, data of AGEs level of liver rats with diabetic after given decoction of unripe *A. altilis* are shown in table 1 and figure 1 below:

Table 1. Effect of treatment Breadfruit A.altilis for 30 days on AGEs of control and experimental groups of rats

Group	AGEs
1	0.724 ± 0.593
2	1.128 ± 0.211[a]
3	0.937 ± 0.230[b]
4	0.805 ± 0.193[b]
5	1.346 ± 0.253[b]

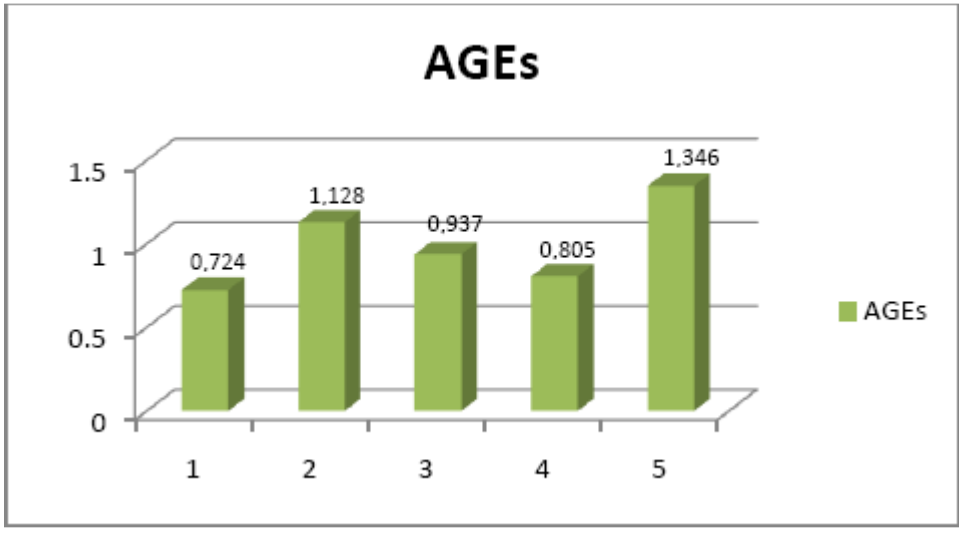

Figure 1. Diagram effect of treatment Breadfruit *A.altilis* for 30 days on AGEs of control and experimental groups of rats

1: control
2: diabetic
3: diabetic+*A.altilis* 0.266 mg/g body weight/day/100ml
4: diabetic+*A.altilis* 0.533 mg/g body weight/day/100ml
5:diabetic +A.altilis 1.066 mg/g body weight/day/100ml

Table 1 and figure 1 show that there is a significant increase of AGEs ($p<0,05$) in the diabetic group compared with the control (a). The given of decoction of unripe *A.altilis* with the concentration of 0.266 and 0.533 mg/g body weight of rats/day/100ml (P3, P4) to the diabetic rats have reduced AGEs significantly compared with the diabetic group (b).

DISCUSSION

Oxidative stress has an important role in diabetic, that is in diabetes condition, OFRs are produced from stimulation of H_2O_2 in cell-β of pancreas. Many researches have shown that concentration of lipid peroxides and hydroperoxide increase in diabetic rats. This indicates the increase of free radicals formation. The increase of lipid peroxides in diabetic may cause oxidative stress as a consequence of the decrease of endogenous antioxidant enzyme system (Kaleem *et al.*, 2005). Results of pre-clinic research have given many convincing evidence on the role of medicinal plant on the decrease of oxidative stress for diabetics.

The decrease of endogenous antioxidants and the increase of peroxidase in mellitus diabetics have emphasized the urgency of keeping the potential of antioxidants. The increasing production in mellitus diabetics are caused by glucose autooxidase, protein glicase, and activation of poliol line (Evans *et al.,* 2002). OFRs are the cause of the increase of oxidative stress of various tissues which indicated by the damage of proteins, lipids and DNA (Atamer *et al.*, 1996).

The high level of glucose in STZ induced rats can cause oxidative stress. STZ works selectively in rats (*R.norvegicus*) by damaging cell- β of pancreas, hence inhibiting the synthesis of insulin hormone creating the hyperglycemia. This is in accordance with the research result of Matsuoka *et al.* (1997) which stated that the increase of reactive oxygen compound in pancreas' cell-β can inhibit the transcription of insulin gene. The given of decoction of unripe *A. altilis* facilitates the decrease of free radicals produced during diabetes. This is supported by the level of AGEs in P3 (dose of 0.266 mg/g body weight of rats/day/100ml) and P4 (dose of 0.533 mg/g body weight of rats/day /100ml) which are significantly lower than P2 group (without the given of *A. altilis*). With the dose, the exogenous antioxidant contained in unripe *A. altilis* is able to show a significant effect in inhibiting the formation of AGEs. Therefore the decoction of *A. altilis* with doses of 0.266 mg/g body weight of rats/day/100ml and 0.533 mg/g body weight of rats/day/100ml can partially reduce imbalance between OFRs and scavenging enzyme activity. Hence the excess formation of AGEs caused by the influence of various glycation reactions can be reduced. It is assumed that this phenomenon is caused by the influence of various antioxidant contained in unripe *A. artilis*, namely some micronutrients such as vitamin B1, vitamin C, and minerals, flavonoid and steroid (Hakim, 2007; Ersam *et al.*, 2003). Micronutrients and flavonoid act as inhibitors for AGEs. Micronutrients as vitamins and minerals are needed by the body to run specific functions i.e. the continuation of metabolism reactions and cellular reactions such as glycolysis, lipid cycle, and acid metabolism to retain the production of body energy. Micronutrients are famous for their role in preventing and dealing with complication that often found in type 1 and 2 diabetes patients (O'Connel, 2001). Derivate of vitamin B1, vitamin B6, pyridoxamin and pyrophosphate thiamin are known to be the inhibition of protein modification. According to Voziyan, there are 2 inhibition mechanisms, that is by electrofilic reaction of –NH2 cluster with dicarbonyl cluster and metal chelating agent (Culbertson *et al.*, 2003).

Flavonoid in A.altilis fruit is a compound discent of prenilased flavonoid as artoindonesianin-F (stilben type), artoindonesianin- AN (arilbenzofuran discent). Flavonoid is one of the contents of medicinal plants which can be function as antioxidants (Hakim, 2007). Flavonoid can reduce free radicals (…), alkoksil, (ROO), and (OH) (Kirsh *et al.*, 2006; Pourmorad *et al.*, 2006). The work mechanism of anti-free radicals consists of (3): (1) reducing of formation of free radicals or reactive oxygen species, either by enzyme inhibition or metal ion chelating which involved in the production of free radicals. (2) scavenging of free-radicals. Based on the research by Culbertson and coworkers (Tuminah, 2003), continuous formation of Amadori product which end with the formation of AGEs in glycocilase can be reduced or limited by giving chelating agent and radical trap by antioxidants.

Vitamin C as a micronutrient contained in unripe fruit of *A. altilis* can inhibit the formation of AGEs through its role as a reduction-equivalent donor (Beckman *et al.,* 2001). The role is done by ascorbate acid which is a form of active vitamin C, as an equivalent donor it is capable of playing role as free-radicals reducer and reacting directly with superperoxide anion (O_2), hydroxyl radical (OH) and lipid peroxide. Vitamin C can inhibit formation of O_2, OH, peroxil radical (ROO), singlet oxygen (O_2) and (H_2O_2). This is in line with the research of Beckman and coworkers (2001). Intake of vitamin C can repair the function of decreasing endothel by hyperglycemia. In vitro, vitamin C also plays a role as a co-antioxidant in the regeneration form of α-tocoferol, gluthation, and β -caroten. The role of the vitamin C is beneficial for maintaining cells membrane integrity. Intakes of exogenous antioxidant of vitamin C and flavonoid which contained in unripe *A. altilis* show the influence of AGEs levels of rats hepar toward group P4 ($p<0,05$) indicated by the decrease of AGEs levels compared with the positive control which STZ induced without the intake of decoction of unripe *A. altilis*.

Diabetes rats with the decoction of unripe fruit of *A. altilis* for group 5 showed an interesting result. Compared with group 4, there is an increase of AGEs levels in group 5. therefore it is suspected that the active compounds in the decoction of unripe fruit of *A. altilis* have potentials as antioxidants in certain range of dosage. However, in higher dosage there is a possibility that the active compounds may change into pro-oxidants which need endogenous antioxidants to neutralize them. In line with this finding, active compounds such as vitamin C will be oxidized fast with the presence of metal catalyst, especially Cu. Vitamin C oxidation inducted by Cu can produce peroxide hydrogen (H_2O_2) and hydroxyl radical (OH). The hydroxyl radical can draw hydrogen atom from the membrane lipid causing lipid peroxidation (Halliwell, 1999). The iron-ascorbate binding even can produce much more hydroxyl radical and activate some enzyme including catalase (Ceriello and Motz, 2004; Ivanova and Ivanov, 2000). From the statement it can be assumed that high ascorbate acid consumption may trigger the formation of high hydroxyl radical (OH).

CONCLUSION

From this research it can be concluded that decoction of unripe A.altilis can reduce free radicals caused by diabetes which is indicated by the decrease of liver AGEs especially at the concentration of 0.266 and 0.533 mg/gbody weight of rats/day/100ml, however, higher concentration showed a potential as pro-oxidant. Therefore the use of decoction of unripe *A.altilis* needs further chemical and pharmacology investigations to be used as an antioxidant supplement to prevent further complication of diabetes.

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ANTIOXIDANT ACTIVITY OF FLAVONOIDS COMPOUND FROM KELOR LEAVES (*MORINGA OLEIFERA*)

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Abstract: Kelor (*Moringa oleifera*) had been investigated that consists of tannins, flavanoids, saponins, and alkaloids which usually have antioxidant activity. The aim of this investigation is to determine antioxidant activity of flavanoid compound from kelor leaves. Antioxidant activity was determined by 2,2-diphenil-2-picrylhydrazyl (DPPH) free radical scavenging method. The result showed that from 13 fraction of ethanol extract, fraction 11 was the most active fraction for antioxidant activity (IC_{50} 147.3 μg/ml). The fraction 11 had been separated by preparative TLC and consists of four components (F 11.1, 11.2, 11.3, and11.4). F11.1 was analysed by TLC and quercetine as a standard, showed that F11.1 gave the same retention factor (Rf) value with quercetine. FTIR Identification showed that F11.1 content –OH stretch (3429 cm^{-1}), C-H aliphatic (2924 cm^{-1}, 2854.2 cm^{-1}), C=O stretch (1637.96 cm^{-1}), overtone or combination bands (2426.45; 2362.47; 2337.98cm^{-1}), C=C ring stretch (1450 cm^{-1}), C-O stretch (1109.90 cm^{-1}).

INTRODUCTION

Moringa oleifera Lam. (horseradish, drumstick tree, kelor, marangghi, moltong, parrongge) is a highly valued plant, distributed in many countries of the tropics and subtropics. It has an impressive range of medicinal uses with high nutritional value. Different parts of this plant contain a profile of important minerals, and are a good source of protein, vitamins, beta-carotene, amino acids and derivate of phenol.

Various parts of kelor are generally known for their multiple pharmacological activities. A leaf extracts show hypocholesterolaemic (Gupta *et al.* 1999). Hypotensive, antioxidant, and anti-ulcerative activity (Siddhuraju and Becker, 2003). The dry pods are adequate to use as a substratum for laboratory animal bedding. The seeds show antifungal and antibacterial (Eilert et al. 1981), antitumor (Murakami et al. 1998), anti-inflammatory, diuretic, antispasmodic and larvicide's activity against the mosquito which transmits dengue and yellow fever. The seeds of this plant are also employed for water purification (Okuda *et al.*, 2001). Gupta and coworkers (1999) showed that the roots were able to depress the central nervous system, cause analgesia and potentate the analgesic effect of morphine.

Phytochemical investigation isolated the bioactive compounds from the seeds of kelor and found to have glycosides such as niazimicin and niazirin, beta-xyterol and 41% moringa oil (which was found to contain high level of unsaturated fatty acids similar to olive oil) (Mukarazami *et al.*, 1998). Phytochemistry analysis of leave's extract demonstrated the presence of the common phytoconstituents like tannin, saponins, flavanoids, terpenoids, and glycoside (Nepolean et al. 2009).

MATERIALS AND METHODS

Plant materials and chemicals

Kelor leaves were collected from Bogor, Indonesia.

Preparation of kelor extract

The dried and powdered kelor leaves were extracted with n-hexane for three days. The residue was extracted with ethanol 70% for three days. The extracts were filtered using Whatman 40 and concentrated in vacuum at 40°C using a rotary evaporator. The extract yields were then calculated.

Fractionated ethanol extract by column chromatography

Sum of 1.5 g of semi-solid masses of ethanol extract were separated by column chromatography using step gradient method (methanol-ethyl acetate as a mobile phase). Each fraction concentrated in vacuum at 40°C using a rotary evaporator to yield semi-solid masses whose weights were determined.

Antioxidant assay (Batubara et al. 2009)

The antioxidant assay used in this study adopted a free-radical-scavenging activity using the 2,2-diphenyl-1-picryl-hydrazyl (DPPH) test. Each fraction were diluted in ethanol to make final concentrations of 25, 50, 100, 150, 200, and 250 µg/ml. 100 µl of DPPH solution (11.8 mg DPPH in 100 ml ethanol) was added to each well plate. After 30 min, the absorbance of the mixture was measured at 490 nm. The positive control was ascorbic acid while ethanol was used as the blank. The inhibitory activity was calculated according to the following equation:

$$\text{Inhibition (\%)} = [1 - (A_{sample} - A_{control})/(A_{blank} - A_{control})] \times 100\%.$$

where A_{sample} is the absorbance of the sample, $A_{control}$ is the absorbance of ascorbic acid as control and A_{blank} is the absorbance of ethanol as the blank. Each fraction concentration of the fractions and positive control were tested in duplicate.

RESULTS AND DISCUSSION

Sum of 13 fractions had been separated from ethanol extract of kelor leaves. The rendement and retention factor (Rf) value of the fractions are presented in Table 1.

Table 1 Rendement and Rf value for the fractions of ethanol extract

Number of Fraction	Rendement (%)	Flavanoid test	R_f value
1	7.06	+	0.12, 0.95, 0.88, 0.75
2	5.3	-	0.98, 0.90, 0.85, 0.78
3	4.58	-	0.96
4	2.99	+	0.93, 0.88
5	1.28	+	0.96
6	21.19	+	0.94, 0.86
7	2.8	-	0.98, 0.91, 0.84, 0.75
8	9.97	+	0.95, 0.85
9	3.15	-	0.94
10	1.61	+	0.93, 0.82
11	5.32	+	0.93, 0.86, 0.81, 0.75
12	12.91	-	0
13	5.87	-	0

Each of fractions was tested with flavanoid test and antioxidant activity. Fraction 11 was the most active fraction for antioxidant activity (IC_{50} 147.3 µg/ml). Relation between fractions concentration and % inhibition are presented in Fig.1.

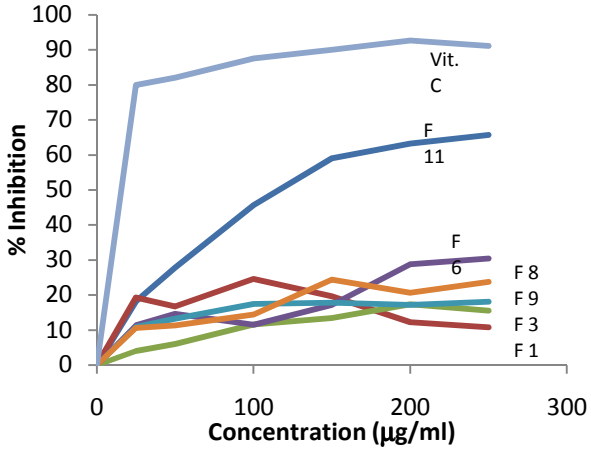

Fig. 1 Relation between fraction concentration and % inhibition

Fraction 11 had been separated by preparative TLC (methanol: ethyl acetate: water as mobile phase). The separation was obtained four components (F11.1, F11.2, F11.3, and F11.4). Each of fraction was analysed by TLC (with quercetine as a standard), F11.1 showed the same resolution with quercetine (Rf = 0.93). FTIR identification showed that F11.1 content –OH stretch (3429cm^{-1}), C-H aliphatic (2924 cm^{-1}, 2854.2 cm^{-1}), C=O stretch (1637.96 cm^{-1}), overtone or combination bands (2426.45; 2362.47; 2337.98cm^{-1}), C=C ring stretch (1450 cm^{-1}), C-O stretch (1109.90 cm^{-1}). FTIR spectrum of F11.1 is presented in Fig 2.

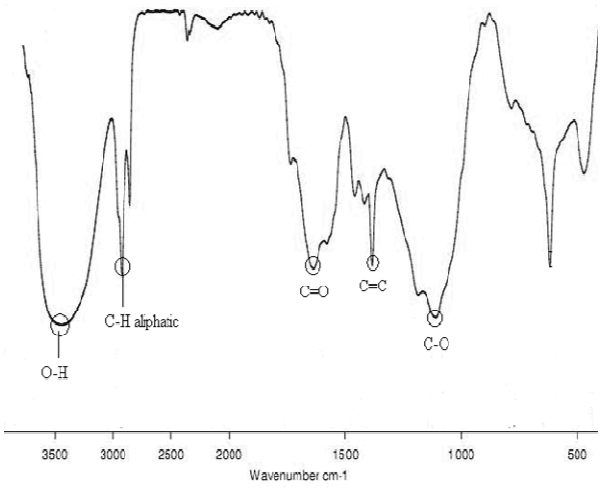

Fig. 2 FTIR spectra of F11.1

CONCLUSION

Fraction 11 from this study demonstrated as the best antioxidant value. Fraction 11.1 was analysed by TLC and had the same resolution with quercetine as standard (Rf = 0.93), FTIR identification of F11.1 showed F11.1 content functional group –OH, C-H aliphatic, C=O, aromatic, and C-O.

ACKNOWLEDGEMENTS

We would like to thank Prof. Dr. Ir. Latifah K. Darusman, Dr. Irmanida Batubara, and Muhammad Yusuf, who have supported us throughout this research.

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DOCKING STUDY AND STRUCTURE MODIFICATION OF ETHYL *P*-METHOXYCINNAMATE ISOLATED FROM *KAEMPFERIA GALANGA* LINN. TO ENHANCE ITS SELECTIVITY ON CYCLOOXYGENASE-2

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ABSTRACT : Ethyl *p*-methoxycinnamate, major ingredient of *Kaempferia galanga* rhizome, have been reported not only has analgesic – anti inflammatory activities like NSAIDs which inhibited cyclooxygenase, but also inhibition of tumor cell proliferation in specimen of mouse epidermis and extent of papilloma. Therefore, it will be strategic and interesting to carry out docking and synthetic studies on the derivates of ethyl *p*-methoxycinnamate to search for novel cyclooxygenase-2 inhibitor lead compounds. We wish to report of structure modification on carboxyl moiety of ethyl *p*-methoxycinnamate and evaluate on their selectivity on COX-2 through molecular docking experiment. Molecular docking experiments were carried out to identify potential COX-2 inhibitors among the derivates of ethyl *p*-methoxycinnamate by Mollegro Virtual Docker program (pdb:1CX2). Isolation of ethyl *p*-methoxycinnamate from *Kaempferia galanga* Linn. rhizome was carried out by percolation with ethanol 96% as solvent. Hydrolysis of ethyl *p*-methoxycinnamate in basic condition was performed to obtain *p*-methoxycinnamic acid. Some thiourea compounds derivates of ethyl *p*-methoxycinnamate was carried out by microwave irradiation. MolDock Score on COX-2 through Mollegro program of aromatic thiourea derivates of ethyl *p*-methoxycinnamate higher than that ethyl *p*-methoxycinnamate. The condition reaction synthesis of derivates of ethyl *p*-methoxycinnamate through microwave irradiation will optimize to improve their yield percentage.

Keywords : ethyl *p*-methoxycinnamate, *Kaempferia galanga*, cyclooxygenase-2, docking study.

INTRODUCTION

Cyclooxygenases (COX) are the key enzymes in the synthesis of prostaglandins (PGs), the ma-in mediators of inflammation, pain and increased body temperature. Prostaglandins are formed from their precursor, arachidonic acid, which cleaved from cell membrane phospholipids by phosphor-lipase A2 (Danneberg *et al,*, 2005; Wu *et al.*, 2004).

There are two isoforms of COX: COX-1 and COX-2, which are differ in many respects. COX-1 is expressed constitutively in most tissues and appears to be responsible for the production of PGs that control normal physiologic functions including maintenance of the gastric mucosa, regulation of renal blood flow and platelet aggregation. In contrast, COX-2 is not detected in most normal tissues. But, it is rapidly induced by both inflammatory and mitogenic stimuli resulting in increased PG synthesis in neoplastic and inflamed tissues (Wu *et al.*, 2004; Dolzhenkol *et al.*, 2006)

Non-steroidal anti-inflammatory drugs (NSAIDs) are dual inhibitors of COX-1/COX-2, and prevent PG synthesis, thus exhibiting analgesic, antipyretic and anti-inflammatory actions. How-ever, NSAIDs have a number of adverse effects, mainly

because of their inhibition of the constitutive isoform of COX. The major adverse effects of NSAID are gastrotoxic effects (*e.g.*, damage of gastric mucosa, gastric bleeding and gastroduodenal ulcers), increased bleeding tendency and delay of the birth process (Dolzhenkol *et al.*, 2006).

COX-2 can be selectively inhibited even though the active sites of COX-2 and COX-1 have si-milar structures. A substitution of isoleucine in COX-1 with valine in the nonsteroidal anti-inflam-matory drug (NSAID) binding site of COX-2 creates a void volume located to the side of the central active site channel in COX-2. Compounds synthesized to bind in this additional space inhibit COX-2, but not COX-1 (Michaux *et al.*, 2006).

In contrast to conventional NSAIDs, selective COX-2 inhibitors do not suppress platelet func-tion and thereby increase the risk of a bleeding complication. Beside that, cyclooxygenase-2 inhi-bitors have been used to block angiogenesis and tumor proliferation process. However, recent publi-cations have suggested that COX-2 inhibitors, like rofecoxib and celecoxib, may be prothrombotic and increase the risk of myocardial infarction (Sulaiman *et al.*, 2008). Consequently, a synthesis of new NSAIDs, that can block angiogenesis and tumor proliferation, with potent anti-inflammatory, analgesic-anti-pyretic action, but with no adverse effects is highly desired.

Ethyl *p*-methoxycinnamate, major ingredient of *Kaempferia galanga* Linn. rhizome, have been reported not only for treate pain as analgesic-anti inflammatory activities like NSAIDs, but also its activity on inhibition proliferation tumor cell in specimen of mouse epidermis and extent of papilloma (Vimala *et al.*, 1999; Xue and Chen, 2001). Therefore, it will be strategic and interesting to carry out a study on the derivates of ethyl *p*-methoxycinnamate and use it as lead compound to search novel cyclooxygenase-2 inhibitor.

RESULTS AND DISCUSSION

Molecular Docking Experiment

Molecular docking experiments were carried out to identify potential cyclooxygenase (COX-1 & COX-2) inhibitors among the derivates of ethyl *p*-methoxycinnamate by Mollegro Virtual Doc-ker (pdb 1CX2). Moldock Score on cyclooxygenase-2 through Mollegro Virtual Docker program of ethyl *p*-methoxycinnamete and its derivates; Celecoxib as standard for COX-2 selective inhibitor :

Table 1. MolDock Score for COX-2 of ethyl *p*-methoxycinnamate and its derivates

No	Compound	MolDock Score for COX-2 (kcal/mole)
1.	ethyl *p*-methoxycinnamate	-95.6337
2.	Celecoxib	-160.535
3.	(*E*)-3-(4-methoxyphenyl)-*N*-(4-methoxyphenylcarbamothioyl) acrylamide	-153.824
4.	(*E*)-*N*-carbamothioyl-3-(4-methoxyphenyl)acrylamide	-103.132
5.	(*E*)-3-(4-methoxyphenyl)-*N*-(4-methylphenylcarbamothioyl) acrylamide	-147.175
6.	(*E*)-3-(4-methoxyphenyl)-*N*-(phenylcarbamothioyl)acrylamide	-141.426

From Table 1. we know that the (*E*)-3-(4-methoxyphenyl)-*N*-(4-methoxyphenylcarbamothioyl) acrylamide has interacted more stable than ethyl *p*-methoxycinnamate and another derivates. This is maybe because its structure more bulky than those, so better interact with active site of COX-2 than those compounds. Celecoxib more stable interact at active site of COX-2 than the other compounds because its structure has sulfonamide group that is able to interact with arginine residue of COX-2 (Simmons, 2004).

Synthesis

Start from ethyl *p*-methoxycinnamate **(1)** from *Kaempferia galangal* Linn., the *p*-methoxy-cinnamoyl isothiocyanates (**4**) were synthesized through the reaction of *p*-methoxycinnamoyl chloride (**3**) and powder ammonium thiocyanate. The final compounds (**6a-6d**) were obtained by the reaction of some primary amines (**5a-5d**) and *p*-methoxycinnamoyl thiocyanates (**4**). (Fig. 1).

Unlike the reaction condition in room temperature (Ekowati, 2007), this reaction is reflux on water bath for about 1 h and separation is convenient. The chemical structures of the synthesize compounds will be confirmed by ^{1}H-NMR, ^{13}C-NMR, IR and HRMS spectral data, the purity will be ascertained by melting point and TLC test.

Experimental

Ethyl p-methoxycinnamate was isolated from *Kaempferia galanga* Linn. under the known method (Ekowati, 2007). *Kaempferia galanga* was collected from Purwodadi Botanical Garden. All reagents and solvent were purchased from standard commercial suppliers. Melting points were measured with a Electrothermal melting point apparatus without correction. IR spectra were recorded in KBr on Jasco FT-IR 5300, and major absorption was listed in cm^{-1}. ^{1}H-NMR and ^{13}C-NMR spectra were obtained on BRUKER instrument, and chemical shift were reported in ppm on the δ-scale from internal Me_4Si. MS spectra were measured with a JEOL JMS 600 spectrometer by using the EI methods. TLC was carried out on glass plates coated with silica gel F_{254} (Merck). Spot detection was performed with UV 254 nm or iodine vapor.

General procedure of Structure Modification of ethyl *p*-methoxycinnamate Isolated from *Kaempferia galangal* Linn.

Ethyl p-methoxycinnamate (2.5 mmol) in 200 ml 5% KOH/ethanol solution was heated for 2 hours by water bath, then acidified with HCl to produce p-methoxycinnammic acid. The crude product was purified by recrystalization using certain solvent and determined its melting point. To 5 mmol of p-methoxycinnamic acid in 10 ml dry benzene and one drop of pyridine, a 5-fold excess of thionyl chloride was added. Care was taken to trap the HCl vapour formed. The mixture was refluxed overnight, the solvent and excess thionyl chloride were removed by rotary evaporation. The addition of benzene and evaporation were repeated several times to remove the last traces of thionyl chloride to give a dark yellow solid. p-methoxycinnamoyl chloride was used in the next re-action without purification. After that, powdered ammonium thiocyanate (7.5 mmol), appropriate p-methoxycinnamoyl chloride (5 mmol), PEG-400 (0.09 g) and dichlorometane (12.5 ml) were placed in a dried round-bottomed flask containing a magnetic stirrer bar and irradiated under microwave 140 W for 60s. Then appropriate amines (ammonia, aniline, p-toluidine and p-anisidine) (5 mmol) was added and the mixture was irradiated under microwave 140W again for 60s. The mixture was filtered off to remove inorganic salts and the filtrate was concentrated under reduced pressure. The resulting solid was recrystalised from ethanol to give respond as p-methoxycynnamoyl thiourea compounds.

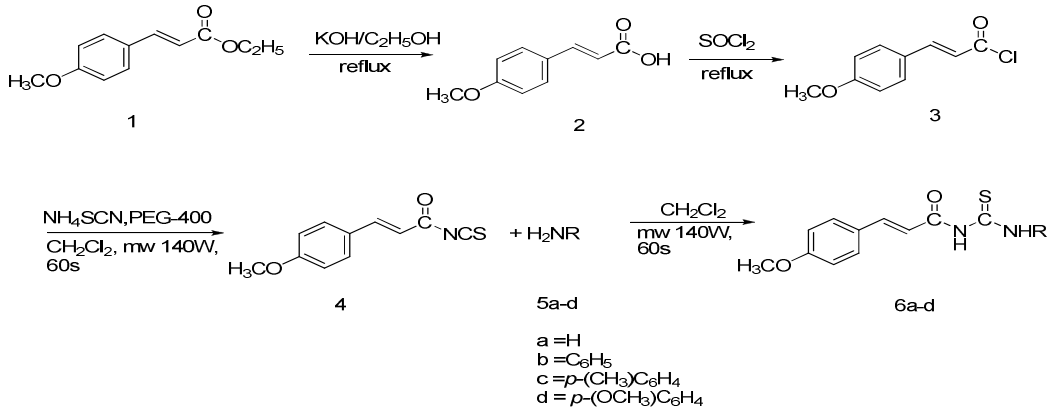

Fig. 1. The schematic representation of compounds 1-6

Transformation ethyl *p*-methoxycinnamate to *p*-methoxycinnamic acid.
p-methoxycinnamic acid (yield 80%) as white crystal (m.p. 169°C). HRMS m/z EI, 178 (M^+). ^{1}H NMR (DMSO) 3.78 (3H, s), 6.60 (1H, d, *J* = 16 Hz), 6.96 (2H, d, *J* = 5.0 Hz), 7.53 (1H, d, *J* = 16 Hz), 7.62 (2H, d, *J* = 5.0 Hz). ^{13}C NMR (DMSO) 55.5 ppm, 114.54 ppm, 116.71 ppm, 127.02 ppm, 130.13 ppm, 143.92 ppm, 161.12 ppm, 168.03 ppm, IR (KBr) 2937, 2843, 2567, 1685, 1624, 1288, 1255 cm^{-1}. Calculated Mass $C_{10}H_{10}O_3$ 178.0630. Measured Mass 178.0617.

Transformation *p*-methoxycinnamic acid to *p*-methoxycinnamoyl chloride
p-methoxycinnamoyl chloride (yield 90%) as yellow solid (m.p. 51°C). HRMS m/z EI, 196 (M^+). ^{1}H NMR ($CDCl_3$) 3.87 (3H, s), 6.51 (1H, d, *J* = 15.2 Hz), 6.96 (2H, d, *J* = 5.0 Hz), 7.53 (1H, d, *J* = 16 Hz), 7.62 (2H, d, *J* = 5.0 Hz). ^{13}C NMR (DMSO) 55.5 ppm, 114.75 ppm, 116.70 ppm, 127.02 ppm, 130.14 ppm, 143.94 ppm, 161.13 ppm, 168.02 ppm. Calc. Mass $C_{10}H_9O_2Cl$ 196.0291. Measured Mass 196.0276.

Synthesis of *(E)*-3-(4-methoxyphenyl)-*N*-(4-methoxyphenylcarbamothioyl)acrylamide.
(yield 26%) as pale yellow crystal (m.p. 181°C) MS m/z EI , 342 (M^+). ^{1}H NMR (DMSO) 3.76 (3H, s), 3.80 (3H,s), 6.86 (1H, d, *J* = 15.80 Hz), 6.95-7.03 (4H, m), 7.51-7.59 (4H, m), 7.70 (1H, d, *J* = 15.80 Hz), 11.43 (1H, s), 12.58 (1H,s). ^{13}C NMR (DMSO) 55.49, 55.61, 114.01, 114.84, 117.23, 125.92, 126.88, 130.33, 130.94, 144.60, 157.59, 161.65, 166.83, 179.23 ppm. IR (KBr) 3235, 3034, 1673, 1593, 1509, 1252, 1150, 825 cm^{-1}. Calc. Mass $C_{16}H_{15}NO_3$ 342.1038. Measured Mass. 342.1031.

Synthesis of (*E*)-*N*-carbamothioyl-3-(4-methoxyphenyl)acrylamide
(yield 15%) as pale yellow crystal (m.p.220°C). MS m/z EI, 236 (M^+). ^{1}H NMR (DMSO) 3.79 (3H, s), 6.82 (1H, d, *J* = 15.60 Hz), 7.00 (2H, d, *J* = 9.20 Hz), 7.55 (2H, d, *J* = 9.20 Hz), 7.64 (1H, d, *J* = 15.60 Hz), 9.40 (1H, s), 9.82 (1H, s), 11.10 (1H,s). IR (KBr) 3357, 3231, 1682, 1593, 1544, 1258, 1159, 821 cm^{-1}. ^{13}C NMR (DMSO) 55.66, 114.88, 117.49, 126.97, 130.32, 144.35, 161.62, 166.31, 182.18 ppm. Calc. Mass $C_{16}H_{15}NO_3$ 236.0619. Measured Mass 236.0601.

Synthesis of (*E*)-3-(4-methoxyphenyl)-*N*-(4-methylphenylcarbamothioyl)acrylamide
(yield 54%) as pale green crystal (m.p. 165°C) MS m/z EI, 326 (M^+). ^{1}H NMR ($CDCl_3$) 2.37 (3H, s), 3.87 (3H, s), 6.31 (1H, d, *J* = 15.40 Hz), 6.93 (2H, d, *J* = 9.60 Hz), 7.21 (2H, d, *J* = 8Hz), 7.78 (1H, d, *J* = 15.40 Hz), 7.54 (4H, m), 8.68 (1H, s), 12.51 (1H,s). IR (KBr) 3224, 3025, 1672, 1590, 1537, 1248, 1151, 827 cm^{-1}. ^{13}C NMR (DMSO) 55.43, 114.51, 115.53, 124.25, 126.42, 129.44, 130.39, 135.10, 146.27, 162.12, 166.08, 178.78 ppm. Calc. Mass $C_{16}H_{15}NO_3$ 326.1095. Measured Mass 326.1089.

Synthesis of (*E*)-3-(4-methoxyphenyl)-*N*-(phenylcarbamothioyl)acrylamide
(yield 50%) as yellow crystal (m.p. 200°C). MS m/z EI, 312 (M^+). ^{1}H NMR ($CDCl_3$) 3.87 (3H, s), 6.31 (1H, d, *J* = 15.60 Hz), 7.30 (1H, d, *J* = 15.60 Hz), 7.71 (2H, d, *J* = 9.60 Hz), 6.94 (2H, d, *J* = 9.60 Hz), 7.50 (2H, d, *J* = 9.60 Hz), 7.41 (2H, t, *J* = 6.80 Hz), 7.28 (1H, t, *J* = 10.80 Hz), 8.69 (1H, s), 12.63 (1H,s). IR (KBr) 3222, 3031, 1671, 1591, 1537, 1244, 1149, 826 cm^{-1}. ^{13}C NMR (DMSO) 55.46 ppm, 114.96 ppm, 124.16 ppm, 126.37 ppm, 126.79 ppm, 128.86 ppm, 130.41 ppm, 137.68 ppm, 146.45 ppm, 162.19 ppm, 166.02 ppm, 178.66 ppm. Calc. Mass $C_{16}H_{15}NO_3$ 312.0932. Measured Mass 312.0928.

CONCLUSION

Mol Dock Score on cyclooxygenase-2 through Mollegro program of aromatic thiou-rea derivates of ethyl *p*-methoxycinnamate higher than that ethyl *p*-methoxycinnamate. The condition reaction synthesis of derivates of ethyl *p*-methoxycinnamate through microwave irradiation will optimize to improve their yield percentage.

ACKNOWLEDGEMENTS

Support for this research was provided by Strategis Nasional Grant from the Airlangga University 2009.

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PROSPECT OF A COMBINATION THERAPY OF HERBS AND PROBIOTIC AS AN ALTERNATIVE CONTROL BACTERIAL DISEASES IN FRESHWATER AQUACULTURE

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Abstract : Antibiotic therapy has been banned in fish disease control because of the emergence of drug-resistant bacteria and the presence of harmful residues to consumers. An alternative therapy directed to the prevention through vaccination and immunostimulant treatment has been introduced. However this approach is not always applicable for an ill fish. Herbs and probiotic combination therapy can be alternative. Immunostimulant herbs and probiotic bacteria are intented to compete with pathogens. Herbs used in this study were Meniran (*Phyllantus urinaria*), Ciplukan (*Physsalis angulata*), Kelor (*Moringa oleifera* Lamk.) and Babandotan (*Ageratum conyzoides*) while probiotics samples were the collection of Research Institute of Freshwater Aquaculture Bogor capable of eradicating pathogenic bacteria *in vitro*. This research was carried out using pathogenic bacteria *Aeromonas hydrophila* that was inoculated at *Tryptic Soy Agar* media. The disc was dipped in the extract of herb and probiotic at various concentrations and was challenged *in vitro*. The test sample demonstrating the greatest effect can be used for disease control in freshwater aquaculture.

Keywords: herbs, probiotic, alternative disease control

INTRODUCTION

As aquacultural production becomes more intensive, the incidence of disease including various infectious diseases has increased as a result of it leading to significant economic losses. Diseases are a crucial factor which inhibits the expansion of aquaculture. One of the bacterial diseases that often attack the disease of freshwater fish was MAS (Motile Aeromonads Septicemia), known also as red spot disease (red spot disease) caused by *Aeromonas hydrophila*. Various chemotherapeutics have been used for treatment or prevention of diseases. However, the use of antimicrobial agents in aquaculture has resulted in more resistant bacterial strains. These resistant bacterial strains could have a negative impact on the therapy of fish diseases or human diseases and the environment of the fish farm. Weston (1996) stated that many aquaculture chemicals are, by their nature, biocide, and maybe released to the surrounding environment at toxic concentrations either through misuse or in some cases, even by following generally accepted procedures for use. Thus, there is a potential for mortality of non target organisms.

Herbs have been widely used in veterinary and human medicine. They are natural products that are not only safe for consumers but also widely available throughout Asia. Nowadays, herbs or herbal products also have a significant role in aquaculture. The aim of this research is to investigate the antibacterial activity at the herbs (*Phyllanthus urinaria*, *Physsalis angulata, Moringa oleifera* Lamk., *Ageratum conyzoides*) and probiotics bacteria against freshwater pathogenic bacteria *Aeromonas hydrophila*.

MATERIAL AND METHOD

The sensitivity of medicinal herbs and probiotics bacteria against pathogenic bacteria of freshwater fish was studied during the period from January to June 2010. Four kind of herbs, meniran (*Phyllanthus urinaria*), ciplukan (*Physsalis angulata*), kelor (*Moringa oleifera* Lamk.) and babandotan (*Ageratum conyzoides*) and two isolates of probiotics bacteria, from mud (L1.C5) and Gouramy intestine (GB.1), were used for this study against pathogenic bacteria, *Aeromonas hydrophila*.

Figure 1. Herbs used

Preparations of crude herbal extracts

The fresh leaves of each of the selected herbs were washed by sterilized distilled water and were dried in oven. The dried leaves were grinded and extracted using hot distilled water and then centrifuged it. Finally, the extract was collected and preserved at 4 ºC for further studied.

Figure 2. (Left) Herbs dried with oven
(Right) Herbs that have been extracted

Sensitivity Test

A suspension of freshly cultured pathogenic bacteria *A. hydrophila* (10^8 cfu/ml) was prepared and 0,1 ml of this suspension was spread over the tryptic soy agar (TSA) plates.Then fine herbal extract of each herbs and probiotics bacteria were dipped in discs and were inoculated in cultured plate to detect the response. Inhibitory response of herbal

extract and probiotics was recorded according to normal growth response of bacteria after incubation at 30 ºC for 18 to 24 h and the extracts were categorized as high, medium and low inhibitory response to observe the sensitive zone. Normal growth was recognized as resistant to extracts and clear zone was recognized as sensitive one.
The research treatment were:

T1	: *P. urinaria* vs Probiotic 1	T9	: *P. urinaria* vs *A. hydrophila*
T2	: *P. urinaria* vs Probiotic 2	T10	: *P. urinaria* vs *A. hydrophila*
T3	: *P. angulata* vs Probiotic 1	T11	: *P. angulata* vs *A. hydrophila*
T4	: *P. angulata* vs Probiotic 2	T12	: *P. angulata* vs *A. hydrophila*
T5	: *M. oleifera* vs Probiotic 1	T13	: *M. oleifera* vs *A. hydrophila*
T6	: *M. oleifera* vs Probiotic 2	T14	: *M. oleifera* vs *A. hydrophila*
T7	: *A. conyzoides* vs Probiotic 1	T15	: *A. conyzoides* vs *A. hydrophila*
T8	: *A. conyzoides* vs Probiotic 2	T16	: *A. conyzoides* vs *A. hydrophila*

RESULT AND DISCUSSION

Results from in vitro from the herb *P. urinaria, P. angulata, M. oleifera* and *A. conyzoides* are as follows:

Table 1. In vitro test of herbs against *Aeromonas hydrophila* and probiotics

No.	Herbal	*A. hydrophila*	Probiotic L1C5	Probiotic GB1
1	*P. urinaria*	Negative	Weak Positive	Weak Positive
2	*P. angulata*	Negative	Negative	Negative
3	*M. oleifera*	Weak Positive	Weak Positive	Negative
4	*A. conyzoides*	Weak Positive	Weak Positive	Negative

P. urinaria yielded did not showed inhibitory effect on *A. hydrophila*, but rather it gave low inhibitory response against probiotics L1.C5 and GB.1. Zainun (2006) suggests the existence of the inhibition extracts of *P. urinaria* against *A. hydrophila*. Zainun (2006) used concentration 10 mg / L was able to inhibit the bacteria *A. hydrophila* with an average inhibition zone of 5.6 mm. Thus, 10 mg/L was the minimum dose that can inhibit the bacteria *A. hydrophila*. Subarnas and Sidik (1993) stated that *P. urinaria* had anti-hepatotoxic activity, hypoglycemic, anti-bacteria, inhibition of angiotensin-converling working enzyme (ACE), diuretics, and inhibition of aldose reductase in lens tissue. This was confirmed by Schwotkowski (2004) stating that filantin, hipofilantin, hipotetralin, nirantin, and nirtetrakin is chemical constituents of *P. urinaria* having activity as anti-bacterial. *Physsalis angulata* did not showed inhibitory effect on *A. hydrophila*, probiotics GB.1 and L1.C5. *Physsalis angulata* expected later can be used to complement probiotic preparations. *Moringa oleifera* and *A. conyzoides* gave inhibitory effect on *A. hydrophila* despite weak positive but both can lysis probiotic L1.C5 but not GB.1. The condition that are expected as potential immunostimulatory herb which will serve to stimulate non-specific immune of host, while probiotics L1.C5 and GB.1 it's proven potentially inhibit pathogenic *A. hydrophila*. So if they would combine later, it will yield two probiotics preparation which are responsible for killing the pathogens and the herbs that are expected to help increase the non specific immune system function so that the fish obtain complete protection for tackling the disease.

The following pictures below show the potential of *P. urinaria* and *P. angulata* on GB.1 probiotic and pathogenic *A. hydrophila*.

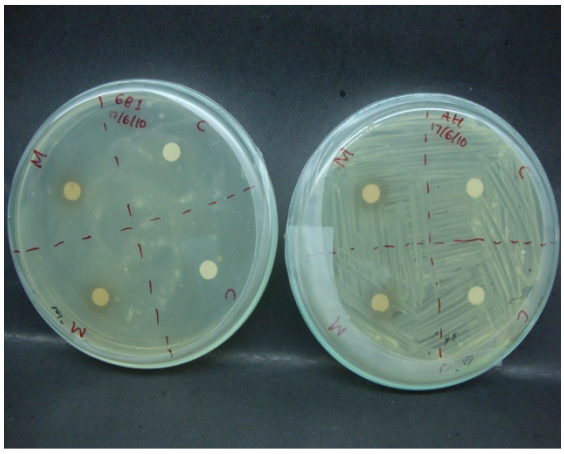

Figure 3. (Left): In vitro test of *P. urinaria* and *P. angulata* on probiotics GB1 (Right): In vitro test of *P. urinaria* and *P. angulata* on *A. hydrophila*

P. urinaria shown in Figure 3 looks inhibit better on the probiotic GB.1 and against the pathogen *A. hydrophila*, another case *P. angulata* cannot inhibit probiotic GB.1 and *A. hydrophila*. *Physsalis angulata* may be as a candidate to be combined with probiotics, although still needs further study in vivo to prove whether *P. angulata* can enhance non-specific immune system. Figure 4 below presents the results of an in vitro *P.urinaria* and *P. angulata* of probiotic L1.C5. *P. urinaria* can inhibit probiotics L1.C5 although weak, but *P. angulata* on the contrary, gave no inhibition against L1.C5. While *M. oleifera* and *A. conyzoides* showed inhibitory effect on probiotic L1.C5 but very weak. The possibility of inhibition ability because of the concentration of bioactive substances that contained in the herb has not been completely extracted or very low bioactive content.

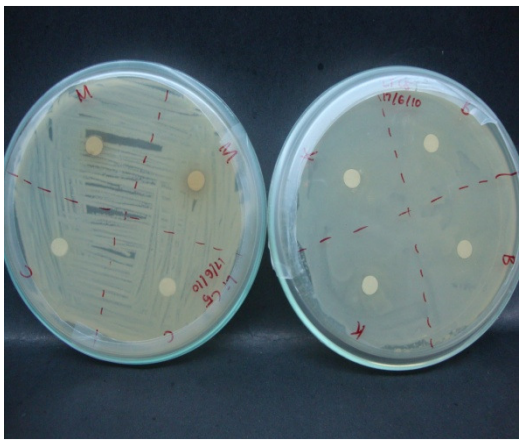

Figure 4.(Left): In vitro test of *P. urinaria* and *P. angulata* on probiotics L1C5 (Right): In vitro test of *M. oleifera* and *A. conyzoides* on probiotics L1C5

Figure 5 below shows *M. oleifera* and *A. conyzoides* may inhibit probiotics L1C5 but were not able inhibit probiotics GB.1.

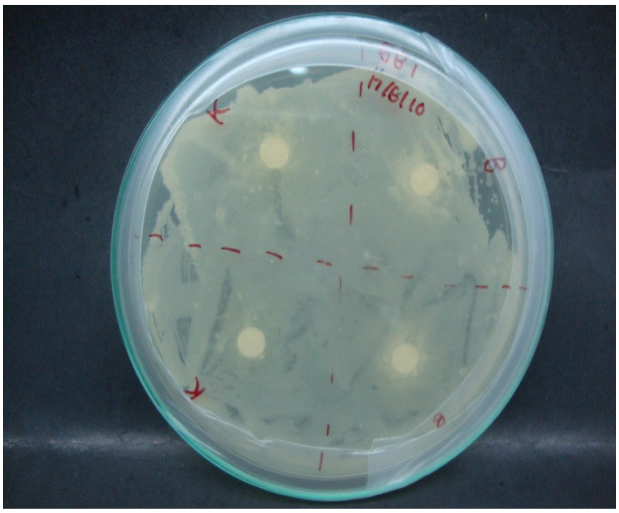

Figure 5. In vitro test of *M. oleifera* and *A. conyzoides* of probiotic GB1

Phyllanthus urinaria included in the Family: *Euphorbiaceae*, with a different name *Phyllanthus amarus, Phyllanthus niruri* or *Phyllanthus debilis*. General name is: Hsieh-hsia Chu (Pearl under the leaf) [China] Chanca piedra (Stone breaker) [Amazonia] Quebra pedra [Brazil] Pitirishi, Budhatri [India] Hurricane weed [Bahamas]. Functions**:** Anodyne, antibacterial, liver protective, anti-inflammatory, antispasmodic, antiviral, carminative, choleretic, diuretic, improves digestion, emmemagogue, laxative, vermifiuge. Energetic functions: Clears the liver, benefits the vision, expels parasites, drainsdamp. Indications: gallstones (urinary tract infection, nephretic edema, Enteritis and diarrhea, infantile marasmus, swollen and inflamed rectum. Prostatitis, gonorrhea, syphillis, vaginitis, hepatitis, jaundice, asthma, bronchitis, cough, tuberculosis fever, influenza, digestive pain, joint pain, conjunctivitis and anemia. The other herbs is ciplukan (*Physalis angulata*). The sinonim is :*Physalis minima* L. The classification: Division: Spermatophyta, Subdivision: Angiosperrnae, Class: Dicotyledonae, Ordo: :Solanales, Family : Solanaceae, Genus: Physalis, Species: *Physalis angulata* L.

Figure 6 The following is an in vitro probiotic GB1 and L1C5 against pathogenic *A. hydrophila* was shown that two probiotic isolates capable inhibit *A. hydrophila.*

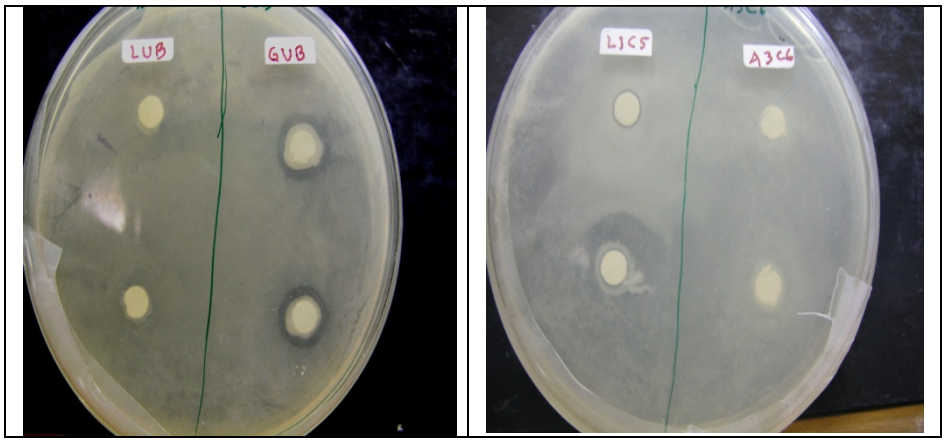

Figure 6. (Left) In vitro test of probiotics GB1 on *A. hydrophila*
(Right) In vitro test of probiotics L1.C5 on *A. hydrophila*

CONCLUSION

Physsalis angulata did not showed inhibitory effects against *A.hydrophila* or probiotics and needs further study to be incorporated as a commercial dosage for enhance non-specific immune to control freshwater fish diseases.

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SUB ACUTE TOXICITY TEST OF ETHANOL EXTRACT OF BETEL PALM (*ARECA CATECHU* L.) ON WISTAR STRAIN ALBINO RAT (*RATTUS NORVEGICUS*)

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Abstract : The usage of medicinal plants as an alternative medicine for people's medication effort is increasing, therefore a research to prove the special benefit and safety of the plant is highly needed. Sub acute toxicity test of ethanol extract of Betel Palm (*Areca catechu* L.) in this research have been done using two parameters; the albi no rat's blood chemistry and it's liver histopathological illustration results. The research laboratory experimental research design with pre test and post test group design. The sample of 40 albino rats were divided into four groups, each consisting of five males and five females. Implementation of subacute toxicity test for 13 consecutive weeks, prior to the test of a complete blood examination, renal and liver function. The control group received only distilled water, treatment group I (P1) were given a dosage areca seed extract at a dose of 60 mg / kg once daily (one time dose). Treatment Group II (P2), a suspension dosage areca seed extract at a dose of 600 mg mg / kg (10 times the dose). Treatment group III (P3) were given suspension dosage areca seed extract at a dose of 1200 mg / kg. During the test development of experimental animals were observed including the food and water intake, the volume of urine and feces, and body weight changes. Further blood, liver and kidney physiological examinations were done at the middle and the last stage of the experiments. At the end of the test performed histopathological examination liver, kidney, heart, lungs and brain. The sub acute toxicity test results generally showed that the administration of multiplication doses of betel palm seed extract up to 20 times of the usual dose did not result in the toxicity illustration, as observed in the blood and urine examinations, and also in the liver, kidney, heart, brain and lung organ histopathological examinations. However, some pathological parameters showed increasing SGPT and thrombocyte. These results can be used as a basis to obtain a clinic test approval (ethical clearance) from the Ethics Commission of the health research center.

***Keywords** : Areca catechu L,* sub acute toxicity, histopathology, clinical pathology

INTRODUCTION

Worm infection on children is still remaining as a public health problem in Indonesia both in rural and also in the urban slums. As a tropical country, Indonesia turns into a high potential area for parasitic worm infection occurrences which are transmitted through soil (Soil-Transmitted Helminthes = STH). One of the infections is the Ascaris lumbricoides (ascariasis) worm infection. This infection is related with poor sanitation and continues environmental pollution by feces all over the year. Research showed that 60% to 80% of the Indonesian residents got infections by one or more of these earthworm type (Soedarto, 1995). Furthermore, school-age children were reported to have the highest prevalence and intensity of ascariasis (Stephenson, 1998). Problems of Reinfection and the low socioeconomic level made a barrier for the usage of modern medicine. Therefore it is necessary to find a practically cheap and easyto- get alternative medicine, to be used as people's self-supporting basis in fighting against the disease. One alternative way of doing this is by using medicinal plants, as they were commonly used in Indonesia. The usage of medicinal plants as an alternative medicine requires some intensive researches to prove their efficacy and safety, with a purpose that the drug can be used safely and effectively afterward. Betel palm (*Areca catechu* L.) has been known by

Indonesian society to cure worm infections, strengthen teeth and gums, treat dysentry, fever and wounds (Muchlisah, 2002).

It has been proved in previous research that areca seed extract contains ovicidal and vermicidal powers against grown-up worms and the Ascaris suum eggs by in vitro, (Muklis, 2007). Areca nuts contain 0.3 to 0.6% alcaloids such as arecolin, arecolidin, arecain, guvacolin, guvacyn and isoguvacyn. Some researches had been carried out both by in vitro and in vivo, resulting a prove that they did not lose effectiveness as compared with the modern worm infection drugs. To prove that the drug would be safe to use as an ascariasis therapy alternative, a toxicity test have to be done in order to see how the test material toxic affects the test animals. Betel palm seed extract (*Areca catechu* L.) acute toxicity test have been carried out by Nuri, *et al.* (2009) on Wistar strain albino rats' liver and kidney during 14 consecutive days. The result of acute toxicity test showed that following the administration of up to 20 times of usual dose, the LD_{50} value could not be determined and there were no specific changes found to the liver and kidney as the consequences. This acute toxicity test was then continued to the subacute toxicity test.

Subacute toxicity tests were essential because sometimes the information or data obtained in acute toxicity tests could not be used to predict what would be happened next after a substance is intentionally or unintentionally admitted into the body repeatedly over a long period (Ngatidjan, 2006). This test was done by administrating some toxic materials consecutively in every day or five times a week, for a period of approximately 10% of the animal's lifetime. This research aims to examine the safety level of the Betel Palm (*Areca catechu* L.) seed ethanol extract through the subacute toxicity test on mice as the experimental animal.

METHOD

A number of 40 rats were divided into four groups, each consisting of five males and five females. Subacute toxicity test execution were started with a complete blood, renal and liver function examination prior to the administration of the traditional medicine, the areca seed extract. Examination results then showed that the experimental animals were under normal circumstances. Furthermore, the areca seed extract were administrated for the next 13 consecutive weeks with doses as follows:

Control groups : negative control group, given a solution of distilled water once a day

Group I : lowest dose group, given the areca seed extract at a dose of 60 mg per kg body weight once a day (1x usual dose)

Group II : given areca seed extract at a dose of 600 mg per kg body weight once a day (10x usual dose),

Group III : the highest dose group, given areca seed extract at a dose of 1200 mg per kg body weight once a day (20x usual dose).

The usual dose measurement in this case was taken from the usual dose used on human being with a certain conversion to mice. During the test, the development of experimental animals were closely monitored including the food and water intake, the volume of urine and feces, and body weight changes. Further blood, liver and kidney physiological examinations were done at the middle and the last stage of the experiments. At the final test, liver, kidney, heart, lung and brain evaluations were done.

RESULTS

Acute toxicity tests have been carried out by Nuri, *et al.* (2009) and resulting that there were no changes in the experimental animals' liver and kidney histopathology during 14 consecutive days of the areca seed extract administration. To achieve a recommendation from the Ethics Commission for proceeding this research further to a clinical test phase, the subacute toxicity test were already conducted during 13 consecutive weeks starting in June 2009 at the Laboratory of Pharmacology Faculty of Medicine of the University of Gadjah Mada. Subacute toxicity test execution started with

a complete blood examination, renal and liver function prior to the administration of the Betel Palm seed extract to the experimental animals (examination I). Examination results then showed that the experimental animals were under normal circumstances.

Furthermore, the experimental animals were then administrated a dose of areca seed extract 1 times (60 mg / kg bw), 10 times (600 mg / kg bw) and 20 times of the usual dose (1200 mg / kg bw) during a 13 consecutive weeks. During the test, the development of experimental animals were closely monitored including the food and water intake, the volume of urine and feces, and body weight changes. Further blood, urine, liver and kidney physiological examinations were done at the middle and the last stage of the experiments. At the final test,liver, kidney, heart, and intestine evaluations were done. The blood and urine test results on rats were as follows:

Table 1. The 1st assessment of liver functions tests in areca seed extract sub acute toxicity test

AST	SGPT	Total Protein	ALB	GLOB
95	81	9.2	4.16	5.04
174	59	6.9	3.79	3.11
220	59	7.9	4.01	3.89
210	84	6.1	3.13	2.97
254	54	8.1	4.06	4.04
176	51	7.7	3.8	3.9
170	60	7.4	3.82	3.58
100	72	7.2	3.32	3.88
264	73	8.2	3.9	4.3
143	72	7.7	3.55	4.15

Table 2. The 2nd assessment of liver functions tests in areca seed extract sub acute toxicity test

AST	SGPT	Total Protein	ALB	GLOB
182	59	8.5	5.19	3.31↑
188	47	7.68	5	2.68↑
144	69	8.64	5.81↑	2.83↑
198	55	7.8	5.69↑	2.11
183	53	7.48	5.47↑	2.02
150	50	7.96	5.29↑	2.67↑
136	52	7.51	5.57↑	1.94
117	53	8.47	5.18	3.29↑
178	70	8.13	4.58	3.55↑
152	50	8.16	6.49↑	2.12

Table 3. The 3rd assessment of liver functions tests in areca seed extract sub-acute toxicity test

AST	SGPT	Total Protein	ALB	GLOB
155	61	8.49	4.27	4.22↑
297	105	9.5	4.72	4.78↑
244	117	7.97	4.21	3.76↑
250	136	8.02	4.3	3.72↑
241	75	8.64	4.2	4.44↑
265	68	9.95	4.47	5.48↑
337	91	8.94	4.1	4.84↑
147	86	9.21	4.3	4.92↑
143	47	8.15	4.03	4.12
195	61	7.81	4.01	3.8

Table 4. The 1st assessment of renal functions yests in areca seed extract sub acute toxicity test

BUN	Creatinine	Uric Acid
34	0.66	4.2
36	0.69	4.4
27	0.72	4.3
26	0.76	4.1
26	0.86	3.8
34	0.69	4.1
27	0.69	4.2
35	0.76	3.8
31	0.83	3.7
31	0.72	3.8

Table 5. The 2nd assessment of renal functions tests in sub acute toxicity test areca seed extract

BUN	Creatinine	Uric Acid
32	0.92	4.4
30	1.04	4.5
30	0.81	4.2
48↑	0.99	4.7
41	0.77	3.9
28	0.88	4.2
39	0.75	3.8
24	0.79	4.4

Table 6. Third examination of renal functions tests in areca seed extract sub-acute toxicity test

BUN	Creatinine	Uric Acid
32	0.6	3.4
38	0.67	3.5
33	0.74	4.6
41	1	4.3
44↑	1.37	6
36	0.82	3.9
39	1.02	4.2
39	0.69	4.3
43	0.9	4.9
38	0.69	4.2

Table 7. The 1st assessment of blood profile in , areca seed extract sub-acute toxicity test

Erythrocytes	Hb	Hmt	Leucocytes	Thrombocytes
5.8	17.4	46	5.2	180
4.3	11.9	36	8.2	260
5.9	16.6	47	4.3	210
6.4	17.1	50	6.8	270
6.6	18.4	52	10.4	200
6.4	16.9	48	8.6	180
5.6	15.7	44	8.9	160
5.7	16.4	48	5.1	190
6.2	16.9	49	10.1	210
5.9	15.5	45	9.6	220

Table 8. The 2nd examination of blood profile in areca seed extract sub-acute toxicity test

Erythrocytes	Hb	Hmt	Leucocytes	Thrombocytes
5.6	15	47	7.8	150
5.7	15.9	47	7.1	190
5	13.9	40	5	140
5.9	16.7	47	11.4	150
5.7	16.4	51	12.3	220
5.4	14.9	46	6.8	160
5.6	16.2	46	7.2	150
6	16.9	48	6.4	360
6	16	53	8.4	190
6.3	18.1	51	7.6	220

Table 9. Third examination of blood profile in areca seed extract sub-acute toxicity test

Erythrocytes	Hb	Hmt	Leucocytes	Thrombocytes
8.6↑	13.7	44	10.3	570↑
7.6↑	16.9	52	4.48	410
6.92↑	12.9	38.9	3.73	530↑
6.8↑	16.4	51	5.73	510↑
10.6↑	16.1	52	5	560↑
8.9↑	16.5	51	9.89	650↑
6.7↑	15.4	48	4.46	600↑
7.8↑	15.6	49	5.5	530↑
10↑	15.8	50	7.3	700↑
6.7↑	15.2	48	8.16	780↑

Examination results in the tables above could be explained as follows: 1) Liver Function Tests On the examination of AST, there were no significant decrease noted on the second examination, then followed by significant increase on the third. The final examination result do not differ significantly compared to the first. On the SGPT check, a not-significantincrease happened at the second check and followed by a significant increase on the third check. Total protein at the third examination showed a significant increase compared to the first and second. Albumin were seen to be increasing significantly at the first, second and third examination, although the increment was still within a normal limit. Globulin levels decreased significantly in the second examination and rose again on the third. 2) Renal Function Tests BUN examination showed a significant increment on the second examination, but on the third one, the increment was not significant and still within normal limits. Creatinine and uric acid did not significantly change from the first until the third examination were done. 3) Blood Profile Examination of leukocytes, hemoglobin and hematocrite did not change significantly from the first up to the last examination. However, erythrocytes and platelets increased significantly at the end of the examination. Histopathological examination of heart, kidney, brain, liver, and, lung were as follows:

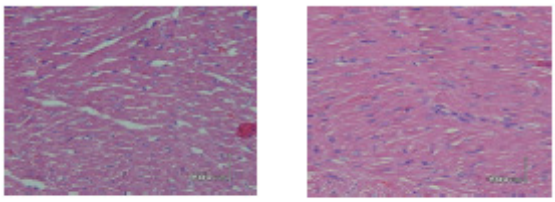

Figure 1. Histopathological picture of the heart (a) control (b) given areca seed extract 20 x of usual dose.

On histopathologic picture of the heart, no specific changes were found after administration of areca seed extract up to 20 times of the usual dose.

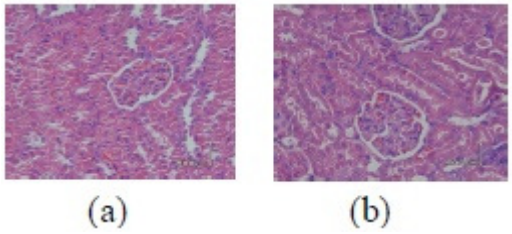

Figure 2. Histopathological picture of kidney (a) control (b) given areca seed extract 20 x of usual dose.

In the description of renal histopathology, no specific changes were found after administration of areca seed extract up to 20 times of the usual dose.

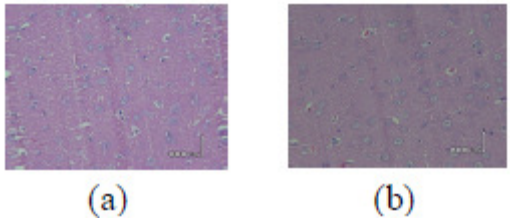

Figure 3. Histopathological picture of the brain (a) control (b) given areca seed extract 20 x of usual dose.

On histopathologic picture of the brain, no specific changes were found after the administration of areca seed extract up to 20 times of the usual dose.

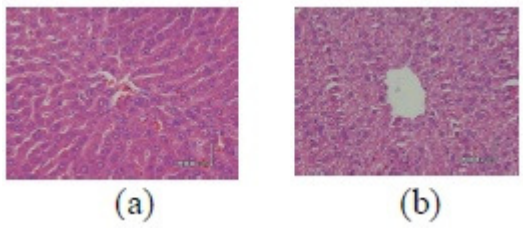

Figure 4. Histopathological picture of the liver (a) control (b) given areca seed extract 20 x of usual dose.

Some of the liver histopathologic illustration indicated the occurrences of congestion. It could possibly caused by the treatment factor or related to the physiological condition of the experimental animals during the treatment, such as stress conditions, increment of blood pressure and so on. The congestion situation could return to a normal state by stopping the treatment. Congestion illustration appears more frequently in the administration of areca seed extract 10 times of the usual dose.

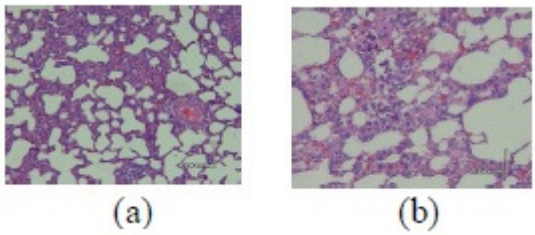

Figure 5. Histopathological picture of lung (a) control (b) given areca seed extract 20 x of usual dose

Some of the lung histopathologic illustrations indicated the occurrences of intestinal and lymphocytic perivasculitis pneumonia. This could be caused by a viral infection during the treatment and did not relate to the treatment itself. Those sub-acute toxicity test results generally shown the conclusions that by administrating areca seed extract up to 20 times of the usual dose didn't result in a toxic illustration, both seen from the blood and urine test results as well as from the histopathologic examinations of liver, kidney, heart, brain and the lung. Nevertheless, some clinical pathology parameters showed an increment of ALT and platelets. These results will be used as the basis for achieving the clinical test approval (ethical clearance) from the Commission on Ethics of health research.

CONCLUSION

From the above research we could conclude that there were no significant hanges shown on clinical pathology examination in the administration of areca seed extract up to 20 times of usual dose, except for ALT and platelets increment at the end of the examination. Similarly, the histopathological examination of the heart, kidneys, lungs and brain didn't indicate any significant changes, while in the liver check some of which are experiencing congestion due to physiological conditions of the experimental animals.

ACKNOWLEDGEMENTS

Thank to the Ministry of Research and Technology for Research and Technology Incentive Grants Year 2009

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SURVEY ON PIPERACEAE FAMILY AT KOTA AGUNG VILLAGE, LAHAT DISTRICT, SOUTH SUMATERA

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Abstract: Piperaceae is a plant family that commonly used as medicinal plants. This family consider had 10 genera and about 1,400 species. In order to inventarize Piperaceae species at Kota Agung, Lahat, South Sumatera a survey was done at April 10 and 11, 2010, at village, forest and riverside area. Samples were collected and identified at Plant Ecology laboratory, Biology Department, Faculty of Science, The University of Sriwijaya,. km 32 Indralaya, South Sumatera. Five species were found; *Piper nigrum, Piper betle, Piper cubeba, Piper aduncum* and *Peperomia pellucidai.* An unknown species was found like Piper, but not identified yet.

Keywords : Piperaceae and species

INTRODUCTION

Piperaceae (pepper family) is a family of order Piperales from subclass Magnoliidae. Field recognition on this family from leaveas alternate, simple, petioles winged, stem jointed, flowers small, bisexual or unisexual in racemes or spikes that are leaf-opposed and appearing terminal.with a small drupe fruit. Habit are woody climbers, shrubs or small trees. A pan-tropical family of about 10 genera and a large but uncertain number of species variously estimated to be from 1400 to over 2000. The bulk of the species belong to only two genera, *Piper* and *Peperomia*. The anatomy Piperaceae are unusual for dicotyledons in their vascular by having scattered vascular bundles (Jones and Luchsinger, 1986). This family considered as the closest taxa to monocotyledons. USDA(2010) noted family Piperaceae had three genera; Piper, Peperomia and Lepianthes. Tjitosoepomo (1987) wrote the genera Heckeria as a Piperaceae family. Other genus is *Macropiper*, found in New Zealand (Anonymous, 2010). Main characteristic of these family is the specific flavour, so peoples use them as medicine and spices. The famous is *Piper nigrum*, as black pepper source; and betel pepper (*P betle*) for masticatory. Van Steenis et al. (1975) said that Piperaceae member family, *P betle and P cubeba* are used by peoples as medicines. Kota Agung, is a villages placed in Kota Agung District, region of Lahat, the province of South Sumatera. It has 1,495 citizen, the second crowd villages in Kota Agung District. The village close to hills and forest area, that conservated by government. Deforestation is a common problem here, eventhough law has protect the forest from logging. Peoples usually use forest area for cultivating plants, i.e., coffee, and pepper. Survey of family Piperaceae needs for species protection and scientific information.

MATERIAL AND METHODS

Survey was done by canfield method, along plantation area of hill slope, deforastation area at top of hill, forest area on hill and riverside from top to down hill. All of specimen that belong to Piperaceae were got and proceed to be herbarium. Photo of fresh specimen taked at field. Identification of samples conducted at Plant Ecology Laboratory, Biology Departement, Faculty of Science, The University of Sriwijaya, during 10-12 April 2010.

RESULTS AND DISCUSSION

Research survey found the spesies as shown at below tabel;

Table 1. Piperasceae species found at Kota Agung, Lahat.

No.	Scientific name	Vernonial name	Habitat
1.	*Piper aduncum*	Pohon sirih	River side
2.	*Piper betle*	Sirih	Planted at garden
3.	*Piper cubeba*	Sesirihan	Stone, river side
4.	*Peperomia pellucida*	Sirih-sirih	Stone, river side
5.	*Piper nigrum*	Sahang	River side

As shown on table above; 5 species were found; and every species could be discuss as follow:

1. *Piper aduncum*
   Found at river side, bush. Habit: trees. Picture 2 below show DR Salni, was taking sample plant of this species while survey was done. Bernard *et al.* (1995) published that *P. aduncum* extract shows insecticidal and growthreducing activity,same with *P. tuberculatum* and *P. decurrens*. T is said that lignans and isobutyl-amides are the active defence compounds in this species. Jose *et al.* (2006) find out 12 compounds in *P. aduncum*, mainly was (E)- nerolidol. On other side, Misni *et al*. (2008) published too about repellent activity of *P. aduncum* essential oil against Aedes aegypti by using human volunteers. *P. aduncum* essential oil on human skin at a concentration of 0.4 g, showed an immedied 100 % reduction in *Aedes aegypti* mosquito bite, with reduced to 70.03 % at hour 8 post-treatment.
2. *Piper betle*
   This is climbing plant with specific smell. Leaves cordate. Picture 3 below shows P betle planted at peoples garden at hill slope in survey area. Van Steenis *et al.* (1975) said P betle used for masticatory and medicine. Commonly peoples in Indonesia know this plant as 'sirih'. Anonymous (2000) published that P betle native to South East Asia, and was planted from 500 years BC. It is said, a mixture of betel leaves and other ingredients is used as a masticatory, which act as a gentle stimulant and is taken after meals to sweeten the breath. The leaves, roots and seeds are all used for medicinal propose in Asia. The leaves are credited with among others; carminative, stimulant, stomachic, expectorant, tonic, astringent, sialagogue, laxative, antihelminthic, and aphrodisiac properties. Peoples at Indralaya, South Sumatera used it as eyes medicine.
3. *Piper cubeba* (see picture 4).
   Just like P betle, but small. Van Steenis et al. (1975) said this plant use for medicine in Indonesia and planted by peoples, but it could find in wild plant. A phrase of Malay says: "*seperti kerakap tumbuh di batu, hidup segan mati tak mau*" (like P cubeba growth on stone, lazy live but doesn't want to die).
4. *Peperomia pellucida*
   Small herb with cordate leaves. Living on soil and stones.(picture 4). Common name is 'shiny bush' or 'silver bush'. Anonymous (2009) said that P pellucida is the plant species that has a history of ethnomedicinal use. Antiimflamantory, chemotherapeutic, and analgesic properties have been found in crude extracts of P pellucida. A scientist of The University of Sriwijaya, DR. Nurtjahjadi, shows me years ago how to increase our body health by consume P pellucida directly, without some treatments. Take and make clear from soil, eat.
5. *Piper nigrum*
   Climbing, cordate leaves. Most popular, trade for spices. (Picture 5). These conference talking about *P. nigrum*, so it is no more comment about it. In this research, *P. nigrum*

planted at coffe trees by farmers. This research find out five species of Piperaceae. Anonymous (2006) said that Botany Departement of Hawaii University survey on Piperaceae found 8 species namely; *Peperomia caperata, Peperomia tetraphylla, Peperomia remyi,* 3 species of other *Peperomia, Piper methysticum* and *Piper nigrum* . This research find less than Hawaii University;because the forest had been disturbed. Picture 1 below show how jungle forest had been logged for plantation/agriculture. Use of insecticide and herbicide in the survey area proved by foud formed bottle of herbicide at river side (Picture 6).

Picture 1. Survey location, note the top of hill with deforestation area

Picture 2. Sampling of Piper aduncum, at river side.

Picture 3. Piper betle, climbing on the tree.

Picture 4. Piper cubeba and Peperomia pellucida on the big stone, at river side

Picture 5. Piper nigrum, planted by people at garden area near little house of hill slope

Picture 6. Herbicide bottle, throw away after using and rolling down by river water

SUMMARY

Five species were found; *Piper nigrum, Piper betle, Piper cubeba, Piper aduncum* and *Peperomia pellucidai.*

ACKNOWLEDGEMENT

We thank The University of Sriwijaya for funding help.

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THE DEVELOPMENT OF TABLET FORMULATION OF *ARTOCARPUS CHAMPEDEN* STEMBARK EXTRACT AS ANTIMALARIAL DRUG

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Abstract: Parasite resistance to antimalarial drug, chloroquine and sulfadoxin-pirimetamin, still become a major problem in malaria control worldwide, therefore, the effort in developing a new and different target of antimalarial drug become a high priority. Our preliminary test revealed that extract from *A. champeden* exhibiedt potent antimalarial activities againts *P. falciparum in vitro* and *P. berghei in vivo.* Several isolated compounds from this plant exhibited antimalarial activity. One of the isolated compound identified as heteroflavon C, a prenylated flavone, have a higher antimalarial activity than chloroquine. Therefore, it is potential to be developed as antimalarial drug. The research was conducted to develop tablet formulation of ethanol extract of *A. champeden* stembark (EEAC). The formula that composed : EEAC 150 mg, lactose 140 mg, cab-o-sil 5%, amilum 46 mg, avicel PH 101 7%, primogel 5%, and Mg stearat 1% was the selected formula. The tablet hardness of the formula has span between 9.0-12.27 kP and the average is 10.78 kP, the disintegration time of formula 12 minutes 47 seconds. A standard 4-days test on *P. berghei* infected mice was used to evaluated *in vivo* antimalarial activity of the tablet. This research revealed that EEAC tablet has antimalarial activity against parasite *P. berghei in vivo.* Oral administration of EEAC tablet at a dose of 10 mg/kg body weight multiple dose (twice a day) inhibited the parasite growth better than 100 mg/kg body weight single dose (once a day). Antimalarial activity of tablet in multiple dose per oral showed inhibition of parasite growth of 73.88 %, while at single dose per oral showed inhibition of parasite growth of 83.32%.

Keywords *: A.champeden,* tablet formulation*, P. berghei, in vivo* antimalarial activity

INTRODUCTION

Parasite resistance to antimalarial drug, chloroquine and sulfadoxin-pirimetamin, still become a major problem in malaria control worldwide, therefore, the effort in developing a new and different target of antimalarial drug become a high priority. *Artocarpus champeden* (family Moraceae) known as ”cempedak”, is widely distributed in Indonesia and has been traditionally used in malarial remedies (Heyne, 1987). Previous study reported that prenylated stilbene from *Artocarpus integer* (syn *A. champeden*) exhibited antimalarial activities againts *P falciparum* (Boonlaksiri et al., 2000). Our preliminary test revealed that extract from *A. champeden* exhibited potent antimalarial activities againts *P. falciparum in vitro* and *P. berghei in vivo.* Several isolated compounds from this plant exhibited antimalarial activity (Hidayati, 2003; Utomo, 2003; Ernawati, 2005). One of the isolated compound identified as heteroflavon C, a prenylated flavone, have a higher antimalarial activity than chloroquine (Widyawaruyanti et al., 2007[a]). Standarized ethanol extract of *A.champeden* stembark (EEAC) also exhibited potent antimalarial activities againts *P. falciparum in vitro* and *P. berghei in vivo.* Therefore, it is potential to develop EEAC as antimalarial phytopharmaceutical product. As phytopharmaceutical product , that requires consistency in the efficacy, safety, and effectivity (Widyawaruyanti *et al.*, 2007[b]; Widyawaruyanti, 2008).

The research was conducted to develop tablet formulation of EEAC and to evaluate the effectivity as antimalarial drug.

MATERIALS AND METHODS

Materials

The stembark of *A. champeden* were collected from Mugirejo, Samarinda, East Kalimantan, Indonesia on October 2009.

The tablet excipients used were lactose monohydrate, amilum manihot as filler, colloidal silicon dioxide (Cab-o-Sil) as absorbent and glidant, microcrystalline cellulose (Avicel PH 101) and sodium starch glycolate (Primogel) as disintegrant and magnesium stearate as lubricant.

Preparation of extract

10 kg powdered cempedak stembark was macerated with ethanol solvent 80% during 2 hour with warm-up at temperature 40^{o}C. Then it was filtered. The residue dried and re-macerated with the same solvent for three times. Extract collected and evaporated to obtain concentrate EEAC.

Preparation of EEAC tablets

The tablet formulation containing EEAC were prepared according to the formula given in table 1. Tablets were prepared by wet granulation method. Extract was dissolved in ethanol for homogenization then mixed with lactose monohydrate, amilum manihot, Cab-O-Sil, Avicel PH101 and Primogel (internal phase disintegrant). The moist mass was granulated by passing them through a 12 mesh sieve (2,100 µm) then dried at 40^{o}C. After the drying process, the granules obtained were resieved through a 18 mesh sieve (1,400 µm). Then the granules were mixed with sodium starch glycolate (external phase disintegrant) and Mg stearate.

The granules were compressed into tablets (final weight 430 mg) by means of spherical punch (1.0 mm in diameter) using laboratory single-punch tablet pump for 1 second with loads of 2 tons. The tablets were evaluated for physical characteristics including tablet hardness, weight uniformity, friability and disintegration time. Then were determined for its antimalarial activity.

Table 1. Formula of EEAC Tablets

Component	Quantity
Internal addition	
EEAC	150 mg
Lactose monohydrate	140 mg
Cab-o-Sil	5%
Amilum manihot	46 mg
Avicel PH 101	7%
Primogel	5%
External addition	
Primogel	5%
Mg stearat	1%
Tablet weight	430 mg

Tablet organolpetic and weight uniformity

Tablets were evaluated for their physical appearance visually and also for its higroscopicity. For tablet weight uniformity, 20 tablets were weighed individually by using O'Hauss miligram balance

Crushing Strength and Friability Tests

The load (kP) required to diametrically break each tablet was determined at room temperature using Erweka Hardness tester. Ten tablets were used for its determination. The friability of the tablets were evaluated for 20 tablets using Erweka Friability Tester which operated at 50 rotation per minute for 10 minutes.

Disintegration Test

The disintegration time of tablets were determined in distilled water at 37 ± 0.5^0C using an Erweka disintegration testing apparatus.

Evaluation of Tablet Properties

a. Physical appeareance, tablets were evaluated for their physical appearance visually and also for higroscopicity.
b. Tablet hardness, it was determined for 10 tablets using Erweka Hardness Tester.
c. Tablet weight uniformity, 20 tablets were weighed individually by using O'Hauss miligram balance. The result should fulfill the requirements.

In vivo antimalarial test

Antimalarial activity of EEAC tablet was determined by modification of the "4-day Supressived Test" originally described by Peters (1980).

For each experiment, mice were randomly assigned to a given treatment group (five mice in each group). The day of infection is termed D0, and succeeding day of infection are termed D1, D2, etc. EEAC tablet was suspended in CMC-Na (0.5%) and was given to mice once a day at dose 100 mg/kg body weight (as single dose) and twice a day 10 mg/kg body weight (as multiple dose). While untreated group received CMC-Na (0.5%) solution. Thin blood smear were made from the tail blood everyday. The level of parasitemia in mice, as seen in Giemsa-stained smears, were assessed.

RESULT AND DISCUSSION

Result of Formulation Study of EEAC Optimation formula of EEAC tablet

EEAC tablets have been successfully prepared and evaluated. The tablets were evaluated for uniformity of weight, hardness, friability, and disintegration time. The results of physical appearance of EEAC tablet prepared using wet granulation method shows good physical appearance and relatively non higroscopic. The result indicate that the tablets fullfill the requirement (398,3 – 462,9 mg mg) which have range of tablet weight between 424,8 - 435,0 mg. A good degree of uniformity of weight was achieved for all of tablet prepared. The percent deviation did not exceed 5%, indicating excellent uniformity of weight in the tablet formulations prepared (Table 2).

The tablet exhibited good mechanical properties with regard to both hardness and friability (Tables 3 and 4). The crushing strength test results interval value of tablets hardness between 9.0 - 12.27 kP with an average is 10,78 kP. In the friability studies, weight loss values of the tablet was less than 1%.

The result of disintegration time of EEAC tablet shows the time required for all tablets to break up into small particle is 11 minutes 56 seconds. This is less than traditional medicine requisite which stated time of disintegration should be less than 20 minutes.

Table 2.Result of evaluation uniformity of weight of EEAC tablet

No	weight (mg)	No.	weight (mg)
1	432.2	11	432.5
	427.8	12	433.3
3	430.6	13	435.0
4	426.7	14	428.7
5	431.3	15	433.0
6	424.8	16	431.7
7	430.9	17	432.1
8	428.7	18	432.5
9	431.9	19	427.4
10	430.7	20	430.0
Interval = 424.8 – 435.0 mg Requisite = 398.3 – 462.9 mg (7.5%)			
Average = 430.6 mg			
Standart deviation = 2.54%			

Table 3. Result of evaluation hardness of EEAC tablet

No	Hardness (kP)
1	9.02
2	10.32
3	10.51
4	10.89
5	11.83
6	10.51
7	9.06
8	12.65
9	12.27
10	10.76
Interval = 9.0 - 12.27 kP	
Average = 10.78 kP	
Standart deviation = 1.21 %	

Table 4. Result of evaluation friability of EEAC tablet

Weight before test	8,624.2 mg
Weight after test	8,607.4 mg
Percentage of weight loss	0.19%

In vivo antimalarial test

Antimalaria activity test result from various therapy models of EEAC tablets showed at fig 1. The result showed that EEAC tablet given as single dose and multiple dose can inhibit the growth of parasite, compare with the untreated group. Three day after the treatment ended, the inhibition percentage for EEAC tablet as single dose was 83.32% and multiple dose was 73,88%.

The dosage of EEAC tablet given twice a day is five times than given once a day. It concluded that multiple dose (twice a day, 10 mg/kg body weight) more effective than single dose (once a day, 10 mg/kg body weight).

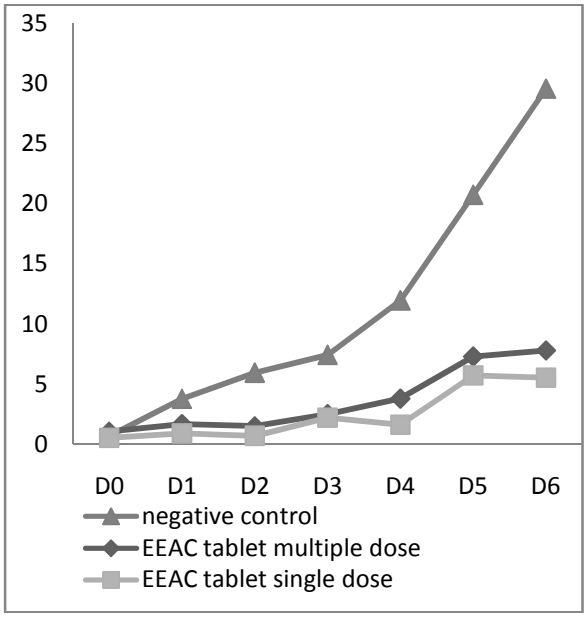

Fig 1. Graphic of parasitemia percentage *P. berghei* infected mice

CONCLUSION

The formula of EEAC tablet that composed : EEAC 150 mg, lactose 140 mg, cab-o-sil 5%, amilum 46 mg, avicel PH 101 7%, primogel 5%, and Mg stearat 1% was active as antimalarial drug. Oral administration of EEAC tablet at dose of 10 mg/kg body weight multiple dose (twice a day) more potential than 100 mg/kg body weight single dose (once a day).

ACKNOWLEGEMENT

This study received financial support from DIKTI RUSNAS No. 171/SP2H/PP/DP2M/V/2009.

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TESTING AND TRANSDERMAL'S FORMULATION OF LEAF EXTRACT *PTEROCARPUS INDICUS* THE SHADE STREET TO LOWER BLOOD SUGAR RATE

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Abstract : The aim of this study was to determining the effect of drug's penetration using transdermal patch. Skin is one of the drug's releases routes in which it has many advantages. The advantages are active drug ingredients that are not resistant acid can not hydrolized by stomach acid, preventing the first pass effect in the liver so it can increase the bioavailability of the drug. The composition of the transdermal patchs consist of HPMC: PG (35%: 40%, 10%: 40%), HPMC: glycerol (35%: 10%, 10%: 10%) with 2 grams of menthol as an enhancer, and extract of leaves *Pterocarpus indicus* as an active ingredient 0.2 grams for each formulation. The method of this study to test pharmacological used enzymatic method than for the penetration in vitro used Franz diffusion cell method. The dosage of patchs formulation with the composition of the HPMC: glycerol (10%: 10%) can penetrates of the drug's releases well, with a linear correlation between the dosage of drug's penetrated against time. Pharmacological effects on extract of leaves *Pterocarpus indicus* dose of 250 mg / kg and 450 mg / kg can be used as an antidiabetic after the seventh day, which was tested on mice experimental animals.

Keywords : *pterocarpus indicus*, transdermal, antidiabetic, penetration

INTRODUCTION

Diabetes or increased blood sugar levels is disease that increasingly getting popular day by day mortality rates are increasingly higher. Diabetes mellitus or commonly called "*mother of all diseases*" is a disease in which the concentration of glucose (simple sugar) in the blood is high because the body can not release or use insulin adequately. According to the diagnostic criteria Perkeni (Endrokrinologi Indonesian Association) in 2006, is said to have diabetes if a person has a fasting blood glucose > 126 mg/dL and the tests when > 200 mg/dL. Various research necessary to find good way of treatment for decreasing blood sugar with minimal side effects. Based on that conducted various studies to find ways of good sugar treatment to lower blood sugar levels with minimal side effects.

There are many functions from *Pterocarpus indicus* such as extract of the bark in the Philippines are used for diabetes therapy, leprosies and flu. Further more, the young leaves are used to accelerate cook boils and water soaked leaves are used to wash in to get the hair to grow better, water decoction of the cola tree is also to stop the diarrhea, or a as gargle to heal cancer sores, and even the sap can be for shampoos (Heyne,1987). Leaf extract Kino and P.incidus was also reported to have a property to control the tumor and cancer (Duke and Wain, 1981). Juice from the root of this plant in Malaysia is used for treatment of syphilis. In Indonesia ,young leaves are used as a treatment ulcher or ulcers (Thomson, 2006). Ironically in Indonesia, this plant is only popular as a shade and ornamental plants in urban roadside.

Substances contained in Pterocarpus indicus consist of isoflavones, flavones, narrin, santalin, angolensin, pterocarpin, pterostilben homopterocarpin, prunetin (prunusetin), formonoetin, isoliquiritigenin, p-hydroxyhydratropic acid, pterofuran, ptercarpol, β-eusdemol (Duke, 1983) and (-)-epicatechin (Takeuchi *et al.*, 1985) that play a role in decreasing blood sugar. Basd on the resuts of the study in vivo between diabetic

ras treated with glibenclamide and antihiperglikemik f bark extracts of *Pterocarpus santalinus* L. (Wich contains lupeol, β-sitosterol,(-)-epicatechin) at doses of 0.25 g/ kg BW obtained result is more effective than glibenclamide (Rao *et al.*,20010, where as Pterocarpus marsupium Roxb.have ability to lower blood sugar in experimental animals within five days and have a compound that contained the (-)-epicatechin (Ahmad *et al.*, 1991). (-)-epicatechin have hypoglycemic effects due to regenerate beta cells, insulin has the effect of such activities and also converting poinsulin to insulin (Rao *et al.*, 2001). *Perocarpus santalinus* L Plant and *Pterocarpus marsupium* Roxb.have been investigated and antihiperglikemik hypoglicemid effect can only be obtained abroad, the using plants alternative *Pterocarpus indicus* Willd that many scattered in the archipelago,which is one clan. Result from various studies angsana leaves can give the effect of blood glucose levels declne. By the Biological Research (1990) about the effects of infusion of leaves of *Pterocarpus indicus* Willd orally 10% no difference with the 50 mg /kg bw of tolbutamide, where as infusion decreased 20% larger than the effects by tolbutamide in effects decreasing the blood glucose level.This research examined the effects of ethanol extract of leaves of Pterocarpus indicus Wild in transdermal preparations.

Intravenous and oral dosage forms of this leaf has less pharmacological effectiveness, where the active ingredients flavonoids from these stocks will experience the hydrolysis in acid (stomach acid). Trasdermal formulations of the extract was used to overcame these problems because the network directly into the blood. In the preparation of matrix type patch, the type of polymer used as matrix plays as an important role in the nature of chemical physics and penetrating patch dosage of active ingredients. In this study, using HPMC matrices. The use of menthol as a penetration enhancer for 0.2 ounces to enhance terabsobsi flavonoids. Propylene glycol at levels of 10% gives the best plasticizer properties of transdermal patches.

MATERIALS AND METHODS

Plant Material

The plant material used in this study are: Sonokembang leaves (*Pterocarpus indicus* W.) taken on the road Dinoya,Surabaya,East Java. Section of young leaves on aereted, until dry and then is pulverized as reseach material. Before being used for research, the plant is determinated at the Botanical Garden LIPI Purwodadi Pasuruan, East Java.

Chemicals

Chemicals used in this study, if not stated, the degree of p.a (pro analysis), including: n-hexane, Acetone, Ethanol 70%, Ethanol 96%, Methanol, Acetic Acid glacial, Aluminium chloride, n-butanol, Silica gel 60 GF254, Silika gel 60 for column chromatography, WFI (Water For Injection) (Brataco Chemika Surabaya, Indonesia), Distilled water, HPMC, Gliserol, Propilene glikol, Menthol, Alloxan, Insulin 100 IU, PGA (Gom Arab), Na2HPO4, NaH2PO4, Rutin, Sodium Hydrochloride.

Specimen

Wistar strain rat skin obtained from male skin rats. Shaved skin of rats with clipped fur, then store in the refrigerator until used.

Research Tools

The tools used for this research is a set of tools perkolator, gram scales, a set of thin layer chromatography instruments, glass instruments, mouisture analyzer MA 30, capililary tube, Oven (Memmert, Germany), porcelain cup, bowls, desiccator, densitometers, a set of tools ash, restrainer, advantagemeter, striptest, strirer, Frans Diffusion Cell penetration tools.

Research Stages:

The first examination of Sonokembang and flavonoid

The first examination of Sonokembang and flavonoid include macroscopic and microscopic leaf slices Sonokembang (*Pterocarpus indicus* W.)

Determination of sample degree

Determination of sample degree are moisture content and ash content determination.

Determination procedure of ash

Angsana leaf powder weight 20 grams, then weight the empty cup. Then the powder inserted into the cup and then heated at temperature of 100°C for one hour. Once completed to ashes, input into the desiccator at temperature of 50°C for one day. Considering the cup and the powder obtained.

Extraction of Sample

Extraction of sample is using the maceration with cold extraction procedure as follows: considering 150 mg powder and dissolve it in 100 ml ethanol 70%, let it stand at room temperature for one day. After a day of filtered and taken it extract.

Extract Standardization

Parameters of solvent soluble compounds include the levels of certain compounds that dissolve in water and levels of soluble in ethanol.

1. Levels of water soluble compounds
   Maceration of 5 grams of extract for 24 hours with 100 ml of water using chloroform LP clogged with pumpkin whipped several times during the six hours and then left for 18 hours. Strain, steamed 20 ml of filtrate to dry in a shallow cup that has been tared, heat the residue at a temperature of 105°C until the weight remains. Calculate the concentration in percent soluble in water, calculated on the initial extract.
2. Soluble content of Ethanol 70%
   Maceration of 5 grams of extract for 24 hours with 100 ml ethanol 70% use the clogged with pumpkin whipped several times during the first six hours and then left for 18 hours. filter by avoid rapid evaporation ethanol. Then steamed 20 ml filtrate to dry in a shallow cup that has been tared, heat the residue at temperature of 105°C until the weight remains. Calculate the concentration in percent soluble in ethanol 70%, calculated on the initial extract.

TLC Examination

Speckled extract on TLC plate 2 µl of GF254, which is used as mobile phase Butanol : Acetic Acid : Water (4:1:5), which has made a day earlier. Chamber saturated with mobile phase and TLC plate inserted to propagate and reach the phase boundary marker.

Testing Blood Glucose

Measurement of blood glucose levels induced in rats before (day 0) and after alloxan induced diabetes and day (2,3,5,7). Before the blood drawn 16-18 hours of fasting rats. The way the treatment of test animals that is 20 white male wistar rats and was made diabetic with alloxan induced by 150 mg/kgBW by intramuscular and then divided randomly into four groups, each of five rats. The first were given Angsana leaf infusion with doses of 250 mg/kgBW in mouth, the second group were given Angsana leaf infusion with doses 450 mg/kgBW in mouth, the third group as a negative control group were given water 5 ml/kgBW in oral, the fourth group as positive control group were given insulin 12.6 IU/kgBW subcutan. Each group was treated once daily for 7 days. Furthermore, the determination of blood glucose levels is doing day (2,3,5,7) by using

enzymatic method, namely ion intermediate hexacyanoferrate (III) ion will be reduced to hexacyanoferrate (II).

Making patches

Making a patch of leaves angsana according with the formula presented in table 1. Angsana leaf levels selected after performing a blood glucose test. The use of menthol as a penetration enhancer of 0.2 gram. Propylene glycol at levels of 10% gives the best plasticizer properties of transdermal patches.

For each patch with a diameter of 4 cm, derived as follows: leaves of Pterocarpus indicus (0.2 grams) along with 0.2 grams of menthol. Solution is added in the base polymer prepared by dissolving HPMC with Propylene glycol and Glycerol in accordance with the formula in 10 ml alcohol. The solution is poured on the aluminium plate and dried at room temperature for 30 minutes to the evaporation of water and get the film layer. After the film layer is formed, wrapped in aluminium foil and stored in desiccator until used. Each formula replicated four times.

Table 1. Composition Leaf Extract patch dossage Angsana

Function	*Composition*	*Formula A*	*Formula B*	*Formula C*	*Formula D*
Stabilizing	HPMC	35%	10%	35%	10%
Platisizer	PG	40%	40%	-	-
Platisizer	Glycerol	-	-	10%	10%
Enhancer	Menthol	0.2 gram	0.2 gram	0.2 gram	0.2 gram
The active ingredients	Angsana leaf extract	0.2 gram	0.2 gram	0.2 gram	0.2 gram
Solvent	Alcohol	10 ml	10 ml	10 ml	10 ml

To select the best patch preparation, to be optimized fourth formula above.

Penetration Test in Vitro

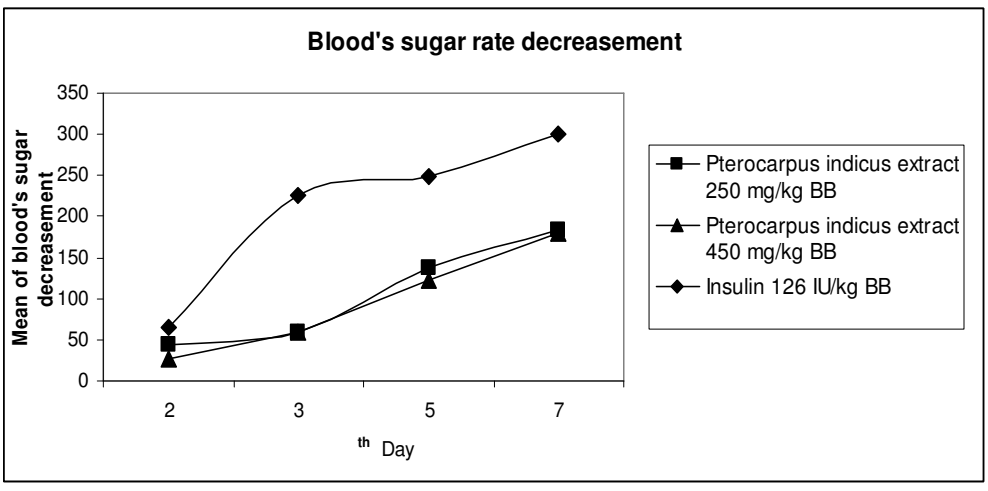
Rat skin obtained from Wistar rats approximately 4 months of age, weight 250-300 gram that had been murdered, her hair shorn using scissors. Skin that has been shaved stored at temperature 4°C in the refrigerator until used.

Penetration tests carried out using a vertical diffusion cell type is modified. Diameter of 4 cm and a volume of 20 ml aceptor compartement. Compartement donor containing a truncated form of circular patches with a diameter of 4 cm and closed. Compartement aceptor contains isotonis pH 7.4 phosphate buffer with the addition NaCl, mixed with 780 rpm speed. Observations were made during six hours, and sample were taken at 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 4, 5, 6, hours of 0.5 ml. Each extract content of the samples tested *Pterocarpus indicus* Willd across the membrane by using densitometry. Marker used were routine. Observations were obtained from the slope of flux on the plot number of *Pterocarpus indicus* Willd root across the membrane vs time.

RESULTS AND DISCUSSION

Before being used for materials research, carried identification that includes the water content is obtained in one gram of crude leaf Angsana at 9.91%, crude ash leaf Angsana 41.38% is obtained, standardized extract assay 70% ethanol soluble extract was 1.97% and grade is water soluble extract 1:47%, then 70% ethanol used as solvent maceration leaves Angsana bulbs.

Fig1. Chart of blood's sugar rate decreasement

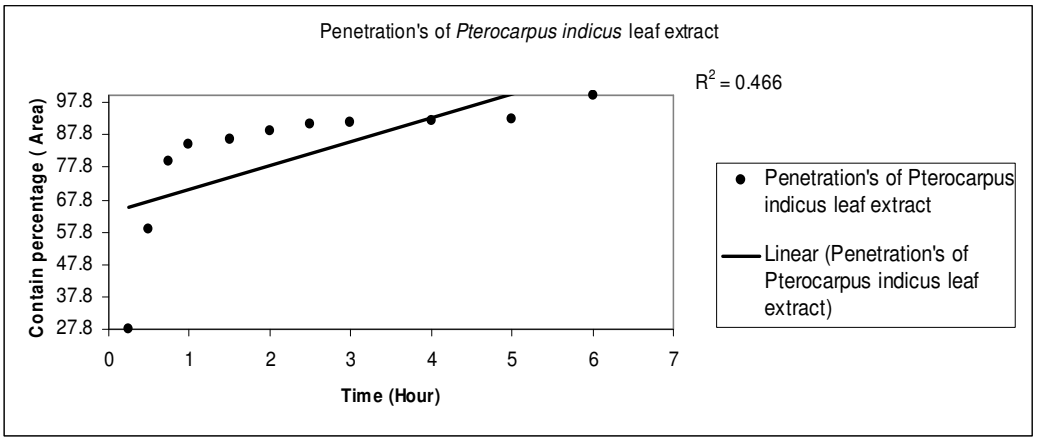


This study aimed to investigate the effect of decreasing blood glucose levels in leaf extracts Angsana. A single dose of 250 mg / kg and 450 mg / kg. These doses were obtained from the journal then continued as the dose to the research mg / kg. This research used as a comparison with the dose of insulin 12.6 IU. Observation of the effect of decreasing blood glucose levels in alloxan method, the results of statistical computations using one way anova gained the ability extract dose of 250 mg / kg and doses of 450 mg extract / kg BW have the same effectiveness or there is no significant difference in the decrease in glucose levels blood, as well as to insulin 12.6 IU no significant difference in the decrease in blood glucose.

On examination of the content of flavonoids by thin layer chromatography (TLC) using mobile phase n-butanol: acetic acid: water = 4:1:5 and stationary phase silica gel GF254. The observation under UV light at 254 nm for comparison and extracts showed Rf value of each is 0.1575 while the Rf value of Pterocarpus indicus extract containing (-)-epicathecin expected around 0.1413. Due to the visible stain on the UV 254 nm. Rf values of extracts were then used in penetration.

At selected patch formula D obtained results are not broken patches, thin, and elastic in accordance with the requirements of a good patch.

Picture 2. Penetration's chart of Pterocarpus indicus leaf extract



Results of penetration on the D patch formula obtained experimental value of r greater than 0.466 at $\alpha = 0.1$ r theoretical table is 0.458. This shows the linear correlation between the amount of leaf extract of Pterocarpus indicus penetrasion against time.

CONCLUSION

1. The dosage of leaf extract *Pterocarpus indicus* Willd are 250 mg / kg and 450 mg / kg can be used as an antidiabetic after 7th day in male rats which given alloxan.
2. The penetration rate of leaf extract *Pterocarpus indicus* Willd has linier correlation against time (hours).

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UTILIZATION OF TRADITIONAL MEDICINE AS STAMINA ENHANCER IN SUNDANESE COMMUNITIES AT CICEMET VILLAGE, BANTEN KIDUL

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Abstracts : Indonesian people has long been known and used traditional medicinal plants as one of efforts to overcome health problems, particularly body stamina. The knowledge was inherited from one generation to generation. A study was conducted at Cicemet Village, Banten Kidul which inhabitants was Sundanese. Data collecting was carried out by using explorative survey through interviewing local communities using the medicinal plants existing at their adjacent areas. There were 21 species of medicinal plants which is used by the communities as stamina enhancer, such as tangkur gunung *(Lophatherum gracile),* ki cantung (*Goniothalamus macrophyllus),* canar bokor *(Smilax leucophylla),* harega *(Bidens pilosa), and* ki urat (*Plantago major).* Ninety percent of the plants was found growing wild at nature, while ten percent have been cultivated. Processing method, parts of the plants used, chemical composition of each species were described in this paper.

Keywords **:** Traditional medicines; Stamina enhancer; Sundanese; Banten Kidul

INTRODUCTION

Stamina, in human health becomes very important due to its role in efforts to improve healthy body condition, because it is relatively more cost-effective and give high impact in improving communities health condition. Whereas definitively, stamina means ability of human body to conduct daily activities without significant tired and still have energy to enjoy the leisure time and for sudden purposes (Anonim, 2009).

Gunung Halimun Salak National Parks, particularly the area lies at altitude of 500-1.500 m asl, has important biodiversity of medicinal plants (Hidayat, 2005). Forests are important parts for communities life at Cicemet Village, Banten Kidul that still relies on natural medication, including plants used as body stamina enhancer, either carried out by themselves or through native healer.

Cicemet Village that belongs to Kasepuhan Ciptagelar area is a custommary village having special characteristics in location and housing form and also tradition that is still adhered strongly by follower communities. The community lived at Kampung Ciptagelar are named as kasepuhan communities.

The study aimed to find out indigenous knowledge of Kasepuhan Ciptagelar communities, particularly communities of Cicemet Village who lives at Gunung Halimun Salak National Parks areas in utilizing plants existing at their surroundings areas, especially plants used as body stamina enhancer.

MATERIALS AND METHODS

Data collecting was done at Cicemet Village, belonging to kasepuhan Ciptagelar, Cibeber Distric, Lebak Regency, Banten Kidul, through direct observation and interviewing custommary leaders, native leader, local communities knowing traditional medication, especially plants as body stamina enhancer. Local name of every plants used was recorded, as well as parts of the plants, processing and its usage. Every plants species was taken its specimen sample to make herbarium, then added alcohol 70%, wrapped by

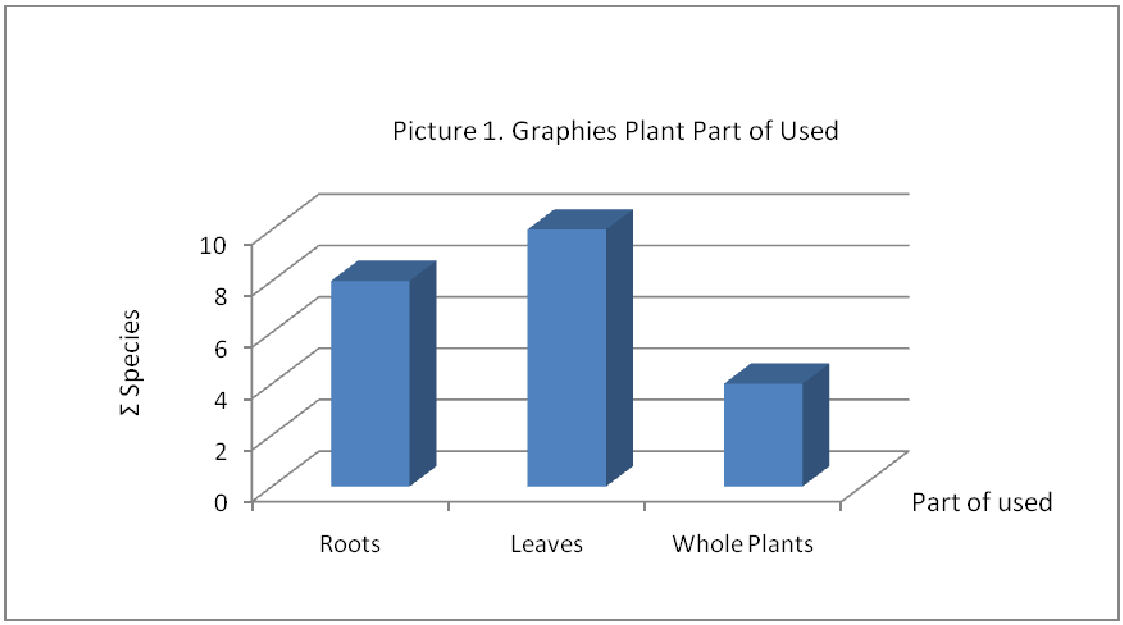
paper and put into plastic bag. The specimen was then processed as common herbarium making and for identification of scientific name purposes.

RESULTS AND DISCUSSION

The study results revealed that there were 21 plant species that belongs to 16 family and 20 genus, used by Sundanese communities at Banten Kidul areas for improving body stamina (Table 1). Parts of the plants that mostly used was leaves, followed by root, and the whole parts of plants (Picture 1).

Table 1. List of plants used as the stamina enhancer in Banten Kidul

No.	Family	Species	Local Name	Part of used
1.	Acanthaceae	*Justicia gendarusa* Burm.f.	Andarusa	Leaves
2.	Amaryllidaceae	*Curculigo latifolia* Dryand.	Parasi	Roots
3.	Annonaceae	*Goniothalamus macrophyllus* Hook.f.& Th.	Ki cantung	Roots
4.	Apiaceae	*Centella asiatica* (L.) Urb.	Antanan	Whole plant
5.	Arecaceae	*Arenga pinnata* (Wurmb.) Merr.	Kawung	Roots
6.	Asteraceae	*Ageratum conyzoides* L.	Jukut bau	Whole plant
7.	Asteraceae	*Bidens pilosa* L.	Harega	Leaves
8.	Asteraceae	*Blumea balsamifera* (L.) DC.	Cape	Leaves
9.	Lauraceae	*Litsea cubeba* (Lour.) Pers	Ki lemo	Roots, leaves
10.	Malvaceae	*Urena lobata* L.	Pungpurutan	Roots
11.	Moraceae	*Ficus deltoidea* Jack	Kayu ara	Leaves
12.	Plantaginaceae	*Plantago major* L.	Ki urat	Leaves
13.	Poaceae	*Imperata cylindrica* L.	Eurih	Roots
14.	Poaceae	*Lophatherum gracile* Brongn.	Tangkur gunung	Roots
15.	Rubiaceae	*Paederia foetida* L.	Kasembukan	Leaves
16.	Rubiaceae	*Psychotria viridiflora* Reinw.ex Blume	Ki kores	Leaves
17.	Smilaxaceae	*Smilax leucophylla* Blume	Canar bokor	Leaves
18.	Solanaceae	*Physalis angulata* L.	Cecenet monyet	Whole plant
19.	Solanaceae	*Physalis minima* L.	Cecenet alit	Whole plant
20	Symplocaceae	*Symplocos odoratissima* (Blume) Choisy ex Zoll.	Ki sariawan	Leaves
21.	Verbenaceae	*Lantana camara* L.	Cente	Roots



Plant species which the whole plants used has habitus as herbs and easy to cultivate, such as *Centella asiatica*, *Ageratum conyzoides*, *Physalis angulata*, and *Physalis minima***.** Sundanese communities at location used the plants as stamina enhancer in form of concoction or mixture of the 21 plants species. The concoction of 21 plant species and parts of the plants used was suitable as Table 1, after washed, all plants were boiled with 1 liter water into 0.5 liter. This decoction was drunk one glass (± 200ºC) everyday in the morning and in evening. The concoction may not consist of 21 plant species, but it may consist of less of the amount, the important thing is the amount should be odd. Of the amount, 11 plant species supported by secondary data wgich stated that the plants used as tonic, that are *Bidens pilosa***,** *Centella asiatica*, *Curculigo latifolia*, *Ficus deltoidea*, *Imperata cylindrica*, *Justicia gendarusa*, *Lantana camara*, *Litsea cubeba*, *Lophaterum gracile*, *Plantago major*, and *Smilax leucophylla*.

Two plant species has been categorized as rare species, namely *Ficus deltoidea* (Yuliani, 2001), and *Symplocos odoratissima* (Mogea dkk., 2001). Therefore, it should be conserved ex-situ and in-situ.

Ageratum conyzoides (jukut bau), in Indonesia, an infusion of the roots is used against fever, and an infusion of the leaves to wash sore eyes, and to treat stomach-ache and wounds. The aerial part contain: 11 chromenes in total, lignan (+)- sesamin and the sesquiterpene caryophyllene epoxide (Rahayu dkk., 1999).

Arenga pinnata (kawung) is used for medicinal purposes: roots are considered stomachic and pectoral, petioles haemostatic, cicatrizant and diuretic, and fresh, unfermented sap is purgative. The main product, however, is the palm sugar obtained from the juice tapped from inflorescence stalks and widely used in all kinds of dishes, sweets, drinks and preserves. It can be fermented to make vinegar or palm wine (Endreswari, 2003).

Bidens pilosa (harega), in Indonesia young shoots and young leaves are eaten raw or cooked as a vegetable. The flowers of *B. pilosa* are used in the Philippines in the production of a kind of wine called 'sinitsit'. In Mexico, the leaves are used as a substitute for tea as a tonic and stimulant (Alonzo & Hildebrand, 1999). The leaves contains alkaloid polina, saponin, bitter compound, essential oil, tanin (Agromedia, 2008).

Blumea balsamifera (cape) is widely used for a number of ailments, mainly as astomachic, antispasmodic, vermifuge and sudorific. It is well known for its medicinally

important essential oil. It yields a high quality camphor oil, known as "ngai camphor". Camphor is used in liniments against rheumatic pains; 3% ethanol solution is used to sooth itching (Alonzo, 1999).

Centella asiatica (antanan), per 100 g edible portion fresh leaves contain: Water 88 g, protein 2 g, fat 0.2 g, carbohydrates 7 g, fibre 1.6 g, Ca 170 mg, P 32 mg, Fe 6 mg, provitamin A 4.5 mg and vitamin C 49 mg (Hargono dkk., 1999). In China, it has been known for many centuries as a medicinal plant with tonic and cooling properties, and (together with *Hydrocotyle* spp.) is known in pharmacology as 'Folia Hydrocotyles'. It is also used in India and Thailand as a tonic and to treat dysentery.

Curculigo latifolia (parasi), a decoction of the powdered rhizomes is used in Chinese traditional medicine as a general tonic and analeptic in the treatment of decline. In the Philippines, Nepal and India, the rhizome is used as diuretic and aphrodisiac, and to cure skin diseases (externally), peptic ulcers, piles, gonorrhoea, leucorrhoea, asthma, jaundice, diarrhoea and headache (Lemmens & Horsten, 1999). Fruits contain: 114 amino acids containing the peptide curculin were isolated.

Ficua deltoidea (kayu ara), distribution of it is Thailand, Peninsular Malaysia, Sumatra, Java, Borneo, the Philippines (Palawan) and Sulawesi; introduced in Indo-China, India and Pakistan. In South Kalimantan, *F. deltoidea* is reported to be an effective remedy against leucorrhoea. As it promotes contraction of the vagina it is also considered an aphrodisiac (Rojo et al., 1999).

Goniothalamus macrophyllus (ki cantung), in Java, mountain dwellers use an infusion of the roots to treat typhoid fevers. In Malaysia, a decoction of the roots is used externally for colds, and likewise a steam bath is used in fevers. Heated leaves may be poulticed on swellings. A decoction is drunk after childbirth (Shaari, 2001).

Imperata cylindrica (eurih), rhizome decoctions of *I. cylindrica* are used as a diuretic in Indonesia, Malaysia, Thailand, Indo-China. In Indo- china and by Chinese throughout the South-East Asia region the rhizomes are considered haemostatic and cooling and are prescribed as a general tonic as well as in cases of acute nephritis, hypertension, epistaxis and haemoptysis (Jonathan & Hariadi, 1999). The rhizomes contain the biphenyl ethers cylindol A and B, the phenolic compound imperanene, the sesquiterpenoid cylindrene, and the lignans graminone A and B. The rhizome has also been found to cantain 19% sugars (saccharose, dextrose, fructose, xylose) and varoius acids (including malic, citric, tartaric, chlorogenic, coumaric and oxalic acid)

Justicia gendarusa (andarusa), in Thailand, the roots are used againts diuresis, diarrhoea and as antivenin; the bark is used as antipyretic, anticough, diuretic and anti-amoebic, in the treatment of wounds and allergy; he leaves are taken internally againts cough, fever and as a cardiotonic, and used externally to treat inflammation, wounds and allergy (Sangat-Roemantyo, 1999).

Lantana camara (cente), in Central America, a decoction is taken as a stomachic and remedy for rheumatism. A decoction of leaves or flowers is considered a remedy for colds or fever and employed for its diuretic and sudorific properties and as an emmenagogue. It is sometimes taken as a tonic and to treat hypertension in Costa Rica (Windadri & Valkenburg, 1999). The leaves contain an essential oil, oleanolic acid, lantadene A, lantadene B, lantanilic acid, icterogenin and camaroside.

Litsea cubeba (ki lemo), traditionally the Dayak Kenyah people of East Kalimantan use the fruits and bark as oral and topical medicine for babies as well as for adults. It is applied in cases of fever, stomach-ache, chestpain and as a tonic. In Java, 2 slightly different essential oils are steam-distilled from the leaves. The oil produced in West Java is called trawas oil, that from Central Java krangean oil. Both oils are used medicinally and in soap perfumes (Azah & Susiarti, 1999). All parts of its contain essential oil. Krangean oil contains mainly 1,8-cineole (50%), citral (10%), citronellal (1%), linalool, α-pinene and β-pinene. Trawas oil contain is 1,8-cineole and citronellal (25%).

Lophaterum gracile (tangkur gunung), known as stamina enhancer has long been used by Sundanese communities at TNGHS areas, by washing its roots, boiling with 4 glasses of water into 2 glasses, and drunk every morning and evening. In Peninsular Malaysia, the tubers of *L. gracile* are eaten as a tonic and to treat chancre. In China, the aerial parts of the plant are applied to sore in the mouth and against urinary problems, the roots and tubers as a cooling medicine to treat urinary problems, and during childbirth to hasten delivery (Uji, 2003). Aerial parts of *L. gracile* contain triterpenoids, arundoin, cylandrin, friedelin, taraxerol and steroids The roots are used also as fragrance of alcoholic beverages or wine (Burkill, 1935).

Paederia foetida (kasembukan), the leaves and stems are used as a diuretic for inflammation of the urethra, or they are mashed and applied for earache, ulcerations of the nose and swollen eyes. In Java, the leaves are applied as a poultice for a swollen belly, distension, herpes or ringworm. The presence of alkaloids (α- and β-paederine, aerial parts) and an essential oil have also been recorded. Monoterpenes such as linalol constitute the major components in the oil (Aguilar, 2001).

Physalis angulata (cecenet monyet), in Malesia, the aerial parts, including the fruits, are used to cure digestive and intestinal problems, and various skin problems such as sores, bols and cut. In Papua New Guinea, constipation is relieved by drinking a decoction of the leaves. The sap of the leaves in water is taken as an abortifacient, although their use to treat sterility is also mentioned. The fruit is eaten as a snack, and the leaves as a salad, although the taste is bitter (Rahayu, 2001).

Physalis minima (cecenet alit), in Java, the root is eaten as a vermifuge and an extract of the root is taken for fevers. In Brunei, chewed roots are applied to the lower abdomen to reduce pain. Pounded leaves are used for headache and itches. In Sabah, a decoction of the roots is drunk to treat hypertension and diabetes. The fruit is edible but poisonous when consumed in large quantities. In Thailand, all parts are used as a diuretic and antipyretic (Rahayu, 2001).

Plantago major (ki urat), the seeds have a great reputation as a remedy for dysentery and diarrhoea. They are considered pectoral, demulcent, quieting, antirheumatic and tonic, and to conduce fertility. Leaves of *P. Major* contain iridoids and phenols: flavonoids, phenolic acids and phenylpropanoic esters of glycosides (verbascoside, plantamajoside) (Pangemanan, 1999).

Psychotria viridiflora (ki kores), in Peninsular Malaysia and Java, the leaves are used for skin complaints such as scabies and the bites of poisonous insects and snakes. In Java, the bark and the juice of the stem are also used for these purposes. In West Kalimantan, the aerial parts are used for itch and skin infections (Ong & Brotonogoro, 2001).

Smilax leucophylla (canar bokor), in South-East Asia, decoctions of *S. Leucophylla* roots and rhizomes are rather commonly used internally to treat syphilis, gonorrhoea, rheumatism and coughs and as a tonic (e.g. after childbirth) and aphrodisiac, and externally to treat skin diseases including psoriasis, wounds, inflammations, swellings, ulcers and boils (Theo, 1999).

Symplocos odoratissima (ki sariawan) contains large amounts of aluminium, up to 50% of the ash, and this is responsible for the action as a mordant. Gallic and ellagic acid are common. Leucoanthocyanins occur in various amounts, and quercetin and caffeic acid have also been demontrared (Astuti, 2003). The leaves contains glucosidasin, plocsin, metil salisilat, aluminium, tanin (Agromedia, 2008).

Urena lobata (pungpurutan), is common on roadsides, in waste places, fallow fields, plantations, secondary growths, teak-forests, from sea-level up to 2000 m altitude, in many locations naturalized as a noxious weed. In india, the root is popular as a diuretic, while the leaves are prescribed for inflammation of the intestines and bladder. An infusion of the roots is given to aid difficult childbirth. A lotion made from the plant is used to treat yaws and headache (Ong, 2001). The seeds contain about 7% of a fatty oil,

which contains the fatty acids malvalic acid (2.4%), sterculic acid (42%), dihydromalvalic acid (0.5%) and dihydrosterculic acid (1.2%). The aerial parts contain the flavonol quercetin.

CONCLUSION

There were 21 plant species used as decoction for body stamina enhancer. Its processing was still traditional. Of 21 plant species, only ±10% which has been cultivated, and the remains (±90%) grow wild in nature.

Two plant species has categorized as rare, that are *Ficus deltoidea* and *Symplocos odoratissima*, therefore ex-situ and in-situ conservation should be done.

Further study was needed to find out proper dosage of the plants in usage as body stamina enhancer.

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EFFECT OF SOME SELECTED HERBAL PLANT EXTRACTS AS POTENTIAL DENTAL PLAQUE BIOFILM INHIBITORS

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Abstract : Dental plaque formation is initiated by mouth cavity microbes built biofilm. Plants have been known as a potential source of antibacterial and biofilm inhibitor compounds. This research aimed to screen the potency of several herbal plant extracts as an anti plaque active agent, based on their by in vitro inhibition activity against the planktonic growth and biofilm formation of *S. mutans* ATCC 21752. Fifteen extracts were obtained by macerating the pulverized-dried samples in petroleum ether for defatting, and the residue was further macerated in 70% ethanol. Antibacterial and inhibition of biofilm formation were measured using micro dilution in polystyrene 96-wells plate at 37°C for 24 h. The percentage of inhibition was calculated by comparing the absorbance of samples to the vehicle (control) measured by plate reader at 595 nm. Samples for biofilm formation inhibition were first stained by 1% crystal violet. The above assays were performed in triplicates, while Listerine® was used as a control. This research revealed that the ethanolic extracts of *Temulawak and Jahe rhizomes* showed potential antibacterial agents and biofilm formation inhibitor towards *S. mutans*. *Jahe* extract showed MIC_{90} at 0.091 % w/v; MBC at 0.91% w/v; IC_{50} at 0.040% w/v; and EC_{50} at 0.013% w/v.

Keywords : biofilm inhibitor, antibacterial, plant extracts

INTRODUCTION

Dental plaque is one major mouth cavity health problem which may lead to caries and dental loss (Marsh, 2006). *S. mutans*, a Gram positive bacteria which is normally found in the mouth cavity, is one major cause of dental plaque formation (Loesche, 1996). This bacteria has capability to convert sugars to extracellular glucans and fructans polymers which can be used for adhere on dental surface (Pratama, 2005). Its fermentation by products cause acidic environment which further demineralize enamels (Islam et al., 2007). Therefore to overcome dental caries it is important to control mouth flora and at the same time control their capabilities to build biofilm.

Developed dental biofilm plaque can consist of many different microbes which protected inside the biofilm layer. Systemic pathogenic microbes such as *Helicobacter pylori* may harbor and hidden from immunological reaction (Nasrolahei *et al*., 2008). Several infection diseases in pneumonia and cardiovascular have been correlated to oral infection (Li *et al*., 2000). Nakano and colaborators (2007) reported the detection of novel serotype *k* of *S. mutans* in infective endocarditis patients.

Mechanical treatments with dental floss and brush may reduce the plaque but significant bacterial counts can be reduced by adding chemicals (Addy et al., 1986; Alexander, 1977). At recent time, available chemicals for dental paste and mouthwash are mostly imported such as chlorhexidine and fluor, not to mention the side effects such as imbalance mouth flora and dental staining (Kidd et al., 1997). On the other hand, herbs are reported to be promising sources of bioactive phytochemicals for antimicrobes, antibiofilm, as well as antioxidants and antiinflamatory, etc. Therefore it is interesting to search for new promising herbal extracts to be developed as oral hygiene product active ingredients. In this reports, fifteen (15) extracts were evaluated for their inhibitory

activity against the planktonic growth and biofilm formation. The herbs selection was based on previous reported data on marked antibacterial activity.

METHODOLOGY

Research materials

Extraction solvent: ethanol, distilled water, petroleum ether, DMSO (technical grades); *Streptococcus mutans* ATCC 21752, Standar McFarland: $BaCl_2$ and H_2SO_4 (Merck), Media for assays: media Nutrient Broth dan Agar (Oxoid), Brain Heart Infusion; crystal violet; Bioautography assay: silica gel F 254 plates, hexane, ethyl acetate (Merck) spray reagent for TLC: $FeCl_3$, anisaldehyde, H_2SO_4 (Merck); Positive control: Listerine®.

Plant materials

Fresh rhizomes of *Curcuma xanthorriza* Roxb. (*temulawak*), *Curcuma aeruginosa* Roxb (*temu hitam*), *Curcuma heyneana* Val. (*temu giring*), *Kaemferia galanga* L. (*kencur*), *Zingiber officinale* Roxb *(jahe and jahe merah)* and fresh leaves of *Elephantopus scraber* L. (*tapak liman*), *Piper betle* L. (*sirih*), *Piper crocatum* (Ruiz & Pav.) (*sirih merah*), *Andrographis paniculata* Burm.f.Hess. (*sambiloto*), *Apium graveolens (seledri)*, *Anredera cordifolia (binahong)*, *Guazuma ulmifolia (jati belanda)*, *Sauropus andorgynus* L. Merr. (katuk), and *Cymbopogon citratus (sereh),* and fruit of *Phaleria macrocarpa* (Scheff.) Boerl (*makutadewa*), were collected on February, 2009 in Yogyakarta, Indonesia.

Equipments

Biorad® plate reader, TLC chamber, UV lamps 254 dan 366 nm, Micropippettes Socorex® (single channel: 0.5 – 10; 5–50; 50-200, 200–1000 µL; multi channel 20 – 200 µL), Autoklave, Drying oven, Microtiter plate flat-bottom and flexible U-bottom PVC 96 wells, inkubator, Laminar Air Flow chamber.

Isolation of the herbal extracts

Fresh materials collected were dried by using oven at 40-60° C. Dried samples were pulverized and macerated with petroleum ether for defatting. The residue were collected and evaporated to dryness and then further macerated using 70% ethanol. The ethanol extracts were collected and evaporated to yield sample extracts.

Bacteria and Culture condition

Streptococcus mutans was cultured in nutrient agar at 37°C for MIC assays. A McFarland 2 standard was used to create inoculum density in physiological saline, and the medium for the assays was nutrient broth. The media used in biofilm assays was in BHI enriched with sucrose 2%, and the inoculum density used was McFarland standard 5.

Determination of the mínimum inhibitory concentration (MIC)

MICs were determined by the microtiter broth method (Amsterdam, 1996) in sterile flat bottom 96-well polystyrene plates. Serial dilution techniques were used to determine the MICs of the essential oils at concentration of 0.6-0.0006% after 24 h growth at 37°C. Negative controls (cells + media), positive control (cells+media+listerine®), vehicle controls (cells + media + metanol), and media controls were included. Blanks were done by performing the same treatment as for samples, without cells added. All tests were performed in triplicate. Optical density readings were taken using plate reader at 595 nm, 24 h post-inoculation. To account for the effect of the essential oils color, a formula for calculating percent inhibition was used. Percent inhibition of replicate tests was used to determine the final MIC values.

% inhibition = $(1 - (\frac{ODs-ODb}{ODv})x100\%$, where ODs = optical density of samples; ODb= optical density of blank; ODv = optical density of vehicle controls – optical density of vehicle controls blank

Ten µL of the suspensions were subcultured on nutrient agar plates to determine if the inhibition was reversible or permanent. MBC was determined as the highest dilution (lowest concentration at which no growth occured on the plates.

Determination of the biofilm formation inhibition

IC_{50}s were determined by the adherence assay in flexible U-bottom 96-well polystyrene plates. Essential oils were diluted in metanol to obtain 0.6% v/v concentration. Serial dilution techniques were used to determine the IC_{50}s of the essential oils at concentration of 0.6-0.0006% after 24 h growth at 37°C. Negative controls (cells + media), positive control (cells+media+listerine®), vehicle controls (cells + media + metanol), and media controls were included. Blankos were done by performing the same treatment as for samples, only without cells added. All tests were performed in triplicate. After 24 h incubation, the contents of the wells were aspired, rinsed 3 times with distilled wáter, and fixed for 10 min. Then, 200 µL of 1% cristal violet stain was added to the wells to stain for 15 min. The excess stain was rinsed off with tap wáter and 200µL metanol was added to the wells, and transfer to plat bottom 96-well plates. Optical density readings were taken using plate reader at 595 nm, 24 h post-inoculation. To account for the effect of the essential oils color, a formula for calculating percent inhibition was used. The mean % inhibition of replicate tests used was the same as for MIC calculation. IC_{50}s were calculated by using probit analyses which is concentration of samples inhibited 50% of biofilm formation.

Determination of the biofilm degradation activity

IC_{50}s were determined by the adherence assay in flexible U-bottom 96-well polystyrene plates. Essential oils were diluted in metanol to obtain 0.6% v/v concentration Serial dilution techniques were used to determine the IC_{50}s of the essential oils at concentration of 0.6-0.0006% after 24 h growth at 37°C. Negative controls (cells + media), positive control (cells+media+listerine®), vehicle controls (cells + media + metanol), and media controls were included. Blanks were done by performing the same treatment as for samples, without cells added. All tests were performed in triplicate. After 24 h incubation, the contents of the wells were aspired. Serial dilution of essential oils in media were added to the wells and incubated for another 24 h in 37° C incubator. Further treatment were as for biofilm formation inhibition determination.

Bioautography assay

Several TLC systems were evaluated to find the best separation of the essential oils components. The best system was used to elute the samples in 1% concentration in toluene, in triplicate. After drying the solvents, one replication of the eluted samples were put on to the nutrient agar containing bacteria in petri dish for 30 min, while others were sprayed with anisaldehyde H_2SO_4 and $FeCl_3$ separately. After incubation in 37°C for 24 h,the diameter of inhibition zones of each spot were measured in mm.

RESULTS

Screening bioactivity of some selected herbal extracts as could be seen in Picture 1. showed an interesting phenomena as can be found in *Kunyit* and *Sambiloto* extracts. These two extracts did not show antibacterial activity but on the other hand show a prominent biofilm inhibition. As the reverse *Sereh* and *Jati belanda* showed moderate antibacterial activity but no biofilm inhibitory effect.

The extracts of *Temulawak*, and Ginger (*Jahe*) showed most prominent inhibition in both planktonic growth and biofilm formation of *S. mutans as* (Figure 1). *Temulawak* has been reported by Kim and colaborators (2008) to possess potential antibacterial and antibiofilm against *S. mutans* due to xanthorrizol. Therefore this research was continued to explore for the activity of ginger extract only.

Results of antibacterial and antibiofilm activity of Ginger extract showed concentration dependant activity (Table 1-3). It means that the higher concentration of extract will increase the activity. MBC was detected at 10 fold of MIC_{90} value, which means that it need that 10 times higher concentration of MIC_{90} to kill the bacteria. The biofilm formation inhibition was calculated as IC_{50} value while biofilm degradation activity was calculated as EC_{50} value by probit analyses by using SPSS seri 17.

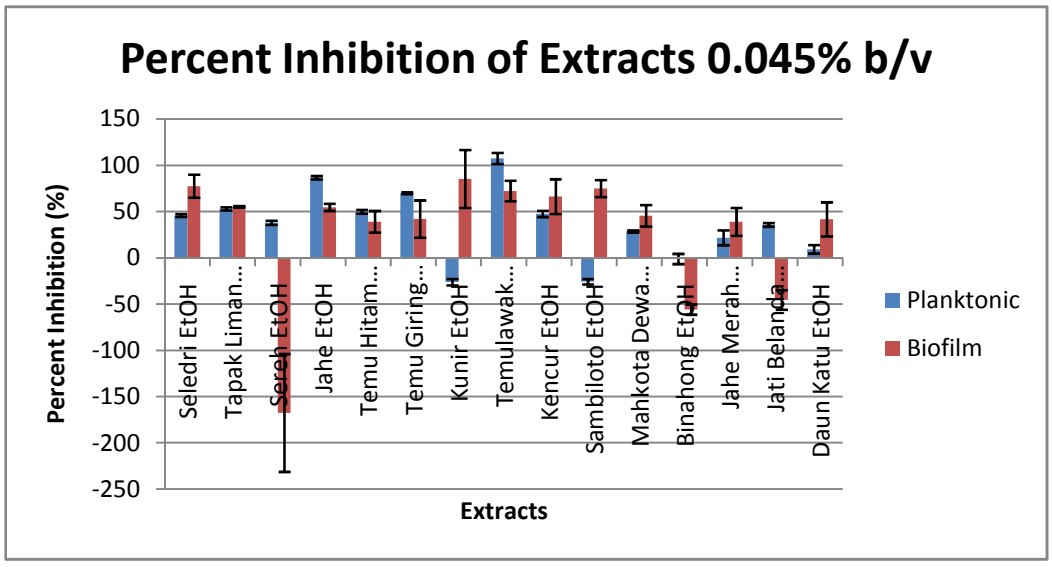

Figure 1. Screening results of herbal extracts inhibitory effects (concentration of 0.045% b/v) towards planktonic growth and biofilm formation of *S. mutans*

The activity results showed that it needs almost the same concentration to exhibit at least 90% of planktonic growth and biofilm formation inhibition as well as biofilm degradation. This phenomenon can be explained that by inhibiting the planktonic growth of the bacteria, bacterial amount will be reduced, and the minimum level of bacterial signal threshold needed to produce biofilm can not be achieved. Therefore, the biofilm formation will be inhibited. On the other hand, less concentration of extract was needed to expose 90% of biofilm degradation. It could be suggested that Ginger extract is more potent as biofilm degradation agent than as antibacterial or biofilm formation inhibitor.

Table 1. Results of Ginger extract antibacterial activity towards *S. mutans*

Concentration (% $^{w}/_{v}$)	Growth Inhibition (%)	MIC_{90} (% $^{w}/_{v}$)	MBC (% $^{w}/_{v}$)
0.023	0	0.091	0.91
0.032	12.28		
0.045	64.02		
0.068	53.73		
0.091	130.99		

Table 2. Results of Ginger extract biofilm formation inhibitory activity towards *S. mutans*

Concentration (% $^{w}/_{v}$)	Biofilm formation Inhibition (%)	IC_{50} (% $^{w}/_{v}$)
0.0045	0	0.040
0.023	23.44	
0.045	47.89	
0.068	83.87	

Table 3. Results of Ginger extract biofilm degradation activity towards *S. mutans*

Concentration (% $^{w}/_{v}$)	Biofilm degradation (%)	EC_{50} (% $^{w}/_{v}$)
0.023	70.27	0.013
0.045	84.07	
0.068	93.57	
0.091	96.87	

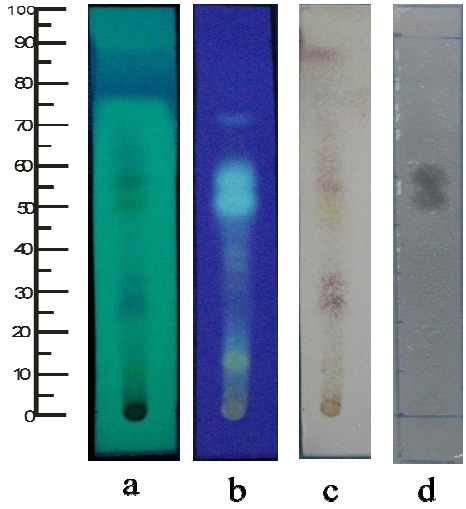

Figure 2. Bioautography profile of Ginger extract
Stationary phase: silica gel F254; mobile phase: hexane-ethyl acetate (7:3 v/v); a: UV 254; b: UV 366; c: Anisaldehyde H_2SO_4; d: bioautography

Table 4. Bioautography profile of Ginger extract
Stationary phase: silica gel F254; mobile phase: hexane-ethyl acetate (7:3 v/v); a: UV 254; b: UV 366; c: Anisaldehyde H_2SO_4; d: bioautography

hRf	**Color changes**			**Inhibitory zone (mm)**	**Predicted substance group**
	a	**b**	**c**		
13	+	Yellowish-brown	Yellowish-brown	-	Long conjugated bond
28	+	-	Purple	-	terpenoid
32	+	-	Purple	-	terpenoid
52	+	Greenish blue	Yellowish brown	5.0	Long conjugated bond
58	+	Greenish blue	Purple	5.2	Long conjugated bond
65	+	-	Redish purple	-	terpenoid

DISCUSSION

Jahe/Ginger rhizome (*Zingiber officinale* Roxb.) chemical constituents reported are essential oils and oleoresin with concentration of 1-4%. The essential oil consists of sesquiterpene hydrocarbon such as zingiberene, ar-curcume, β-sesquiphellandrene and β-bisabolene, while the oleoresin consists of gingerols and its dehydration products shogaols (WHO, 1999).

Ethanolic extract of Ginger was reported to exhibit antibacterial activity towards Gram negative-anaerobic oral bacteria related to human periodontic disease (Park et al., 2008); antibacterial towards *S. aureus* (Azu *et al.*, 2007). The biological activity of red ginger essential oils was reported as antibacterial and antibiofilm active towards *S. mutans* (Hertiani et al., unpublished).

The Ginger ethanolic extract in this research has been freed from essential oils, by defatting method using petroleum ether, and the residue then extracted with ethanol 70%. It is interesting to note that 2 (two) substances which have long conjugated bond exhibited growth inhibition of *S. mutans* in the extract (Fig.2, table 4). These compounds showed similar properties of which suggest related chemical structures. By doing a comparative study of the TLC profile with the literature (Natural Remedies, Research Centre, 1999) showed that these two spot are not gingerols. It showed higher hRf value which means lower polarity. Therefore it is suggested that these two compounds might be the dehydration products of gingerols, i.e. shogaol (Fig. 3)

Based on the above results showing the potency of Ginger extract as antibacterial and antibiofilm against *S. mutans* as well as other properties of gingerol and shogaol as antiinflamatory as well as antioxidative agent, supports Ginger extract application as active constituent in mouth hygene products. The fact that Ginger is also an edible plant with a pleasant taste will add the benefit of its usage.

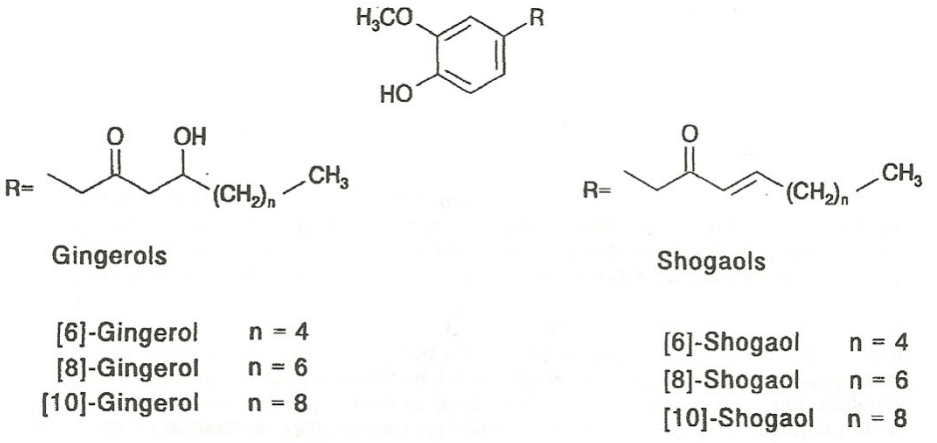

Figure 3 Chemical structure of gingerols and shogaols (Wagner and Bladt, 1984)

CONCLUSION

This research revealed that the extracts of *temulawak,* and *jahe rhizomes* showed most potential antibacterial and biofilm formation inhibitor towards *S.mutans*. *Jahe* rhizome extract showed MIC_{90} value at 0.091% w/v, MBC at 0.91% w/v, IC_{50} at 0.040% w/v and EC_{50} at 0.013% w/v.

ACKNOWLEDGEMENT

Authors thank to Mr. Joko Santosa for herbal species identification and to DAAD for financing the Conference contribution and travel expenses.

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ANTI INFLAMMATORY ACTIVITY OF ETHANOLIC EXTRACT OF LEAVES OF JARAK PAGAR *(JATROPA CURCAS* LINN.) AND NEUTROPHILS PROFILE IN RATS FOOT INDUCED CARRAGEENAN

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Abstract : The research was aimed to identify the anti inflammatory effect of ethanolic extract of leaves of jarak pagar in white rats induced caragenin 1%. Rats were divided into 5 groups. The first group was given caragenin 1 % subplantar (negative control), the second group was given diclofenac sodium (positive control). The third, the fourth and the fifth group were given ethanolic extract of leaves of jarak pagar at doses of 200, 300, and 500 mg/kg BW orally. The parameter test was ~~o~~edema volume that was measured every 0,5 hour~~s~~ during 3 hours with pletismometer. The data were analyzed the percentage of increasing of ~~o~~edema volume, Area under the curve (AUC) and the percentage of the anti inflammatory potency. The statistical data analysis was performed based on the analysis of varian test and t-LSD test with a confidence level of 95 %. Tissue histopathology tests performed on different treatment taken at the third hour, to support the pharmacological data. Tissue histopathology results were observed and then analyzed qualitatively. Results showed that the treatment doses of 200 mg / kg BW, 300 mg / kg BW and 500 mg / kg BW showed anti-inflammatory effects with AUC values 83.89 ± 1.27 % hour; 76.02 ± 2.67 % hour dan 45,21 ± 9,32 % hour respectively, under the AUC value of negative control groups. The anti inflammatory potency of diclofenac sodium, extract at doses of 200, 300 mg/kg BW and 500 mg/kg BW were 53.95 ± 2.47 %; 22.98 ± 1.17 %; 23.14 ± 2.70 % and 54.29 ± 9.43 %, respectively. From the results of its anti-inflammatory percentage of ethanolic extract of leaves of jarak pagar (*Jatropa curcas* L.) started to show its ability to reduce edema at dose of 200 mg / kg BW. Histopathologic observations indicated by the recruitment of neutrophils in inflamed tissue. The number of neutrophils in rats treatmented with ethanolic extract of leaves of *Jatropha curcas*, dose of 500 mg / kg BW was fewer than the treatment dose of 300 mg / kg BW and a control group which were induced by caragenin. Based on these results, it can be said that the leaves of jarak pagar (*Jatropa curcas* L.) has anti-inflammatory effects.

Keywords : *Jatropa curcas*, anti inflammatory, edema volume

INTRODUCTION

Inflammation is described as the principal response of the body invoked to deal with injuries and its hallmarks include swelling, redness, pain and fever (tumor, rubor, dolor and calor) (Soesatyo, 2002). Inflammation is a reaction of the microcirculation that is characterized by the movement of serum proteins and leukocytes (neutrophils, eosinophils and macrophages) from the blood to the extra-vascular tissue. Anti-inflammatory molecules, such as aspirin and its derivatives and other non-steroidal anti-inflammatory drugs (diclofenac sodium, aspirin, ibuprofen, naproxen), at low therapeutic doses, irreversibly inhibit the activity of COX-1 and COX-2 (Dahlan, 1999).

The modern drugs have dangerous side effects such as gastrointestinal complaints, renal disorders, allergies, toxic to the liver and the relatively expensive price Alternative to the use of anti-inflammatory drugs are directed at the plant containing the active compounds in pharmacologic. One of the traditional medicines used by people of Indonesia are the leaves of *Jatropha curcas*. This plant grows throughout Southeast Asia compounds containing saponins, polyphenols, and flavanoid (Hutapea, 2004). This leaves

have medicinal properties as an ulcer, mouthwash, medications edema, anti-swelling, and rheumatic pain (Amijoyo.S., 1998 and Mardisiswoyo, 1995). Castor oil used as medicine edema, liniment, rheumatism and skin diseases(Heyne,1987).

Although this palnt has many useful claims, the mechanism of its medicinal effects are not understood. The objectives of this study were to evaluate the effect of Jatropha curcas as antiinflammatory on carrageen-induced foot paw edema in rats**.**

MATERIAL AND METHODS

MATERIAL

Plant Material

The fresh leaves of J. curcas was collected locally, authenticated by Taxonomist of Department of Taxonomy, Faculty of Biology, Jendral Soedirman University.

Chemicals

Carrageenan, Normal saline solution, Diclofenac sodium, aquadest, formalin, paraffin and hematoxillin-eosine.

Animals

Male wistar rats (2-3 month, 150–200 g) were used to study the antiinflammatory activity. The animals (five per cage) were maintained under standard laboratory conditions (light period of 12 h/day and temperature 27°C ± 2°C), with access to food and water ad libitum

PROCEDURES

Extraction of compound

The sun-dried leaves was crushed into fine powder. Pulverized leaves 500 mg was extracted with ethanol (95%) at room temperature, 3 x 24 hous. The ethanolic extract was filtered and evaporated with a rotary evaporator.

The anti-inflammatory activity

The anti-inflammatory activity of jarak pagar was determined using a carrageenan-induced paw edeme test. Twenty five male rats were randomly divided into 5 groups. The ethanolic extract of leaves of jarak pagar at doses 200, 300, and 500 mg/kg was administered orally to rats for 15 minutes after subcutanes injection of 0,1 ml carrageenan 1% into the plantar surface of the left hind paw. The control group received an equivalent volume of normal slaine solution and the positive control group received diclofenac sodium 40 mg/kg. The paw edema volumes were measured at 0.5, 1, 1.5, 2, 1.5, and 3 h using a plethysmometer. Edema volumes was expressed as the mean increase in paw volume relative the control animals. The difference between the two readings was taken as the volume of edema and the percentage antiinflammatory activity was calculated as the percentage inhibition of rat paw edema between control animals and rats pretreated with the The ethanolic extract of leaves of jarak pagar using the ratio = (Control mean – Treated mean) x 100 / Control mean. Inflammatory tissue, normal tissue and tissue that has been getting treatment on the hour into three isolated and fixed in 10% formalin made preparations crosswise, then performed with hematoksili-eosin staining which was subsequently examined under a microscope.

Statistical analysis

Numerical results are expressed as mean ± SD, unless otherwise stated. One-way analysis of variance (ANOVA) was usedfor statistical comparison; $p < 0.05$ being the criterion for statistical significance. The significant treatment means were further subjected to LSD test.

RESULT AND DISCUSSION

Carrageenan-induced paw edema is a suitable experiment animal model for evaluating an anti edematous effect. Edema developed following injection of carrageenan serves as an index of acute inflammatory changes, was and can be determined from differences in the paw volume measured immediately after carrageenan injection and then every 0,5 hours for 3 hours. Then analyzed into the increasing of edema volume (%) can be seen in Table 1.

Table 1. The increasing of edema volume in various treatments measured at 0.5, 1, 1.5, 2, 2,5, and 3 h after carrageenan 1% injection

Treatments	The increasing of edema volume (%) (n=3) at hour-						
	0	0.5	1	1.5	2	2.5	3
I	5,90	16,58	28,74	35,54	35,72	36,68	41,62
II	8.68	14.52	20.22	18,23	16,47	13,01	10,64
III	8,68	17,46	29,84	38,22	29,58	24,86	23,50
IV	11,43	22,14	30,83	27,55	23,51	21,48	20,82
V	10,92	16,10	23,21	15,47	8,90	11,97	9,31

Information :

I. : Normal saline solution (negative control)
II. : Diclofenac sodium (positive control)
III. : Jarak Pagar (*Jatropa curcas*) 200 mg/kg
IV. : Jarak Pagar (*Jatropa curcas*) 300 mg/kg
V. : Jarak Pagar (*Jatropa curcas*) 500 mg/kg

From Table 1, *Jatropa curcas* at 200 mg / kg, 300 mg / kg and 500 mg / kg was demonstrated to inhibit inflammation in the carrageenan induced rat paw edema model. *Jatropa curcas* at 500 mg/kg significantly decreased edema volume. From the curves at figure 1, it appears that the group treated with doses of 500 mg / kg BW and diclofenac sodium 40 mg / kg BW curve is below the curve of negative control group.

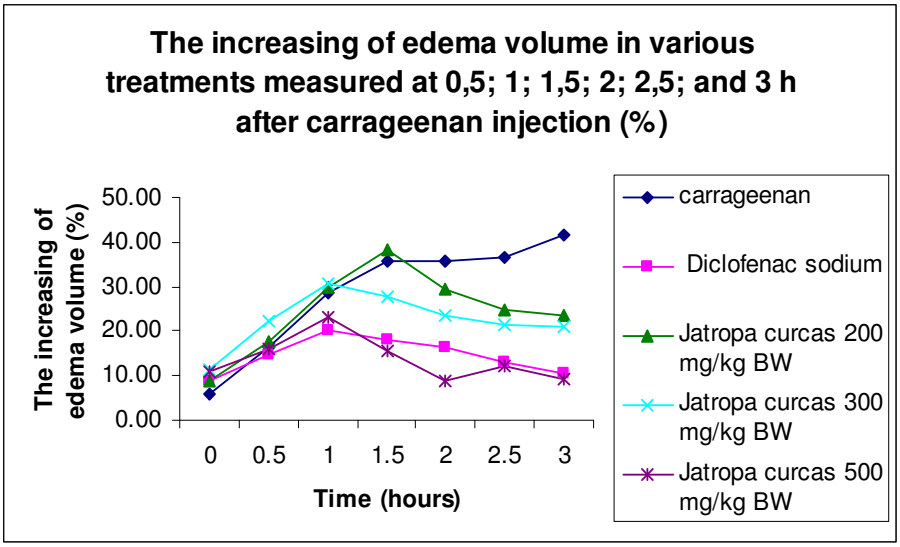


Figure 1. The increasing of edema volume (%) in various treatments measured every 0.5 h for 3 hours after carrageenan injection

Then, this curves was calculated as the Area Under The Curve at 0 h until 3 h. The animals which have a large value of AUC_{0-3} were demonstrated rat paw edema. The area under the curve (AUC_{0-3}) calculation results can be seen in table 2. The treatment doses of 200 mg / kg BW, 300 mg / kg BW and 500 mg / kg BW showed anti-inflammatory effects with AUC values 83,89 ± 1,27 % hour; 76,02 ± 2,67 % hour dan 45,21 ± 9,32 % hour respectively, under the AUC value of negative control groups.

Table 2. ***Area Under The Curve*** **(AUC_{0-3}) from The increasing of edema volume vs time in various treatments measured at 0.5, 1, 1.5, 2, 2.5, and 3 h after carrageenan injection**

Group	Treatment	AUC_{0-3}±SD (n = 3) (% hour)
I	Normal saline sol.	98.91±3.01
II	Diclofenac sodium	48.72±5.19
III	Jatropa curcas 200 mg/kg	83.89±1.27
IV	Jatropa curcas 300 mg/kg	76.02±2.67
V	Jatropa curcas 500 mg/kg	45.21±9.32

To demonstrate the validity of the carrageenan induced paw edema test, rats were administered diclofenac sodium 40 mg/kg. As expected , diclofenac sodium significantly ($p<0,05$) decreased edema compared negative control group. *Jatropa curcas* L. at 500 mg / kg showed the most small of value of AUC_{0-3}, this suggests that these doses can reduce the inflammation was higher than the other treatments.

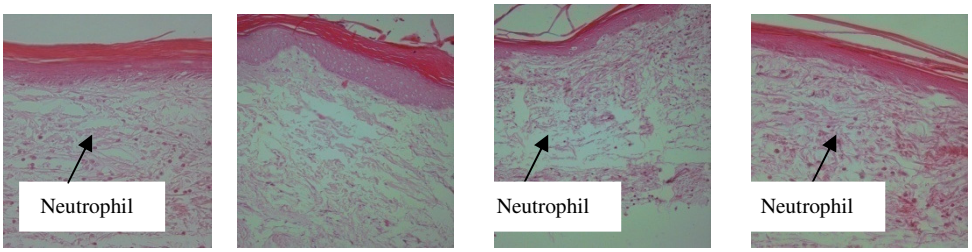
Furthermore, the value of AUC_{0-3} was calculated as the anti-inflammatory potency (%), can be seen at Table 3. From the calculation of anti-anti-inflammatory potency of ethanolic extract of leaves of Jarak pagar (*Jatropa curcas* L.) showed that anti-inflammatory effect at doses of 200, 300 and 500 mg/kg .

Table 3. Antiinflammatory potency (%) of *Jatropa curcas* L. 200, 300, 500 mg/kg and diclofenac sodium 40 mg/kg

Treatment	Antiinflammatory potency ±SD (n = 5)
Diclofenac 40mg/kg	53.95±2.47 %
Jatropa curcas 200 mg/kg	22.98±1.17 %
Jatropa curcas 300 mg/kg	23.14±2.70 %
Jatropa curcas 500 mg/kg	54.29±9.43 %

The anti inflammatory potency of diclofenac sodium, extract at doses of 200, 300 mg/kg BW and 500 mg/kg BW were 53.95 ± 2.47 %, 22.98 ± 1.17 %. 23.14 ± 2.70 % and 54.29 ± 9.43 %, respectively. The antiinflammatory potency of *Jatropa curcas* at 200 and 300 mg/kg but not 500 mg/kg was significantly different from the control ($p < 0.05$). Treatment of ethanol extract of leaves of *Jatropha curcas*, a dose of 500 mg / kg BW showed the greatest inhibition of edema than diclofenac sodium 40 mg / kg and the other treatments.

Histopathologic observations indicated by the recruitment of neutrophils in inflamed tissue The number of neutrophils in rats fed the foot tissue of ethanol extract of leaves of *Jatropha curcas*, a dose of 500 mg/kg were fewer than 300 mg/kg. The neutrophils profile can be seen at figure 2.



(A) (B) (C) (D)

Figure 2. Neutrophils Profile in Rats Foot Induced Carrageenan in various treatments

A. Rat foot induced carrageenan 1%
B. Normal rat foot
C. Rat foot with treatment of *Jatropha curcas* 300 mg / kg
D. Rat foot with treatment of *Jatropha curcas* 500 mg / kg

Based on these results it can be said that the leaves of jarak pagar (*Jatropa curcas* L.) has anti-inflammatory effects and the mechanism of the anti-inflammatory effects of leaves of *Jatropha curcas* involves reduction of neutrophils

CONCLUSION

In this study, the ethanolic extract of leaves of Jarak Pagar *(Jatropa curcas* Linn) was demonstrated to inhibit inflammation in rats. The anti-inflammatory potency of the ethanol extract of leaves of *Jatropa curcas* L. was 54.29 ± 9.43%. The mechanism of antiinflammatory effect of *Jatropha curcas* involves reduction of neutrophils.

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PDMAA COATED CAPILLARIES IN REDUCING PROTEIN ADSORPTION

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Abstract : The performance of polydimethylacrylamide (PDMAA) coated capillaries was examined with regard to the analysis of proteins by capillary electrophoresis. The PDMAA coated capillary (internal diameter: 75 µm) was prepared by the polymerization of dimethylacrylamide (DMAA) inside the capillary. For routine protein analysis, the PDMAA coated capillaries showed a good stability in long-term protein separation and can effectively reduce protein adsorption on the capillary wall. It also provides improved separation efficiency and better reproducibility of the EOF mobility compared to bare fused-silica capillaries in single use and the linear polyacrylamide (LPA) coated capillaries in multiple uses, especially at a pH close to the p*I* of the protein. Even though the PDMAA-coated capillaries can minimize the protein-wall interaction, it cannot prevent it completely.

Keywords : PDMAA coated capillary/CZE/protein adsorption

INTRODUCTION

Capillary Electrophoresis (CE) is considerable as powerful technique for the analysis of proteins, because it is not a single technique which has different mechanisms of separation. Many techniques of CE and their combinations can be used for the analysis of proteins, such as capillary zone electrophoresis (CZE), capillary isoelectric focusing (CIEF), micellar electrokinetic chromatography (MEKC), sodium dodecyl sulphate-gel CE (SDS-gel CE), etc. The choice of a CE technique depends on the aim of analysis.
Many publications discuss about CE techniques to improve the separation of proteins. As a result, it can be used for the analysis of proteins in real samples, such as biological tissue, protein pharmaceuticals, food and agricultural samples. Many topics such as the electrophoretic migration of proteins, sample pre-treatment, choosing the CE techniques and various forms of detection have been reported to be able to support an improvement of proteins separation (Dolník, 2006).

Based on three dimensional conformations, a protein is determined by the sequence of amino acid components and their interactions with each other. As a consequence, proteins differ from one another in shape and size. Heterogeneity of the protein surface is also found, *i.e.* it has possibly hydrophilic, hydrophobic, cationic and anionic patches at the same time. The distributions of these different patches on the protein surface are greatly affected by environmental factors such as pH, ionic strength, temperature and interaction with organic molecules or a solid surface. Because of these factors, electrostatic force, hydrogen bonding, charge-transfer, and/or hydrophobic interactions possibly happen and it can cause protein adsorption.

The interaction between positively charged proteins and negatively charged silanol groups on the capillary surface is a major problem for the analysis of proteins by capillary electrophoresis, especially if the proteins are separated at pH values lower than their isoelectric points (p*I*). Consequently**,** adsorptions at the capillary wall frequently happen. This can cause peak broadening and asymmetric peak shapes, low efficiency, low recovery of analysis, irreversible protein adsorption, a drifting EOF and irreproducible migration times (Wätzig *et al.*, 1998; Corradini and Cannarssa, 1996).

Several strategies have been proposed to prevent the problem of protein adsorption. In CZE, the choice pH buffer is able to influence the charges of analytes. The

addition of high concentrations of alkali salts, zwitterions or other additives to the buffer solution can be used to suppress the electrostatic interaction between the capillary wall and the proteins. The high concentrations of positively charged ions compete positively charges of the protein to interact with the negative silanol groups of the capillary wall. Zwitterions perform ion pairing with the proteins, thus protein-wall and protein-protein interactions can be reduced. The use of buffer additives is also useful for masking the activity of silanol groups. Nevertheless, in the addition of ionic salt, the applied field strength should be controlled to avoid high current that may possibly lead to denaturation and precipitation of proteins (Wätzig *et al.*, 1998; Corradini and Cannarssa, 1996).

In order to deactivate the silanol groups, the use of coated capillaries in CZE is preferable to reduce the wall interactions of protein molecules. The ideal coating can provide separation efficiency, better protein recovery and reproduciblility of EOF and migration time of analytes. Yet, significant adsorption of proteins is still observed in recent days using coated capillaries (Verzola *et al.*, 2000; Graf *et al.*, 2005).

In this experiment, the performance of polydimethylacrylamide (PDMAA)-coated capillary (IMTEK, Albert-Ludwigs Universität Freiburg) was examined with regard to the analysis of proteins. This capillary with i.d. 75 µm was prepared by the polymerization of dimethylacrylamide (DMAA) inside the capillary that is previously coated with a silane having a polymerizable group (MPS, methacryloyloxypropyl trimethoxy silane).

EXPERIMENTAL

Chemicals

ß-lactoglobulin from bovine milk (pI:4.83) and neostigmine bromide were purchased from Sigma-Aldrich, Steinheim, Germany; disodium hydrogen phosphate-2-hydrate and potassium phosphate monobasic from Riedel-de Haen (Sigma-Aldrich, Seelze, Germany); sodium acetate anhydrous, sodium chloride and acetanilide from Fluka (Sigma-Aldrich, Steinheim, Germany); acetic acid (analytical reagent) from Merck (Darmstadt, Germany).

The sample solution was content of 100 $\mu g.ml^{-1}$ of acetanilide as EOF marker, 500 $\mu g.ml^{-1}$ of neostigmine bromide as internal standard and 35 $\mu mol.L^{-1}$ of ß-lactoglobulin as a model protein. ß-lactoglobulin was dissolved in the isoosmotic NaCl solution first before added to the sample solution. Phosphate buffer, 50 $mmol.L^{-1}$, pH 7.0, 6.5, 6.0 and acetate buffer, 50 $mmol.L^{-1}$, pH 5.5 was prepared freshly.

Instrument

Protein analysis using CZE technique was carried out on UniCAM Crystal 310 CE. The detection was performed by UV at wavelength 210 nm. PDMAA coated capillaries with the ID 75 µM (IMTEK, University of Freiburg, Germany) which were cut to an effective length of 48 cm from the total length 60 cm.

Methods

The PDMAA coated capillaries was rinsed by buffer with the pressure 1200 mbar for 30 minutes. The initial of separationmethod was buffer rinsing for 2 minute and then was followed by hydrodynamic injection of the sample by applying a pressure of 30 mbar for 12 s. The protein separation was performed by applying a voltage of 20 kV and an additional pressure of 40 mbar for for PDMAA coated capillaries. The integration was done by an integration program K.I.S.S. (Würzburg, Germany) (Schrim and Wätzig, 1998).

DISCUSSION

The application of the PDMAA-coated capillaries for protein analysis was performed under variation of the buffer system using CZE. The ability of the capillary

coating surface on reducing the interaction with proteins was investigated by evaluating the performance of a PDMAA coating in the protein separations by CE. Therefore, this study was focused on the protein adsorption behavior of the capillary wall. A change of apparent EOF mobility or migration time of the EOF marker can give information about the interaction of proteins and the capillary wall.

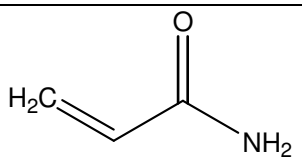
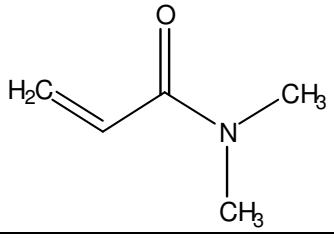
The stability of the PDMAA coating was also investigated by measuring the apparent EOF mobility in a long-term use of protein separation under pH conditions close to the p*I* of the protein. In this case, the interactions between protein analytes and polymer coatings affect the stability of PDMAA-coated capillary.

The comparison of PDMAA to bare fused-silica and to LPA-coated capillaries was also discussed. The structure of the monomers used to coat the bare fused-silica capillary, including acrylamide and *N*,*N*-dimethylacrylamide (DMAA) is shown in Table 1. If proteins were separated using coated capillaries, additional pressure was applied to perform the analysis in a reasonable time frame. Therefore, the apparent EOF is calculated from the measured migration times and is higher than the real occurring EOF. For practical reasons, the apparent EOF was discussed.

In this experiment, ß-lactoglobulin that served as a model protein was separated at different pH values, especially close to its p*I*. Acetanilide that remains uncharged under any applied conditions was chosen as an EOF marker. Neostigmine bromide was used as internal standard, because it provides good stability under any applied conditions.

In order to estimate the precision of protein separation, 30 runs of sample solution containing protein, internal standard and EOF marker were performed. This longer series (n=30) was performed to assure the statistical certainty in order to investigate protein adsorption. RSDs% (relative standard deviation) of migration time, peak area or apparent EOF mobility were used as measurement parameters.

Table 1. Structures of acrylamide and DMAA[#]

Monomer	Chemical structure
Acrylamide	
N,*N*-dimethylacrylamide	

[#]Doherty *et al.*, 2002

ß-lactoglobulin analysis at different pH

In order to evaluate the effect of protein adsorption on the capillary wall, the analysis of ß-lactoglobulin that was done at pH 6.5 using bare fused-silica and PDMAA-coated capillaries was discussed. Figure 1 shows the electropherogram of ß-lactoglobulin analysis using bare fused-silica capillary. An asymmetric peak of ß- lactoglobulin that was observed at this pH indicates interactions between the protein and the capillary wall. As discussed before that the protein adsorption on the capillary wall influences the peak shape of protein. Meanwhile, ß-lactoglobulin analysis by the use of PDMAA-coated capillary at the same pH showed a symmetric peak shape (Figure 2). It proved that the usage of PDMAA-coated capillary can prevent protein adsorption on the capillary wall. Preventing protein adsorption is also demonstrated by PDMAA coating with an excellent

reproducibility of the apparent EOF mobility (measured by migration time of acetanilide) during the protein analysis, with relative standard deviation (RSD) values of 1.209% for 30 consecutive runs (Figure 4). Based on this result, under the same conditions at this pH, the PDMAA coating can provide better performance of protein separation compare to bare fused-silica capillaries.

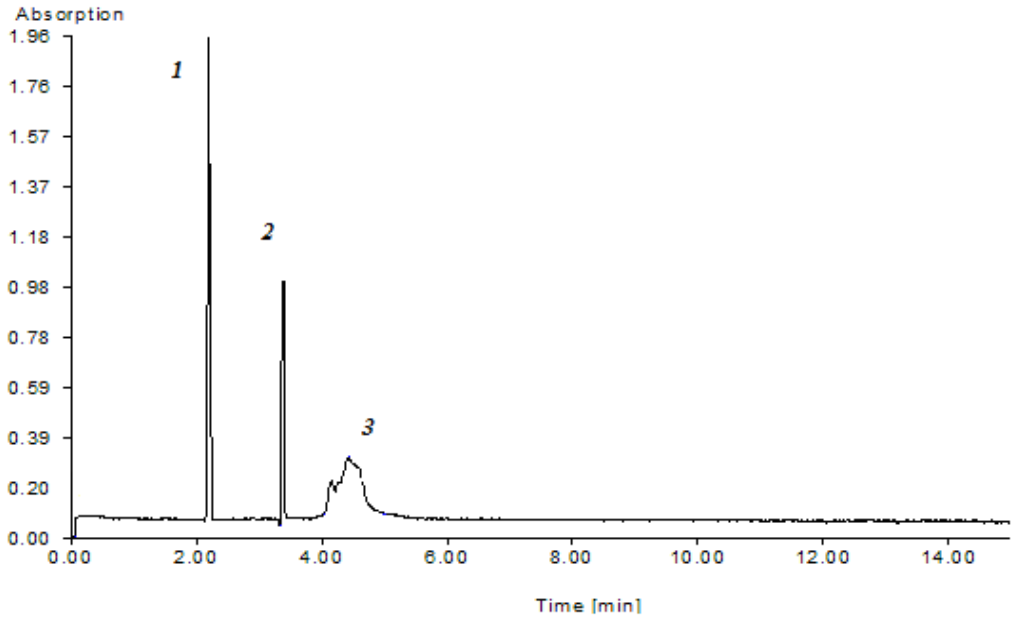


Fig. 1. The electropherogram of protein analysis at pH 6.5 using a bare fused-silica capillary. Peak 1: neostigmine bromide; peak 2: acetanilide; peak 3: ß-lactoglobulin. Phosphate Buffer pH 6.5 (50 mM), V = 25 kV, I ~ 62.4 µA

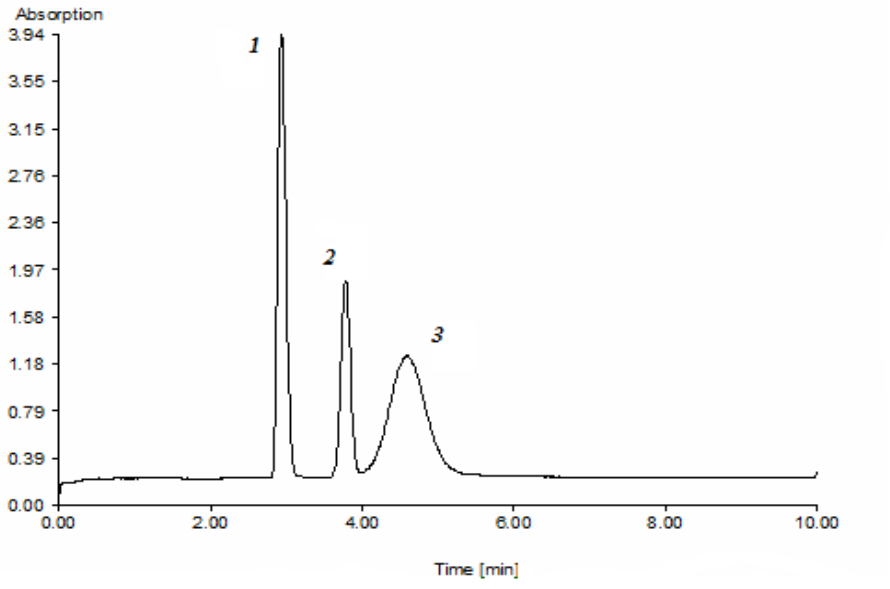


Fig.2. The electropherogram of protein analysis at pH 6.5 using a PDMAA-coated capillary. Peak 1: neostigmine bromide; peak 2: acetanilide; peak 3: ß-lactoglobulin. Phosphate Buffer pH 6.5 (50 mM), V = 13 kV, P = 40 mbar, I ~ 106 µA

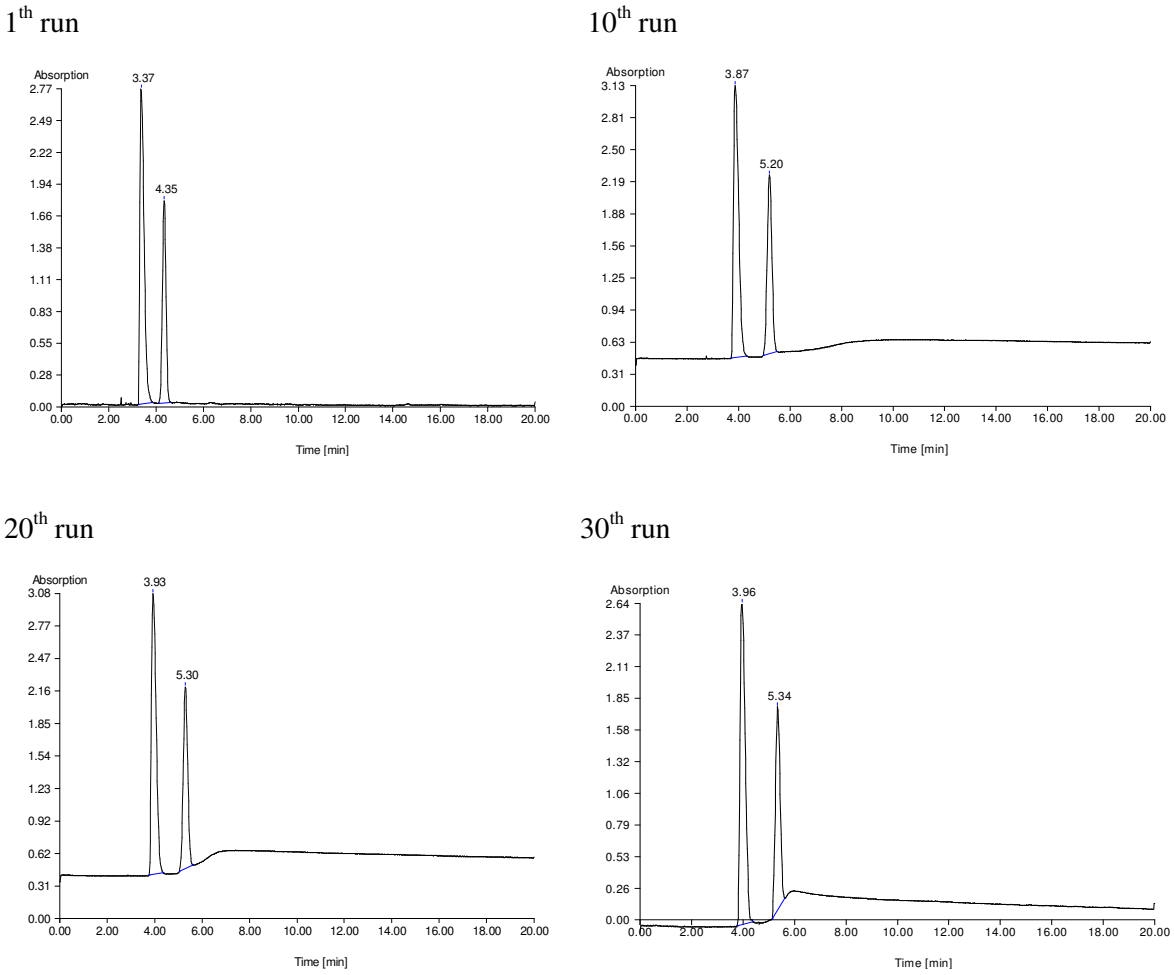


Fig. 3. The electropherogram of protein analysis at pH 5.5 using a PDMAA-coated capillary at the 1th, 10th, 20th, and 30th runs. Peak 1: neostigmine bromide; peak 2: acetanilide; peak 3: ß-lactoglobulin. Acetate Buffer pH 5.5 (50 mM), V = 13 kV, P = 40 mbar, I ~ 19 µA

Figure 3 shows the electropherogram of ß-lactoglobulin analysis at pH 5.5 using PDMAA-coated capillaries at the 1st, 10th, 20th, and 30th runs. The irreproducibility of ß-lactoglobulin peak that indicates the protein adsorption on the capillary wall is observed in this electropherogram. Under this condition, the irreversible adsorption of protein was resulted in runs with no detectable protein peaks at the beginning of series. The adsorbed proteins on the capillary surface may perform aggregation, unfolding or denaturing. This process possibly causes protein aging. After protein aging has been found on the capillary wall, they were more difficult to be removed from capillary surface and completely covered the capillary surface. As a result, ß-lactoglobulin with low recovery was observed after several runs with a un-uniformity in peak shape and migration time.

Based on this result, when PDMAA-coated capillaries are used, strong interactions between positive charges on the protein and negative charges on the wall still take place during electrophoresis at pH 5.5, which this pH is very close to ß-lactoglobulin's p*I*. The existence of protein adsorption on the capillary wall was also demonstrated by the significant changes in the apparent EOF mobility during protein separation. As shown on Figure 4, the decrease of the apparent EOF mobility was observed since the beginning of series and became much slower after 5th runs. This may

indicate that the capillary surface was already completely covered by adsorbed protein. The observed smaller decrease in the following could then be due to ageing processes.

For protein separation at pH 5.5, even the use of PDMAA-coated capillaries was not successful to resolve and detect a protein peak. The irreversibility of protein adsorption could be due to an inhomogeneous coating of the capillary. Some areas of the fused silica that not covered by polymer coating cause protein adsorption. A small thickness of coating layer could be another reason that allows the charges of the silica surface to interact with the proteins on the polymer layer. In general, even though proteins were analyzed using PDMAA-coated capillaries which can efficiently suppress the EOF, the proteins can still be attracted to the surface. This condition influences on the overall separation performance.

Using the same PDMAA-coated capillary, firstly, one series of protein analysis was performed at pH 7.0, and then continued with lower pH values respectively. Figure 4 and Table 2 shows the reproducibility of migration time and peak area of ß-lactoglobulin and acetanilide and apparent EOF mobility at the different pH values. Within a small pH range close to the p*I* of the protein, the adsorption properties change extremely. At a pH of more than 6.0, the PDMAA coating was much more stable and it was reproducible for 30 consecutive runs, whereas a strong adsorption occurred at pH 5.5. This was proved by the strong decrease of the EOF mobility (Figure 4) and the absence of protein peaks on the protein analysis electropherogram (Figure 3) during the protein analysis at pH 5.5. Nevertheless, PDMAA-coated capillaries can be applied to reduce protein adsorption on the capillary wall, and its stability for long-term use proves the high quality of this coating for protein analysis by CZE.

Table 2. RSD (%) of EOF mobility, migration time and peak area using a PDMAA-coated capillary

pH		EOF marker		
		RSD% t_{mig} (min)	**RSD% Peak Area**	**RSD% $\mu_{EOF\ app}$**
7.0	Protein	1.801	1.61	1.79
6.5	Protein	1.21	1.36	1.21
6.0	Protein	3.29	3.85	3.303
5.5	Protein	4.35	4.035	4.86

pH	PROTEIN	
	RSD% t_{mig} (min)	**RSD% Peak Area**
7.0	1.987	4.996
6.5	1.260	2.778
6.0	4.024	4.493
5.5	-	-

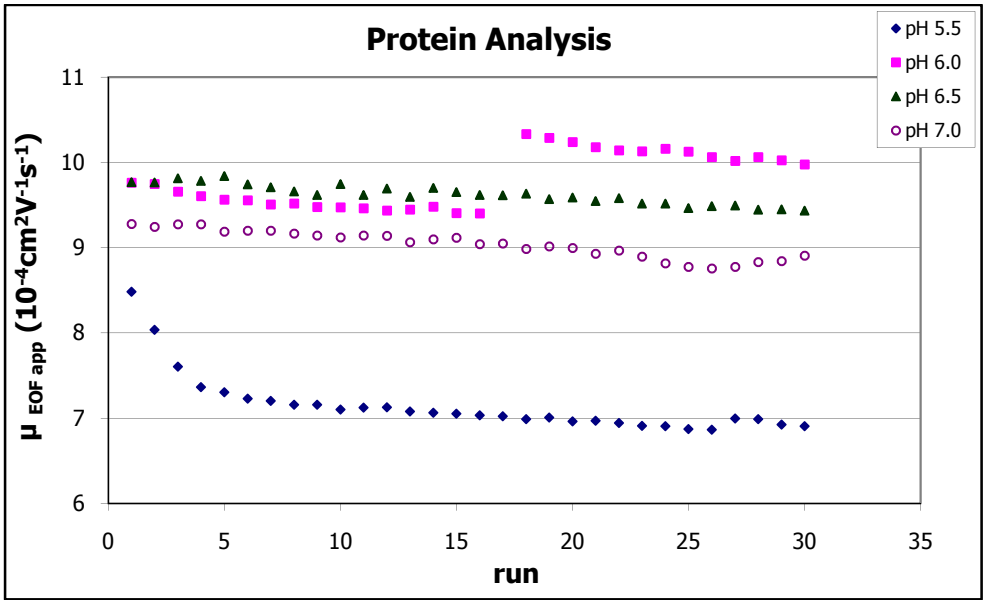


Fig. 4. Apparent EOF mobility of protein analysis at each pH using a PDMAA-coated capillary

Table 3. Migration time reproducibility (n = 30) of the EOF marker separated in bare fused-silica and polymer-coated capillaries at different pH

pH	Bare fused-silica (single use) [8]		PDMAA-coated capillary (multiple use)		LPA-coated capillary (multiple use) [8]		LPA-coated capillary (single use) [8]	
	Increase in t_{mig} of EOF marker (%)	Adsorption	Increase in t_{mig} of EOF marker	Adsorption	Increase in t_{mig} of EOF marker	Adsorption	Increase in t_{mig} of EOF marker	Adsorption
7.0	< 2	0	< 2	0	<2	0		
6.5	< 11	+	< 2	0	< 9	+	< 2	0
6.0	< 16	++	< 4	+	< 19	++	< 10	+
5.5			< 5	+				
5.25								
5.0	< 30	+++						

ß-lactoglobulin analysis on the different types of capillaries

In order to evaluate the effectiveness of PDMAA coating for protein analysis, the comparison between bare fused silica and LPA coating performed by Graf was discussed [8]. The peak areas obtained from experiments using the coated capillaries cannot be compared to those obtained by the bare fused-silica capillary even under the same conditions, due to additional pressure that was applied for the polymer-coated capillaries contributes to the analytes velocity. Therefore, the comparison of the migration times, especially those of acetanilide as EOF marker was discussed.

As discussed above, the PDMAA-coated capillaries showed a good stability in a long-term use and can also effectively reduce protein adsorption on the capillary wall. The performance of these capillaries was also compared with bare fused silica and LPA-coated capillaries (Table 3).

Systematically, the behavior of the protein adsorption on the capillary wall in the different types of capillaries during the protein analysis (such as bare fused-silica, LPA, and PDMAA-coated capillaries) is shown on Table 3. In general, the LPA and PDMAA-coated capillaries reduce protein adsorption on the capillary wall compared to bare fused-

silica capillaries. It is demonstrated by the excellent reproducibility of migration times of acetanilide during protein separation. It is also proved by the results in which the bare fused-silica capillaries do not provide a good reproducibility at pH below 7.0. Meanwhile, the LPA and PDMAA-coated capillaries even give a better reproducibility at pH 6.0. In case of the comparison between the LPA and the PDMAA-coated capillaries, the PDMAA-coated capillaries show better migration time reproducibility of acetanilide than the LPA-coated capillaries in multiple uses, especially at a pH values close to the p*I* of the protein. When using a new capillary for each series, better results were obtained in general. Therefore, using a new coated capillary for each serie in protein analysis is recommended to provide superior reproducibility. However, the PDMAA and LPA-coated capillaries can decrease but cannot a completely prevention of protein adsorption even when using fresh capillaries.

CONCLUSIONS

The protein separation was studied in capillary zone electrophoresis for preventing protein adsorption on the capillary wall. Some strategies were applied to reduce peak broadening, asymmetric peak shapes, low efficiency, low recovery of analysis, irreversible protein adsorption, a drifting EOF and irreproducible migration times that are caused by protein adsorption. Deactivation of the silanol groups by coating capillaries is preferable to minimize the wall interactions of protein molecules. The application of PDMAA as a coating for silica offers improved separation efficiency and better reproducibility of the EOF mobility compared to bare fused-silica capillaries, especially at a pH close to the p*I* of the protein. The stability of the PDMAA coating was also achieved in long-term protein separation. Even though the PDMAA-coated capillaries can minimize the protein-wall interaction, it cannot prevent it completely.

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MEDICINAL PLANT BIODIVERSITY IN DAYAK COMMUNITIES LIVING IN KAHAYAN HULU UTARA, GUNUNG MAS REGENCY, CENTRAL KALIMANTAN

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Abstracts : Central Kalimantan has 15.38 millions areas with various ecosystems and considerable biodiversity particularly medicinal plant species. The species were considered as one of forest commodities groups with fast genetic erosion. This was due to habitat damage caused by legal or illegal exploitation. There were 78 species of medicinal plants found that used as aphrodisiac (7 species), postparturition medicine (18 species), tonicum (9 species), liver (5 species), diarrhoea medicine (4 species), medicine for malaria, cancer and increase of mother milk content others (3 species), low back pain (lumbago), break bone, cosmetic and headaches others (2 species), and others each of 1 species. Four of those species were categorized as endangered species, such as tikang siau (*Eurycoma longifolia*), sesendok (*Fius deltoidea*), plawi (*Alstonia scholaris*), and bajaka bahendak (*Arcangelisia flava*). Further study was still required for finding out appropriate dosage; quality analysis; and its phytochemical properties.

Keywords : Medicinal plants; Biodiversity; Traditional medicines; Dayak tribes; Central Kalimantan

INTRODUCTION

Indonesian tropical forests estimated reaching 143 million ha, is considered as growing sites of 80% of world medicinal plants where 28,000 species of medicinal plants grown and of 1000 species have been utilized as medicine (Pramono. 2002). Indonesia is categorized as megabiodiversity countries. Biodiversity utilization for human prosperity has been conducted traditionally, historically and through modern technology application, hence there are many potential forests which have not been explored to be developed as phytopharmaceutical or modern medicine sources (Ohistein *et al*., 2000).

Indonesia forest area is approximately 75% of Indonesian land and has not been used optimally as medicine source. Potential forests is characterized by vegetation diversity as the most dominant resources of forest component, having various functions and easy to be used.

Central Kalimantan with area of 15,380,000 ha (7.93% of Indonesian land), has various ecosystems and considerable biodiversity, such as fruits, orchids and medicinal plants. On the contrary, most of population and potential medicinal plants were wasted and threat to extinction due to forest clearance, natural forest convention, or being overexploited (Rijai, 2003).

Traditional uses of medicinal plant have been performed, however exploration and further development of the plants were still limited. Therefore, the plants grown in Central Kalimantan forests are regarded as medicinal plants biodiversity sources which its potential and uses have not been described. The aim of study was to reveal indigenous knowledge of local communities on medicinal plants uses before the plants threat to extinction.

METHODS

The study was carried out by exploratory methods (Rugayah *et al*. 2004) of each selected location by collecting data on plant species having potential as medicine found at the location. Observation was also performed using ethnoscience approaches (Friedberg, 1990), this approaches could reveal local communities knowledge on medicinal plants biodiversity, uses, the way of utilization, the way of obtaining the plants, and specific information on its plant management. Specimen sampling was carried out by using tools, such as scissors, cuttings scissors, and cutting stick to take specimen. Every plant specimen collected was labelled by collection number. Information at location, morphological characteristics, habitat, local names, use, and methods of utilization were recorded.

Plant collection specimen was made into herbarium using alcohol 70%, for identification of its scientific name at Herbarium Bogoriense, Pusat Penelitian Biologi-LIPI.

Information on plants uses was obtained from primary and secondary data. Primary data was collected by interviewing selected local communities, i.e. local customary leader, traditional healer (mongongundam/belian), local midwife (monoyatakit), village secretary as translator, and communities who use medicinal plants for their daily life. Secondary data was obtained from related references.

General Description of Research Location

The study was conducted at several villages, namely Tumbang Mesukih, Tumbang Anoi, Renga Hiran, Haruwu, and its surroundings of Bukit Karung protected forest (S.0.34.117; E.113.42.430) with altitude of 200-1000 m asl., administratively lies in Tumbang Napoi Village, District of Miri Minasa (Kahayan Hulu Utara), the community was called Dayak Ot'Danum tribe; Tumbang Menjangan, Tumbang Tembirah (Kuala Kurun) and forest areas surroundings of Sirat Village (S.1.05.194; E.113.51.400), Kahayan Hilir, and the community was called Dayak Ngaju tribe. Both location administratively belonged to Gunung Mas Regencyu Central Kalimantan.

The observation sites took more than 6-7 hours from Palangka Raya – Kuala Kurun using speedboat. Kuala Kurun-Tumbang Napoi was about 1- 2 days travelling when water tides, if Sungai Kahayan lessened the travelling took about 1 week. This caused by gold mining along Kahayan River resulting in water shallowness, hard to be passthrough, and the river has a fast current. The community religion was Christiank and Hindu Kaharingan. They earned money for living by farming (shifting cultivation), backyard farming, and hunting.

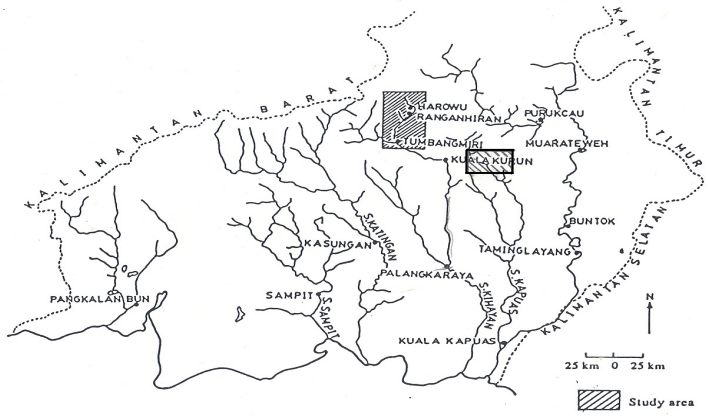
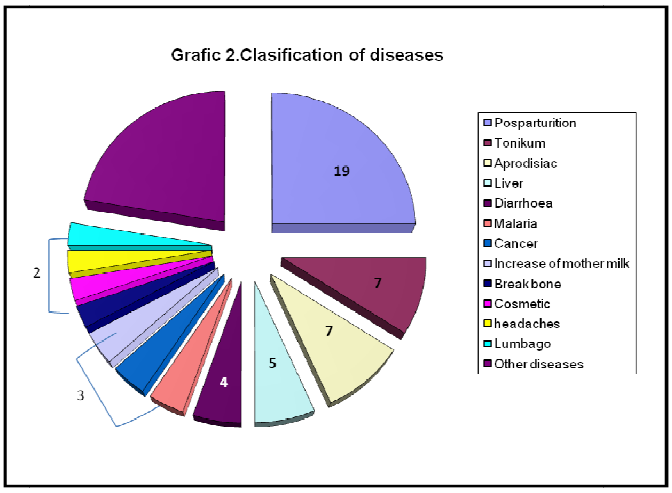


Fig. 1 Study area and central Kalimantan province.

RESULTS AND DISCUSSION

The study has successfully collected more than 77 plant species known by local communities as medicinal plants for curing various diseases, such as aphrodisiac (7 species), postparturition medicine (18 species), tonicum (9 species), liver (5 species), diarrhoea medicine (4 species), medicine for malaria, cancer and increase of mother milk content others (3 species), low back pain (lumbago), break bone, cosmetic and headaches others (2 species), and others each of 1 species. Four of those species were categorized as endangered species, such as tikang siau (*Eurycoma longifolia*), sesendok (*Fius deltoidea*), plawi (*Alstonia scholaris*), and bajaka bahendak (*Arcangelisia flava*). Indication of these species rarity was presumed highly related with imbalanced population caused by habitat damage, forest conversion for oil palm plantation, legal and illegal logging, difficult to be cultivated and these species breeding was still limited.

Observation results of medicinal plants biodiversity showed that about 90.91 % of the plants was gathered at its natural habitat, and the rest was cultivated medicinal plants (9.09 %).



Dayak Ot' Danum and Dayak Ngaju tribes used bajakabahendak (*Arangelisia flava*) as medicine for liver, malaria fever and stamina maintenance. The uses was as follows the stem was cut about one-thumb size (5 pot), dried, poured by hot water, sieved, and drunk. Stem sap of the plants was collected to cure fever, cough, and scorbut. Stem of bajakabendak at Pusian Village, North Sulawesi (Wardah. 2009) was used to cure TBC disease, with dried the stem, rolled like making cigarette, burnt the edge, and inhaled slowly untill the smoke enter through the nose into lung. Stem stalk, and root of this plant contained alkaloid, namely berberin, 8-hydroxyberberine, imacine, olumbamine, jatrorrhizine, palmatine, thalifendin, dehydrocorydalmine, piknarrhin dan homoaromaline (Hernani. 2001). The plants used as medicine was its wood. The wood was collected by cutting the whole tree. Therefore, the plants were threating to extinction, slow growing, and unsecure regeneration. The rarity and threat status were classified as endangered (Diah Sulistiarini.1992).

Of 77 medicinal plant species recorded, the highly use (18 species) was for postparturition medication (Table 1). Midwife (monoyatakit) plays an important role in daily life of Dayak communities due to difficult transportation to reach town and supporting by inadequate economy. Besides as postparturition medicine, the species were recorded as birth spacing or for foetus abortion. Flower shoot of kanahurung (*Fagaraea cf. racemosa*), mixed with fruits of *Areca cathecu* + *Piper betle* + lime with betel leaves were chewed.

Table 1. Medicinal plants pecies diversity tribal society Dayak in the North Upper Kahayan, Gunung Mas District, Center Kalimantan

No.	Local name	Family/nama jenis	Uses	Potency	Keterangan
		Acanthaceae			
1	Ketambat	*Justicia gendarussa*	Rt, Lv	Postparturition	Decoction of root
		Actinidiaceae			
2	Dinding seribu	*Saurauria* sp.	Bark	Tonikum	Prevent or anti body
		Amaranthaceae			
3	Buuyuk	*Amaranthus specious*	Leaves	Postparturition	Leaves are eaten decoction
		Annonaceae			
4	Urui	*Dracontomelon dao*	Bark	Postparturition	Decoction of stem bark
5	Mahawal air	*Mitrephora glabra*	Bark	Anti sengatan	Pounded of bark
		Apiaceae			
6	Antanan	*Hydrocotyle javanica*	Fruit	Postparturition	Fresh eaten
		Apocynaceae			
7	Plawi	*Alstonia scolaris*	Bark	Malaria, Postparturition	Decoction of bark
8	Dangou	*Willughbeia firma*	Bark	Diarrheae	Decoction of bark
		Araceae			
9	Jerangau	*Acorus calamus*	Leaves	Anti rabies	Leaves for pain medication Petiole cold children
		Arecaceae			
10	Ume menyame	*Calamus sp*	Ubt	Postparturition	Umbut for vegetable
	Pinang	*Areca borneensis*	Fruit	Afrodisiac	Fruit or root decoction
12	Pinang	*Areca cathecu*	Fruit	Strengthen and healthy of teeth	Eaten fruit
13	Kelapa	*Cocos nucifera*	Fruit	Liver	Water of unripe fruit drunk
		Aspleniaceae			
14	Paku bunut	*Diplazium asperum*	Allplt	Stomach	Decoction of all plants
		Compositae			
15	Segaallao	*Elephantopus scaber*	Leaves	Interanal diseases	Squeeze of leaves
16	Tamborak	*Ageratum conyzoides*	Leaves	Flour albus	Decoction of leaves
17	Mambung	*Blumea balsamifera*	Leaves	Postparturition, Ginjal	Decoction leaves
		Caricaceae			
18	Papaya	*Carica papaya*	Rt, Lv	Malaria	Decoction of leaves
		Hyperiaceae			
19	Patindis	*Cratoxylum sumatranum*	Leaves	Headaches	
		Commellinaceae			
20	Bak. utan	*Mapania cuspidate*	Root	Tonikum	The root decoction
21	Paku hauwi	*Forrestia mollissima*	Allph	Diarrhea preventive	Plant decoction
		Dilleniaceae			

22	Hantangan	*Dillenia pentogyna*	Cb	Gastrictis medicine	Fiiltrant of cambium
		Ebenaceae			
23	Raja mandak	*Diospyros buxifolia*	Fruit	Lumbago	The fruit of juice
		Euphorbiaceae			
24	Sopang	*Claoxylon longifolium*	Leaves	Tonikum	Decoction of leaves
25	Lampahung	*Baccaurea lanceolata*	Fruit	Tonic, hypertension	The fruit are eaten
26	Tate	*Bridelia glauca*	Leaves	Break bone medication	The pounded of leaves
27	Kayu tumtung	*Codiaeum variegatum*	Leaves	Break bone medication	The pounded of leaves
28	Katuk	*Saurapus androgynus*	Leaves	Increase of mother milk	The leaves for vegetable
29	Uruhandala	*Phyllanthus niruri*	Allp	TBC	Decoction of all plants
		Fabaceae			
30	Tangetar	*Galearia filiformis*	Leaves	Furgative	The leaves of decoction
31	Tapanggan	*Koompassia excelsa*	Cmb	Eye medication	Water from cut stem
32	Sarentak	*Vigna sinensis*	Lv,Pod	Increase of mother milk	Vegetable young leaves
33	Sasating	*Cassia* sp	Seed	Vermifuge	Eaten
		Flagellariaceae			
34		*Flagellaria indica*	Leaves	Lumbago	Decoction leaves
		Hemionitidaceae			
35	Pakuraung	*Taenitis blehnoides*	Allp	Postparturition	Decoction of all plant
		Lamiaceae			
36	Kumis kucing	*Orthosiphon aristatus*	Allp	Diuretic medication	Decoction of all plant
37	Dilem	*Pogostemon cablin*	Ys lv	Shampoo anti dandruff	The young leaves squeeze
38	Kemangi	*Ocimum basilicum*	Leaves	Headaches, painfully tired	Eaten
		Lauraceae			
39	Samakawao	*Heckeria umbellata*	Root	Postparturition	Root of decoction
40	Akar rahwana	*Cinnamomum javanicum*	Root	Woman aprodisiac	The root of decoction
		Leeaceae			
41	Tekam kapas	*Leea indica*	Root	Postparturition	Decoction root
		Liliaceae			
42	Tawang	*Cordyline fruticosa*	Rt/ys 1	Postparturition	Decoction roof or young leaves
		Loganiaceae			
43	Kanahurung	*Fagraea cf. racemosa*	Flower	Postparturition	Fresh young flower eaten
		Malvaceae			
44	Bunga sepatu	*Hibiscus rosa sinensis*	Lv/rt	Postparturition	The leaves f decoction
45	Raja lumpang	*Urena lobata*	Rt	Postparturition	The root of decoction
		Menispermaceae			
46	Bajaka	*Arcangelisia flava*	Stem	Liver, malaria	Decoction of stem

	bahenda			tonic	
47	Penawar gantu	*Tinospora crispa*	Stem	Malaria, snack poison	Decoction of stem
48	Pengarit out	*Tinomiscium phytocrenoides*	Lv	Woman aphrodisiac, KB	The leaves of decoction
		Melastomataceae			
49	Kr munting	*Melastoma affini*	Rt/lv	Postparturition, diarrhoe	The root & leaves of decoction
		Meliaceae			
50	Tlatak manuk	*Dysoxylum excelsum*	leaves	Cancer	Decoction leaves
		Moraceae			
51	Mosae	*Morus alba*	Lv, rt	Liver	Decoction leaves or root
52	Sesendok	*Ficus deltoidea*	Lv	Women aphrodisiac, KB	The leaves of decoction
		Poaceae			
53	Purun	*Eulalia trispicata*	Rt	Tonikum	Decoction of root
54	Tebu kuning	*Saccharum offcinarum*	Stem	Tonikum	Fresh of stem eaten
55	Jelai batu	*Coix lachrymal-jobi*	Rt	Postparturition	Decoction of root
56	Urulewu	*Eleusin indica*	Allp	Postparturition	Decoction of all plant
57	Uru balanda	*Paspalum cojugatum*	Rt	Postparturition	Decoction of root
58	Purun	*Elulalia trispicata*	Rt	Tonicum	Decoction of root
		Polypodaceae			
59	Tarinting	*Microsorium* sp	Allp	Increase mindful capacity	Decoction of all plant
60	Paku raung	*Taenitis blechnoides*	Allp	Postparturition	Decoction of all plant
		Rhamnaceae			
61	Tembalikangin	*Alphitonia cincana*	Rt,bk	Liver, rheumatism	Decoction root and the bark
		Rubiaceae			
62	Taiya	*Nauclea sp*	flower	Cosmetic	Face powder, prevent skin
63	Panahan	*Myrmeconauclea strigosa*	Rt	Tonic, backache	Root decoction
64	Ginseng hutan	*Psychotria leptothyrsa*	Rt	Aprodisiac	Decoction of root
65	Mengkudu	*Morinda citrifolia*	Fr	Liver	Juice fruit
		Rutaceae			
66	Seluang belum	*Luvunga eleutherandra*	Rt/st	Aprodisiac	Decoction of root
		Scrophulariaceae			
67		*Lindernia crustacea*	Lv	Postparturition	Decoction of leaves
68	Tisikpeang	*Curanga fel-terrae*	Allp	Anti cancer	Decoction pf plants
		Sapindaceae			
69	Matananda	*Lepisanthes amoena*	Fr	Cosmetic	Face powder
		Simarubaceae			
70	Tikang siau	*Euycoma longifolia*	Rt	Aprodisiac	Decoction of root
		Verbenaceae			
71	Genari	*Geunsia farinosa*	Lv	Diarrhoe	Decoction leaves
72	Tangundi	*Vitex trifolia*	Lv	Tonsil, rheumatisan	Decoction leaves

		Vitaceae			
73		*Pterisanthes cissoides*	Rt	Cancer	Decoction of root
		Zingiberaceae			
74	tewukak	*Costus speciosus*	Y.st	Diarrhoe	Decoction young stem
75	Suli bahewai	*Nicolaia*	Yst	Baby bruised medicine	Squeeze stem
76	Kunyit merah	*Curcuma domestica*	Rhz	Appendicitis medication	Fresh juice
77	Temu lawak	*Curcuma xanthorrhiza*	Rhz	Anti deodorant	Powder rhizoma

Note: Lv= leaves, bk = bark, rhz= rhizoma, ys= young stem, rt= root, fr= fruit, st= stem, allp= all plant, ys lv= young leaves, sd= seed, cmb= cambium, ubt= umbut, pt= petiole, fr all = fresh all plant

Currently, there are many chemical aphrodisiac sold at market such as 'viagra'. Aphrodisiac medicine formulated by Dayak Ot Danum and Dayak Ngaju tribes was made of 7 species, namely ginseng hutan (*Psychotria leptothyrsa*), tikang siau (*Eurycoma longifolia*), seluang belum (*Luvunga eleutherandra*), akar rahwana (*Cinnamomum javanicum*), sesendok (*Ficus deltoidea*), akar patilak (*Pterisantes* ***cissoides*****),** pengarit out (*Tinomiscium phytocrenoides*), and pinang (*Areca borneensis*) root which need further studied (such as active ingredients, dosages, etc.).

Seluang belum (*Luvunga eleutherandra*) is lianas with hooklike or (in juvenile plants) straigh axillary spines. Leaves alternate, leaflets ovate to lanceolate. Inflorescence a dense axillary panicle or raceme. Flowers bisexual, regular. Fruit an ellipsoid or globose berry. Seeds embedded in a mucilagenous substance. This plants were used as aphrodisiac, medicine for kidney and waist pain. The leaves od the plants was applied for the same purposes. In Thailand the roots are used as a diuretic, and Peninsular Malaysia a root decoction is taken as a post-partum protective medicine (Mutiatikum. 2003). Continuous collecting of the plants without any cultivation activities decreased its population At local market in Central Kalimantan, root of seluang belum was sold of Rp 20,000-25,000/bundle, and in small mug form of Rp 50,000 each.

Tikang siau (*Eurycoma longifolia*), besides as aphrodisiac, was also used as medicine for toothache, postparturition, malaria, tonic, cold, and anthelmintics. The root of tikang siau was sold at local market in shavings form, root cutting, and placed in glass. At Bukit Karung protected forest areas (Haruwu), Dipterocarpaceae forest mixed with *Eurycoma longifolia* were found in form of seedlings. Bigger stand was hardly found. This was caused by increasing demand of the root since its potential as aphrodisiac revealed. Increasing use of the root caused its population decreased, moreover natural habitat of the plant was damage due to logging. Therefore, the rarity and threat status was categorized as eroded species (Rifai, 1992). The plants should give a high priority in research, breeding, development, and uses. Chemical compound contained at the plants was *eurckomalacton, lauricolacton A,B, dehiddroeurikomalakton, euricomanon*, euricomanol, benzoqui-nonsterol, saponin, and asam lemak ester sterol fatty acid.

Ginseng hutan (*Psychotria leptothyrsa*) is shrubs or small trees, climbers, rarely epiphytes or herbs, normally evergreen. Leaves opposite, simple. Inflorescence a terminal cyme, or spiciform. Flower bisexual, corolla tube usuall straight. Fruit a berry or drupe, seed small. Ginseng hutan was believed used for increasing sexual desiree (aphrodisiac). The root was cut with 5 cm size, boiled and then drunk. For better use, roots of ginseng hutan + *Eurycoma longifolia* + *Luvunga eleutherandra* were all boiled, sieved and drunk in the moring and afternoon**.** The leaves and roots of *P. Mindorensis* Elmer and *P.*

extensa Miq (*P. leptothyrsa*). In Peninsular Malaysia, any part of the other used for infected eyes, skin, eruptions and ulcers, crushed as a poultice (Ong. 2001).

Sesendok (*Ficus deltoidea*), an evergreen epiphyte or small shrubis up to 2 m tall; leaves obovate to elliptical or obdeltoid, base broadly cuneate, apex blunt to truncate, rarely pointed, rusty or yellow-olive below; figs axillary, solitary, globose to oblong, 5-10mm in diameter, ripening to orange or red; male flowers dispersed, with 2-3 free tepals and 2 stamens, female flowers sessile to subsessile, with 3-4 tepals. The common name of this plant was known as tabat barito (Indonesia); sempit-sempit, apiapi telinga kera (Sabah); ara tunggal, ara jelateh (Malaysia). In Central Kalimantan (Haruwu and Kuala Kurun), sesendok was believed as women aphrodisiac, boiled water of whole plants as postparturition medicine and uterus tightening. Beside, it is applied for cure white vaginal discharge and menstruation cycles. *F. deltoidea* was highly believed to maintain younger, tonic for men and women, and for diabetes mellitus, pneumoniae, hypertension, and dermatitis medication. In South Kalimantan *F. deltoidea* is reported to be an effective remedy againt leucorrhoea. As it promotes contraction of the vagina it is also considered an aphrodisiac. The latex may have been applied as fish poison. In Thailand *F. deltoidea* is used as an ornamental (Rojo, *et al*. 1999.). In related to the application of 'tabat barito' product formula, based on activty and inhibit zone from bioassay test, extract produced is potential to be developed as base extrat for antiseptic products (Hesty, 2003). Because of uptake directly in their natural habitat, the population in nature is hard to find**.** This plant is classified as one of 41 endangered plant species and it should be conserved (Yuliani, 2001; Mogea, 2001).

Pengarit out' (*Tinomiscium phytocrenoides*) was used by Dayak communities in Kuala Kurun as medicine for vaginal strength, birth controlling, women aphrodisiac, and increase stamina after parturition. Leaves were dried, poured by boiling water, and then drunk. At several locations of Central Kalimantan, such as at forest areas of Kuala Kurun, the plants were hardly found, this might be caused by continuously collecting, and its forest has been legally and illegally exploited**.** In Malaysia, the roots of *Tinomiscium phytocrenoides* are boiled and applied externally for rheumatism, whereas a decoction of the roots may be drunk for the same purpose. In Sumatra, the leaves are used as a dressing for severe cuts. Properties, plant material from Bornean the tetrahydroprotoberberine alkaloid 1–isocorypalmine has been isolated (Brotonegoro. 2001). Root of rahwana *(Cinnamomum javanicum)* besides as aphrodisiac, was used for foetus abortion, and birth spacing.

Plants having potential as postparturition medicine were 17 species, namely *Justicia gendarussa*, *Amaranthus speciosus*, *Alstonia scholaris*, blumea balsamifera, *Hydrocotyle javanica, Calamus* sp, *Taenitis blehnoides, Heckeria umbellata, Leea indica, Hibiscus rosa sinensis, Urena lobata dl* (Tabel1.). These plants were formulated solely and sometimes used two or more mixtures. Knowledge of formulating and blending the medicinal plants was still traditional, differed with local communities in Kasepuhan Cisungsang Taman Nasional Gunung Halimun Salak which blending 40 plant species for postparturition medication (Wardah. 2005). Plawi (*Alstonia scholaris*) which used as medicine for postparturition, applied as malaria medicine and tonic as well. The stem bark is peeled, and the bark is cut and boiled with 3 glasses of water into 1 glass, sieved and drunk twice a day in the morning and afternoon. In the Philippines, a decoction of the bark is used as a febrifuge and tonic, as an emmenagogue, anticholeric and vulnerary. The latex is applied to ulcers and for rheumatic (Teo, 2001).

The plants used as tonic (7 species) were *Saurauria* sp, *Mapania cuspidata*, *Claoxylon longifolium, Koilodepas brevipes, Eulalia trispicata, Saccharum officinarum, dan Myrmeconauclea strigosa* (Tab. 1). Whereas as liver medicines (5 jenis) were coconut (*Cocos nucifera) water*, root, bark of *Alphitonia cincana*, *Morinda citrifolia* fruit and leaves or roots of *Morus alba*. In Vietnam the root bark is used as a diuretic,

antitussive and expectorant and prescribe in oedema, high blood pressure, cough, bronchitis and asthma.

Medication to cure diarrhoea done by Dayak Ot Danum community was using bark of *Willughbeia firma*, whole parts of *Forrestia mollissima*, young stem of *Costus speciosus, and Geunsia farinosa leaves.* *Geunsia farinosa* in Peninsular Malaysia is used to treat vertigo. In Sumatra ground root bark has been applied against swelling (Lemmens. 2003).

Plawi (*Alstonia scholaris*) was used for malaria fever medicine, with boiling its bark, and then drunk. This plant, besides for malaria medicine, has potential as medicine for postparturition, diabetes mellitus, and diarrhoea. Bark of the plant was used for swollen lymph, dysentry, diarrhoea, diabetes mellitus, and malaria (Wardani, 2001). In the Philippines, a decoction of the bark is used as a febrifuge and tonic, as an emmenagogue, antichpleric and vulnerary. The latex is applied to ulcers and for rheumatis (Teo, 2001). Plawi has also potential as medicine for reducing muscle painful, appetite rising, cleaning stomach mucous, stomach inflation, cleaning postparturition blood, and its sap as wound medicine of animals. Chemical compound on its bark was, ethitamine(ditaine), alstonidine, alstonine, akuammicine, akuammidine, tubotaiwine, picrinine, ditamine, echitenine, dan alstonamine**.** Its uses still depend on natural stands, so the population was continuously decreasing, particularly at location where its roots were collected for medicine. Therefore, rarity and threat status was rare (Mogea, *et al*. 2001).

CONCLUSION

Observation conducted at Dayak Ot Danum communities in North Kahayan Hulu and Dayak Ngaju in Kuala Kurun, Gunung Mas Regency, Central Kalimantan showed there were more than 77 plant species known by local communities as medicine for curing several diseases, such as; postparturition medicine (18 species), tonicum (9 species), aphrodisiac (7 species), liver (5 species), diarrhoea medicine (4 species), medicine for malaria, cancer and increase of mother milk content (3 species), low back pain (lumbago), break bone, cosmetic and headaches (2 species), and others each of 1 species. Four of those species were categorized as endangered species, such as tikang siau (*Eurycoma longifolia*), sesendok (*Fius deltoidea*), plawi (*Alstonia scholaris*), and bajaka bahendak (*Arcangelisia flava*).

Further study on active ingredients of various medicinal plants should be done to find out appropriate dosages and evaluate the role of the medicinal plants in medication process.

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SOFTCORAL (*SINULARIA DURA, LOBOPHYTUM STRICTUM, SARCOPHYTON ROSEUM*) FRAGMENTATION IN THOUSAND ISLAND AS POTENTIAL SOURCE OF NATURAL PRODUCT

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ABSTRACT: Research aim was to determine survival rate and growth rate of fragmented-soft coral (*Sinularia dura, Lobophytum strictum,* and *Sarcophyton roseum*). Fragmentation and transplantation of soft coral were carried out onshore and underwater. Well prepared-research will support the success of transplantation. *S. dura* and *L. strictum* showed 100% survival rate. However *S. roseum* indicated low survival rate. This was caused by (a) soft morphological structure of *S. roseum*, hence it was vulnerable to swift away before attaching firmly to the sediment, (b) relatively strong current, (c) unsuccessful acclimatization, (d) predator. Growth rate of *L. strictum* was faster than that of *S. dura*. The result of the research revealed that *S. dura* and *L. strictum* were able to be as raw stock for further research on their bioactive substance content, due to the success of their transplantation.

Keywords: Transplantation, fragmentation, *Sinularia dura, Lobophytum strictum, Sarcophyton roseum*

INTRODUCTION

Background: Coral reef is a unique ecosystem of tropical waters with high level of productivity, high biotic diversity and high aesthetic value, but including one of the most sensitive to environmental changes. Biophysical role of coral reef ecosystems is diverse, such as shelter, feeding and breeding for diverse marine life. Besides acting as the retaining waves, coral reef funtions as producer of the biological resources of high economic value. Reef area is one of important tropical waters producing natural resources has a great potential. Indonesia has the marine living resources with high diversity, but has not been used optimally.

There are a number of marine resources which have the potential to be harvested their bioactive compounds, such as: microalgae, macroalgae, soft corals, echinoderms, molluscs, crustaceans, fish and sponges. Soft corals are part of an important coral reef ecosystems (Benayahu, 1985; Sammarco and Coll, 1998), the second largest component after a hard coral (Manuputty, 1996), playing an important role in the ecology of coral reefs. In the Thousand Islands, the kind of soft corals found are 103 species from four families, and spread over 11 islands from south to north Thousand Islands (Manuputty, 1992). The biochemist gave the attention to soft corals as producer of bioactive compounds. Furthermore, new compounds are expected to be encountered for industry and pharmaceuticals (Weinheimer *et al*., 1977).

Research on marine natural products is a relatively new research field. Cultivation with transplant technique is a measure for providing soft corals stock instead of harvesting from the wild.

Aims: a. Assessing the survival rate of soft coral *S. dura, L. strictum,* and *S. roseum* of artificial fragmentation.
b. Analyzing the growth rate of soft coral *S. dura, L. strictum* and *S. roseum*.

METHODS

Soft coral transplantation research with artificial fragmentation was conducted eight months (June 2007 - October 2007), at Pramuka Island, Kepulauan Seribu (**Figure 1**).

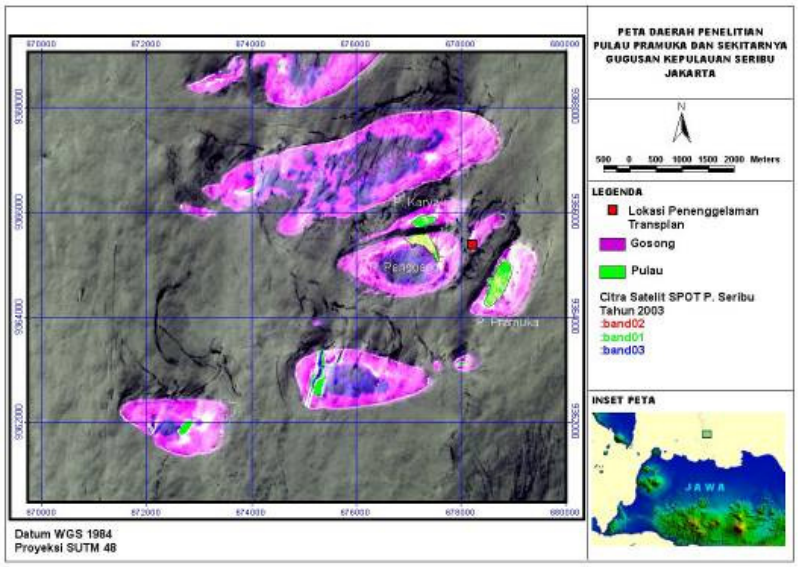


Figure 1. Location of softcoral transplantation at Pramuka Island, Kepulauan Seribu. (red spot: location of transplantation)

Research was conducted on soft corals (Octocorallia: Alcyonacea) *Lobophytum strictum, Sarcophyton roseum* and *Sinularia dura* (Bayer, 1951; Manuputty, 2002; Mather and Bannet, 1993; Verseveldt, 1982) (Figure 2).

Seedlings were taken from the soft coral around the Pramuka island waters at a depth of 3-7 m. High abundance of large soft coral colonies was selected as seed for transplant, placed on a rack of transplant (Figure 3). Stages of the research activities are:

Lobophytum strictum *Sinularia dura* *Sarcophyton roseum*

Figure 2. Fragmented softcorals.

- Determination of softcoral planting locations based on physical factors that support the success of transplantation.
- Search soft corals as the parent seed and the process of acclimatization to avoid stress from the cutting of softcoral (fragmentation).
- Planting seeds of coral transplants on an open system in Kepulauan Seribu.

- Monthly monitoring of survival rate and growth of softcorals and physical appearance of soft coral.

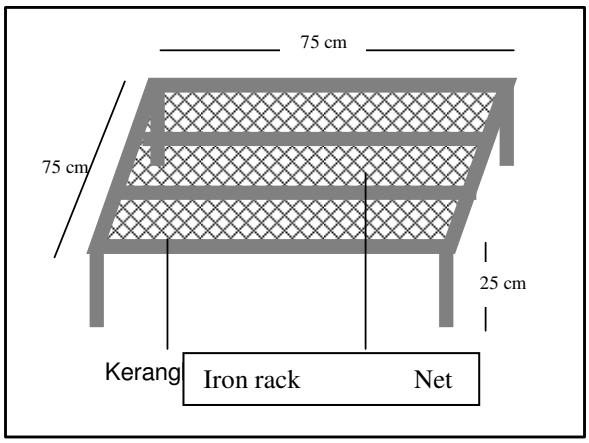


Figure 3. Transplantation rack with net.

Softcoral Survival Rate

Softcoral growth was measured every month by using caliper. Survival rate of transplanted soft coral colonies are measured with the following formula:

$$SR = (Nt / No) \times 100 \%$$

SR = Survival rate (%)
Nt = Number of life soft coral colonies at the end of experiment
No = Number of soft coralcolonies at the onset of experiment

Calculation of survival rates is needed to determine the percentage success rate of transplants by knowing the number of living corals since the study began.

Softcoral Growth

Growth achievement of transplanted softcoral was measured by the following formula:

$$\beta = L_t - L_o$$

β = Growth achievement of transplanted softcoral
Lt = Average size increment after month - t
Lo =Average size of the initial research

To maintain data accuracy, measurements were done on the colonies that have been characterized and mapped previously. Increment of vertical was done by measuring the highest bud. Increment of horizontal was done by measuring the widest bud. Measurement of the transplanted coral growth rate is done by using the formula:

$$\beta = \frac{L_{t+1} - L_1}{t_{i+1} + t_1}$$

β = The rate of accretion of coral fragments transplanted
Lt +1 = Average length or width of the fragment at time i +1
Lt = Average fragment length or width of fragment at time i
ti +1 = Time i +1
ti = Time i

RESULT AND DISCUSSION

Preparation of materials and tools is one of the supporters of success in transplantation activity. Stages of preparation were carried out on land, and some in the bottom waters by SCUBA diving equipment. Preparation experiment rack assembly, preparation and installation of the substrate can be seen in **Figure 5.**

Figure 5. Assembling of substrate and rack.

Softcoral Aclimatitation

Softcoral seed collection was conducted in the waters having a good bottom substrate. Soft coral usually appears in surrounding coral reefs, rocks, and dead coral, and adheres to the substrate (Benayahu and Loya, 1981; Sorokin, 1993). Cutting was performed in the bottom waters to reduce stress (**Figure 6**). Transportation affects the success of transplantation. Transportation on the deck of the ship which is protected for one hour, is not significantly different with water transportation. When soft corals exposed to air for two hours, the success rate between 50-90% and when exposed to air for three hours, the level of success will be 40-70% (Harrior and Fisk, 1988).

Figure 6. Softcoral strain selection

To reduce stress, soft corals to be transplanted were carefully removed and placed in perforated plastic containers and transportation process was carried out in water. This operation should only spend 30 minutes for each pile of rocks to be moved. Soft corals try to maintain the stability of their body metabolism by secreting mucus as consequence of cutting wounds. To reduce stress on the reef, an adjustment effort is necessary (Clark and Edwards, 199; Quinn and Sammarco, 1988).

Survival Rate

Survival rate depends on the accuracy of the method, especially in the treatment of fragments, biological factors such as physiology of transplanted corals and response to environmental conditions (Arvedlund, 2001; Clark and Maldive, 1995). Survival rate was calculated by the percentage of live soft corals from each treatment. Survival rate is calculated starting from the first month until the end of the study.

Soft coral survival rate is relatively high. *S. dura* and *L. strictum* had survival rate of 100% during four months of observation (**Figure 7**). *S. roseum* showed low survival rate (26.7%). This is because (a) very soft physical form, thus *S. roseum* was vulnerable to swift away before attaching firmly to the sediment, (b) the current was quite strong, (c) failure of acclimatization process, and (d) presence of predators (**Figure 8**) (Nybakken, 1992; Tursch and Tursch, 1982). Soft coral *S. dura* and *L. strictum* have strong spicule shape, compared with *S. roseum*.

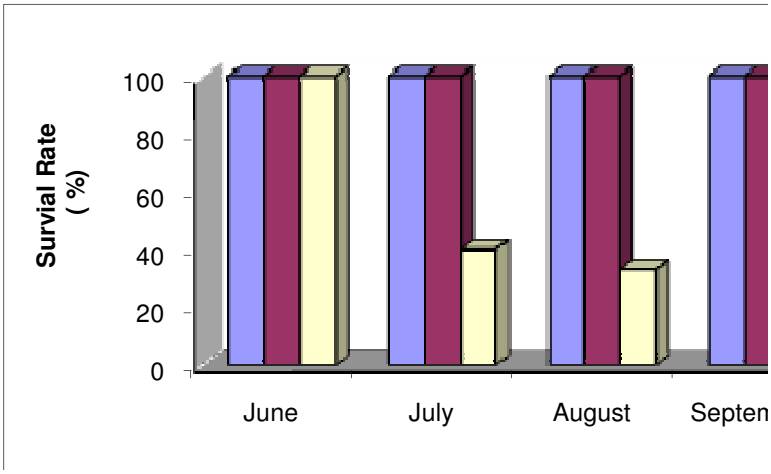
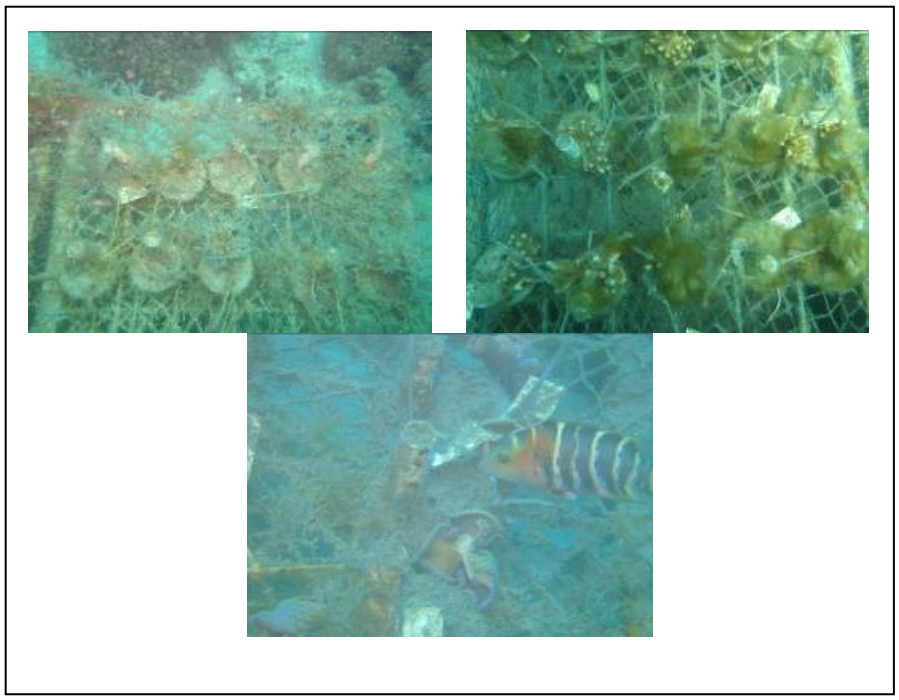


Figure 7. Softcoral survival rate (%).

Generally expressed a successful transplant from a biological standpoint, if the survival rates of various treatments ranged between 50-100%, when transplanted to the similar habitats in which they were collected (Harriot and Fisk, 1988).

Softcoral Growth

In general, growth is defined as the change in the length, width, and weight versus time. Measurements were performed on the growth of *S. dura* and *L. strictum*, since it has a high survival rate. For *S. roseum* only survival was analyzed. It should be further studied the method of good binding of *S. roseum*. Softcorals can grow from each section of fragments.



Growth observations were performed four months (June - October 2007). Growth in the early phase of maintenance was slow, but then growth faster (Effendie, 1997). This is because in the first and second weeks underwent physiological disorders. This physiological disorder was due to wounds caused by cutting. *L. strictum* is soft leather coral, which has skin and a lot of spicules (Fossa and Nilsen, 1998). This is evidenced by the straight and solid fragments after attaching to the substrate.

Sinularia dura

Observations of softcoral growth for four months, starting from the soft coral fragments were bound to the substrate. Planting transplants were performed at the depth of 3 m and 10 m.

Length of soft coral growth rate varied from 0.77 cm/month, 0.34 cm/ month, and 0.32 cm/month, width growths were 0.80 cm/month, 0.32 cm/month, and 0.61 cm / month, for three softcoral (**Figure 9**). Decrease the rate of growth (July-August) can be expected is an implication of the acclimatization and stress from the cutting (fragmentation).

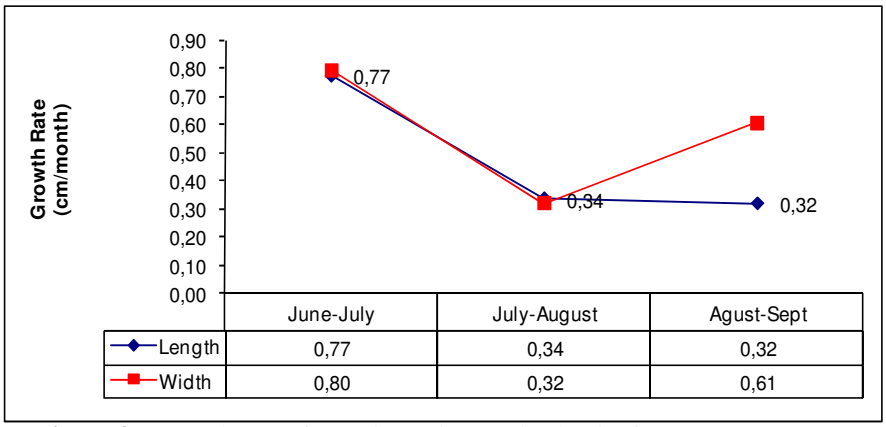


Figure 9. Growth rate of *Sinularia dura* at the depth of 3 m.

Stress is a condition caused by a change in the ecosystem or factors that cause decline in productivity. Stress period fragments of *S. dura* at a depth of 3 m lasted for two weeks, after which this species again showed their colors. Stress causes the color of *S. dura* pale and shrunken. When soft corals began to bloom and color, they have passed phase of acclimatization.

When the cuts were made on soft corals, artificial fragments react by producing lots of mucus. This mucus will isolate the fragment from the water, thus preventing gas exchange, which in turn disrupt the process of photosynthesis (Benayahu and Loya, 1981; Clark and Edwards, 1995).

At the depth of 10 m, growth rate of length and width in the first month was less than those at the depth of 3 m. Length growth rate during the first month was 0.64 cm/month, second month of 0.23 cm/month, and the third month of 0.39 cm/month (**Figure 10**).

S. dura adaptation processes in the depth of 10 m lasted 14-25 days, this proves the existence of a less supportive environment pressure during acclimatization process.

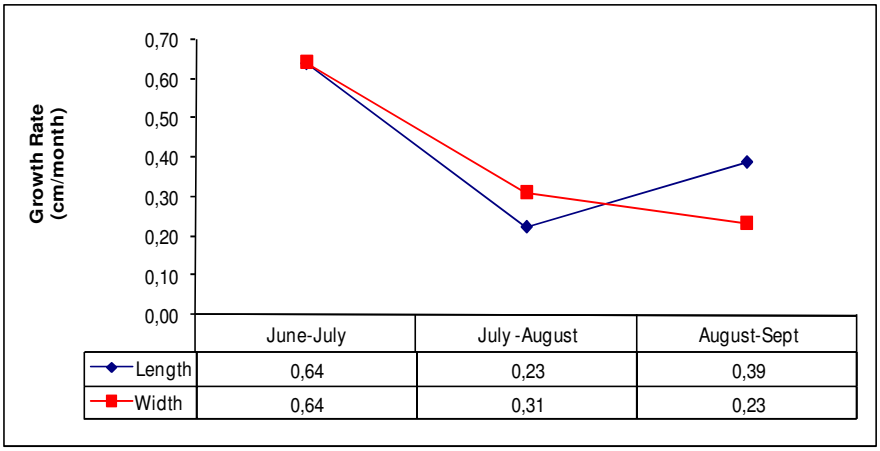


Figure 10. Growth rate of S*inularia dura* at the depth of 10 m.

Environmental pressure caused by fish and other predators biota, and physical factors caused several *S. dura* stress and shrink in the second month of observation. To reduce stress it is needed adjustment effort. If successful, the acclimatization process will bring soft corals back in homeostatic conditions, but if not successful then the biota will stress again with the possibility of even greater stress. Most likely *S. dura* at a depth of 10 m experienced longer stress.

Lobophytum Strictum

The growth rate of *L. strictum* at the beginning of the study was 0.67 cm/month and 0.88 cm/month. Length growth rate has increased over the next month and then decreased to 0.50 cm/month. Width growth has increased again in three months (0.65 cm/month) (**Figure 11**). In general, the growth rate of *L. strictum* was better than *S. dura*. This shows that survival of *L. strictum* was better used as animal transplants.

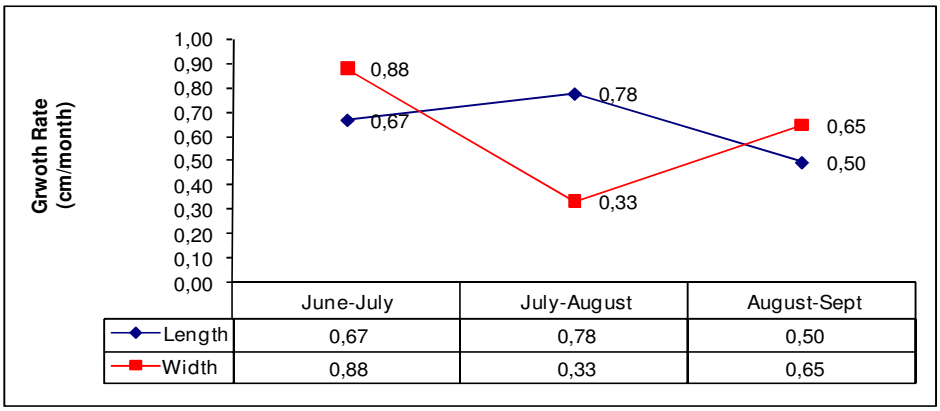


Figure 11. Growth rate of *Lobophytum strictum* at the depth of 3 m.

Differences in growth rate were made possible by the existence of differences characteristics among species. There are polyps that can be withdrawn or outstretched. This is a morphological characteristic that can distinguish between genera (types) with each other. Another difference is anatomically, ie the content of spiculation/sklerit which is a proponent and shaper of the body texture (Manuputty, 1996; Fossa and Nilsen, 1998).

From four months observation the length and width growth of *L. strictum* at the depth of 10 m was not much different (**Figure 12**). At the beginning of the study length growth rate of 1.09 cm/month and width of 0.76 cm/month. Then at the end of the study length growth rate of 0.76 cm/month and width of 0.81 cm/month.

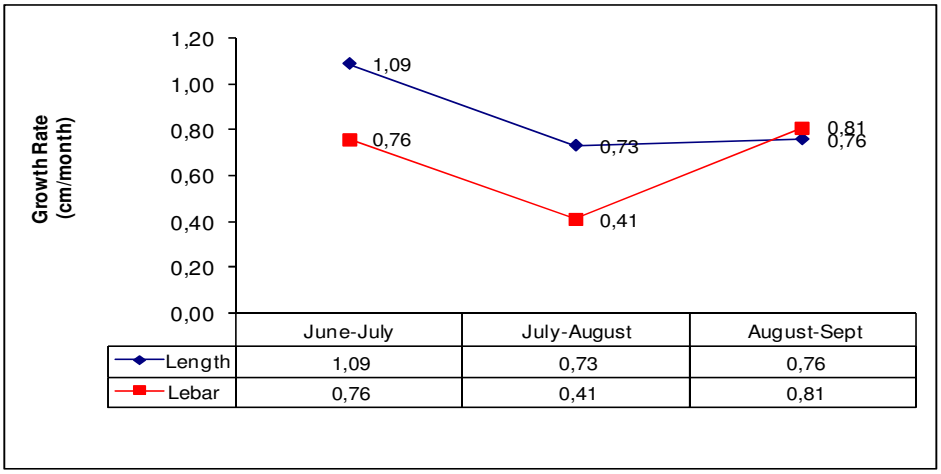


Figure 12. Growth rate of *Lobophytum strictum* at the depth of 10 m.

Based on **Figure 12**, in the second month, the growth rate of coral declined. After going through the phase of acclimatization to the environment, soft corals were back to normal condition. It can be seen from the shape and color of coral fragments were returned to normal. Octocorallia colonies generally have a beautiful color. These colors are produced by a number of zooxanthellae that live inside coral tissue, which produces the brown pigment, yellow, green and so forth (Manuputty, 1996). *S. dura* and *L. strictum* stock can

be used as preparation for bioactive substance research, because of the success of their transplantation process (Manuputty, 1991; Weinheimer *et al*., 1977).

CONCLUSION

Transplantation activities with artificial fragmentation of soft corals were made up of various activities on land and at sea. Proper preparation will support the success of the soft coral transplantation

S. dura and *L. strictum* showed 100% survival rate. However, *S. roseum* showed low survival rate. Growth rate of *S. dura* and *L. strictum* on first and second weeks declined, this is the period of tissue repair on the soft coral fragments. Growth of *L. strictum* was faster than *S. dura*.

S. dura and *L. strictum* stock can be used as preparation for bioactive substance research, because of their success of transplantation process.

ACKNOWLEDGMENTS

This research was funded by the Directorate of Research and Community Service, Directorate General of Higher Education, through competitive grant.

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ETNOPHARMACOLOGY STUDY AND IDENTIFICATION OF CHEMICAL COMPOUNDS OF HERBAL MEDICINES FROM SOUTH SULAWESI AFFECTING THE CENTRAL NERVOUS SYSTEM

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ABSTRACT: Utilization of various herbal medicinal as ingredients in traditional medicines in Indonesia comes from knowledge, traditions and customs of indigenous people (ethnic), known as etnomedicine / etnopharmacology. Known as the South Sulawesi province in Indonesia that almost the entire area is surrounded by ocean, mountains with many endemic flora, this region is inhabited by three ethnic Bugis, Makassar, and Toraja. Especially for ethnic Bugis-Makassar, the use of traditional medicines from natural substances have been recorded since the early 15th century known for sure lontarak pabburak. Some herbal medicinal that affect the central nervous system by the people of South Sulawesi is used *Piper betle leaf* , *Zingiber officinalis Rhizome*, and *Ficus septica* leaf as an analgesic, antipyretic and antiinflammatory, Mimosa pudica , and *Citrus limonis* for sedative hypnotics treatment. To explore the cultural heritage, especially in developing traditional medicine and scientifically prove the truth of his usefulness, a research of medicinal plants etnopharmacology which affects the central nervous system in the region of South Sulawesi. This research was conducted in three ethnic groups in South Sulawesi, Makassar, Bugis, and Toraja. Collecting data and samples on three ethnic conducted using questionnaires. Made herbarium and crude drug samples, then identified the chemical components using reagent. Results were obtained with the data a few plants that affect the CNS include Permot Herb (*Passiflora foetida* L.) containing a chemical component that is steroids, tannins, and flavanoid. Water spinach leaf (*Ipomea aquatica*) containing a chemical component alcaloids, and latex. Bitter Herbs (*Andrographidis paniculata*) contains chemical components of alcaloids and tannins. Fruit Chilli (*Capsicum annum*) contain chemical components of Essential oils, and alcaloids. Princess Shame Herb (*Mimosa pudica*) contains chemical components of Mimosin, essential oil. Papaya (*Carica papaya)* contains chemical components of alcaloids, the enzyme papain, glycosides, saponins. Bitter melon leaves (Momordica charantia), containing the chemical components of saponins, alcaloids, amylum, resin. Red onion (Alium cepa): Essential oils, saponin, starch, glycosides.

Keyword : Etnopharmacology, South Sulawesi, CNS.

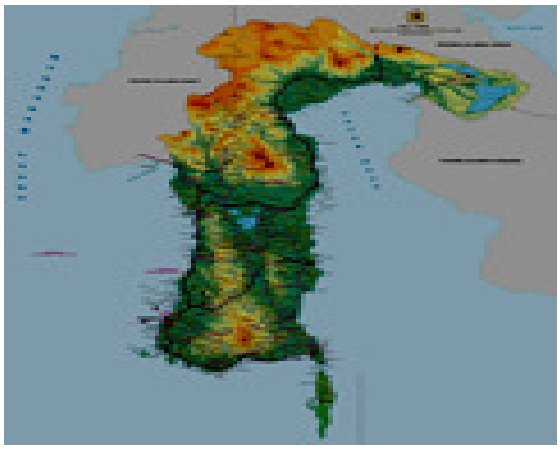
INTRODUCTION

Utilization of various medicinal herbs as ingredients in traditional medicines in Indonesia comes from knowledge, traditions and customs of indigenous people (ethnic) who applied were hereditary. Knowledge known as etnomedicine / etnopharmacology has contributed big in the world economy, especially health and medical industry in terms of helping to identify plants that provide nutritious medicine and pharmacology activity become guidelines in modern drug discovery (synthetic). Geographically, South Sulawesi has a very strategic position because it is located in the middle of the Indonesian archipelago and is an area that is famous for a variety of plants or natural substances that have not been processed. It is known that almost the entire area of South Sulawesi province in Indonesia is surrounded by ocean, mountains with many endemic flora of potential. Verbeek mountainous region stretching across the northern, mountainous region which stretches Latimojong Quarles and in the middle, and a few mountains that

are still strong with the still green jungle also holds several unique species of flora (Hidayat, 2005).

South Sulawesi before the proclamation of the Republic of Indonesia, consists of a number of areas that stand on their own kingdom, and inhabited by four major ethnic groups namely; Bugis, Makassar, Mandar, and Toraja. There are three great kingdoms of Luwu widely influential, Gowa and Bone, which in the XVI and XVII century reached its glory. The first kingdom to follow La Galigo Bugis is Wewang Nriwuk, Luwuk and Tompoktikka. Luwuk get special status because he is regarded as the head of the Bugis. In the late 15th century, several new kingdoms fought against kingdom Luwuk. Among them is Gowa (Makassar), Bone and Wajo'. After Dewaraja's death, a king Luwu, it was causing a scramble for the ruling dynasty Tana Ugi. Allied with the Bone, Gowa and Luwu captivating at the same time have great influence over South Sulawesi. According to Mattulada, Bugis ethnic tribes occupied most of the region of South Sulawesi. They inhabit fourteen of the twenty three districts of Bone County, Soppeng, Wajo, Luwu, Sidenreng Rappang, Bulukumba, Sinjai, Pinrang, Polewali Mamasa, Enrekang, Pare-Pare, Pangkajene and Maros. To the last two districts are transitional areas whose inhabitants use the language both Bugis and Makassar. Enrekang is a transition area where the population Bugis and Toraja, often referred to Duri and Massenrengpulu have a special dialect, the dialect of Duri and Enrekang (Tang, 2005), while the ethnic group inhabiting Makassar Makassar, Gowa, Takalar, mild, Bantaeng, Selayar, Maros and Pakajene. In general, the lives of Makassar and Bugis people mingle, with a population located on the coast and the Gulf of Bone, and around Mount Lompobatang. In socio-cultural ethnic Bugis-Makassar hold siri' principle and pesse in his life. Siri' may mean respect, honor or dignity. Communities will be protected from making all that should in facing the challenges of dignity as a highly respectable member of society. Regarding siri they sacrifice everything, including their lives, for the sake of siri' (Abdullah, 1985). Siri 'as good to walk arm in arm pesse that (which) means the painful feelings, to empathize with the distress implied by others (Pelras, 1996). Implementation of siri' in the (in) everyday social life are stated at the five principles (Said, 2004):

1. There Tongeng (truth in speech)
2. Lempuk (honesty)
3. Getteng (fortitude)
4. Sipakatau (mutual respect)
5. Ri Mappesona gods seuwae (submission to the will of God)



South Sulawesi is inhabited by four ethnic Bugis who inhabit the central and eastern areas, inhabit the southern Makassar, Toraja and Mandar inhabit the northern region inhabited western regions, but with Law Number 26 Year 2004 happens to be the provincial division of the western area of West Sulawesi and the Mandar ethnic group separate from South Sulawesi, so in this study only focused on the three ethnic groups namely Bugis, Makassar, and Toraja.

South Sulawesi includes 45519.24 km^2 total area reaches bordered by region:
- north by the Province of West Sulawesi and Central Sulawesi.
- the east: the Gulf of Bone and Southeast Sulawesi province.

- In the south: the Flores Sea
- the West: Makassar Strait.

Especially for ethnic Bugis-Makassar use of traditional medicines from natural substances have been recorded since the early 15th century known for "sure lontarak pabburak" which contains species of plants, the properties and how to use it, but the publicity and popularity of this reference as if buried along with the progress of the age with the death of traditional leaders , and the shaman / Sanro, South Sulawesi as a result of society itself seemed to lose the guidelines in the use of medicinal plants are nearby. Therefore necessary to study etnopharmacology in South Sulawesi to explore the region's cultural heritage, especially in developing traditional medicine and scientifically prove the truth of his usefulness.

Nervous system, together with the endocrine system, conduct most of the regulatory function for the body, in general, the nervous system that regulate body activities such as kontraks fast muscle, visceral events are changing quickly, and even the speed of secretion of several endocrine glands. The nervous system is unique in terms of the complexity of setting action that can be done, he received thousands of small information from different sensory organs to determine the body's reaction should be done (Arthur 2002). The nervous system consists of two coordinate systems are the central nervous system and peripheral nervous system. Central nervous system including brain (ensefalon) and spinal cord (spinal cord). Both are very soft organ, with a very important function it needs protection. Besides the skull and spinal cord sections, the brain is also protected by three layers of the meninges membrane. When the membrane was exposed to infection will occur inflammation called meningitis. Awareness of pain is formed from two processes, namely acceptance of pain in my big brain stimulation and emotional reaktion of the individual. Analgesic affect the first process by enhancing the awareness of pain threshold, while the narcotics suppress psychological reactions caused by painful stimulation. Diseases other CNS function can be suppressed entirely by non-specific by relief substances such hypnotic and sedatives. As a result of awareness for the exogenous impulses derived and reduced physical activity and mental health. These drugs do not affect the specific behavior (Ganiswarna, 1995).

Drugs acting on central nervous system, among others:

1. Psychotropic, which includes:
   a. Psycoleptic, which is a type of drug is generally suppressif or inhibit certain functions of the CNS, namely hypnotic, sedative, and tranquilizer, and antipsycotropic.
   b. Psychoanaleptic, type of drug that stimulates the central nervous system, namely antidepresive and psycostimulans
2. Types of drugs for neurological disorders, such as antiepyleptic, multiple sclerosis, and Parkinson's disease
3. Pain block like analgesics, general and local anesthetics
4. Vertigo drugs and type of migraine drug (Hoan *et al.*, 2003).

For the prevention and treatment of diseases associated with nervous system such as analgesic, antipyretic, sedative hypnotics, antiinflammatory, and adrenolitika adrenergic, people of South Sulawesi has utilized plants as medicines, such as Leko (*Piper betle*), layya (*Zingiber officinalis*), and Tobo tobo Leaf (*Ficus septica*), Tangan-tangan kanjoli /papaya leaf (*Carica papaya*), guava leaves *(Psidium guajava)*, Tebbarita (*Alstonia scholaris*), as an analgesic, antipyretic and antiinflammatory, Bunga jabe (*Mimosa pudica*), and Lemo kapasa (*Citrus limonis*) for sedative hypnotics, but data about medicinal plants that affect the nervous system in three ethnic groups is still very limited, especially species of plants , how to use and usage rules, and the truth of therapy, the research conducted for the inventory of medicinal plants which affects the nervous system based on three ethnic etnopharmacology in South Sulawesi.

AIM TO RESEARCH

a. To get inventory the medicinal plants that affecting the central nervous system from three ethnic groups in South Sulawesi.
b. To Identify the chemical compounds that can provide therapeutic activity in the central nervous system of etnopharmacology plants in the three ethnic groups in South Sulawesi

METHODS

Research design : Observational reasearch with explorer etnopharmacology of medicinal plant in South Sulawesi.

Research Location. This research was conducted in three ethnic groups in South Sulawesi: Makassar, Bugis, and Toraja. Makale Representing Toraja Ethnic, Soppeng Representing Bugis Ethnic, and Jeneponto Representing Makassar Ethnic.

Procedure Research

a. Observation

This research was conducted through a series of procedures including; preparation of guidelines for the interview (interview guidelines), the informants, conducting field research (observation and interviews). Collecting data on three ethnic conducted using questionnaires and interview. Determination of Informants with qualitative studies that rely on interviews as a strategy to obtain the data, thus the target of research is the number of informants who were intentionally set (purposive) to know the criteria etnopharmacology (especially directed at traditional healers / sanro).

b. Identification of Chemical Component

Material

FeCl3, KOH 10% P w / v in ethanol (90%) P, sulfuric acid, phosphomolybdate P, methanol, reagent Mayer, reagent, Bauchardat Lieberman, Reagent Mollisch, Reagent Luff, Reagent Fehling Fehling A and B, iodine, zinc powder, HCl,

Methods

Plants efficacious drugs acting on central nervous system levels of the chemical determined by the chemical identification methods using chemical reagents. First sample was dried, and extracted with polar solvents (alcohol, and n-Butanol), and non-polar solvents (diethyl ether), each test extract at levels of the chemical by a color reaction or precipitation reaction according to Pharmacopoeia of Indonesia and Indonesia Medika material, include : Identification of tannin, glycoside, alcaloid, starch and aleurone, saponins, carbohydrate, phenol, and flavanoid,

RESULTS AND DISCUSSION

Result

Table 1. Data etnomedicine medicinal plants affecting to central nervous system in three ethnic from South Sulawesi

No	Ethnis	Plant	Crude plant	Pharmacology effect	Preparation	Dosage
1	Toraja	*Carica Papaya*	Leaf	AP	Boiling	Twice dayli
		Alium cepa	Root	AI,A,AP	Juice	Twice dayli
		Mamordica caranthia	Leaf	AP	Boiling	Twice dayli
		Andrographis paniculata	Herb	AI,A,AP	Boiling	Twice dayli
		Ipomea aquatica	Herb	HS	Vegetable	Once dayli
		Capsicum annum	Fruit	AI,St	Vegetable	Once dayli
2	Bugis	*Passiflora foetida*	Herb	AP, AI	Tea	Twice dayli
		Ipomea aquatica	Herb	HS	Vegetable	Once dayli
		Capsicum annum	Fruit	AI,St	Vegetable	Once dayli
		Mimosa pudica	Herb	AI,A,AP	Boiling	Twice dayli
		Ficus septica	Leaf	AI,A,AP	Patch	Twice dayli
		Zingiber officinalis	Rhizome	AI,A,AP	Patch	Twice dayli
		Piper betle	Leaf	AP	Patch	Fourth dayli
		Citrus limonis	Fruit	AP	Juice	Fourth dayli
		Alstonia scholaris	Bark	AP	Boiling	Twice dayli
		Klenhopia hospital	Leaf	AP	Tea	Twice dayli
		Lannea coromandelica	Bark	AI	Patch	Once dayli
3	Makassar	*Alium cepa*	Root	AI,A,AP	Juice	Twice dayli
		Carica papaya	Leaf	AI,A,AP	Boiling	Twice dayli
		Ipomea aquatica	Herb	HS	Vegetable	Once dayli
		Capsicum annum	Friut	AI,St	Vegetable	Once dayli
		Mimosa pudica	Herb	AI,A,AP	Boiling	Twice dayli
		Ficus septica	Leaf	AI,A,AP	Patch	Twice dayli
		Zingiber officinalis	Rhizome	AI,A,AP	Patch	Twice dayli
		Piper betle	Leaf	AP	Patch	Fourth dayli
		Citrus limonis	Fruit	AP	Juice	Fourth dayli
		Alstonia scholaris	Bark	AP	Boiling	Twice dayli
		Klenhopia hospita	Leaf	AP	Tea	Twice dayli
		Lannea coromandelica	Bark	AI	Patch	Once dayli

AI=Antiinflammotory ;AP=Antipyretic:A=Analgetic,HS=Hypnotic-sedative;St=Stimulans

Table 2. Data chemical identification medicinal plant of etnopharmacology in 3th etnic from South Sulawesi

No	Name of plant	Crude plant	Chemichal compound
1	Papaya	*Carica papaya Leaf*	Alcaloid, enzym papain, glycoside,
2	Red onion	*Alium cepa Root*	saponin
3	Bitter melon	*Mamordica carantia*	volatile oil, saponine, starch,
4	Bitter	*Leaf*	glycoside
5	Chilies	*Andrographide Herb*	Saponine, alcaloid, starch, resine
6	Princess Shame	*Capsicum annum Fruit*	Alcaloid dan tannin
7	Water spinach	*Mimosa pudica Herb*	volatile oil , alcaloid
8	Permot	*Ipomea aquatica Herb*	Mimosin, volatile oil
9	Ginger	*Passiflora foetida Herb*	Alcaloid, latex
10	Herbaceous	*Zingiber officinalis*	Steroid, tannin, flavanoid dan
11	swamp	*Rhizome*	saponine
12	Piper	*Ficus septica leaf*	volatile oil , glycoside
13	Alstonia Bark	*Piper betle Leaf*	Alcaloid, tanin, glycoside
14	Paliasa	*Alstonia scholaris Bark*	volatile oil , alcaloid
15	Ayu Jawa	*Klenhopia hospita Leaf*	alcaloid.
	Awar-Awar	*Lannea coromandelica*	flavanoid, glycoside
		Ficus septica	glycoside, ssaponin, sellulose, tannin
			flavanoid, glycoside, tannin,
			saponin.

DISCUSSION

Province of South Sulawesi there are three ethnic Makassar ethnic culture, ethnic Bugis and Toraja ethnic group, the third of this ethnic diversity of different cultures, as well as on alternative medicine with the use of natural ingredients for traditional medicine.

Treatment of central nervous system that is on the awareness of pain is formed from two processes, namely acceptance of pain in my big brain stimulation and emotional reaction of the individual. Analgesic affect the first process by enhancing the awareness of pain threshold, while the narcotics suppress psychological reactions caused by painful stimulation. On the other hand CNS function can be suppressed entirely by non-specific by relief centers substances such as hypnotics and sedatives. As a result of awareness for the exogenous impulses derived and reduced physical activity and mental health. However, natural ingredients that affect drug use must be careful because it can lead to addictive, and even used only for research purposes.

Inventory etnopharmacology use of medicinal plants in the central nervous system as a traditional medicine was conducted in three ethnic groups of South Sulawesi, Makassar ethnicity in the district ie mild, ethnic Bugis Soppeng district and ethnic Toraja. Collecting data using questionnaires and interviews with the local community about the use of medicinal plants have an effect on the treatment of central nervous system tradisidonal.

From the results of interviews and questionnaire data showed that the use of medicinal plants in the treatment of central nervous system in three ethnic groups of South Sulawesi was obtained: *Ipomoea aquatica* leaf and *Capsicum annum* fruit. *Alium cepa* root and *Carica papaya* leaf used by ethnic Toraja and Makassar, while *Ficus septica* leaf, rhizome *Zingiber officinalis*, *Piper betle* leaf, and *Citrus limonis* fruit were used by ethnic Bugis and Makassar for the treatment of CNS. *Passiflora foetida* Herb, *Mimosa pudica* herb, *Ipomea aquatica* leaf, and *Citrus limonis* fruit for sedative hypnotics treatment. *Carica papaya, Mamordica caranthia, Alstonia scholaris,* and *Klenhopia hospita* to treath of fever and head pain (antipyretic). *Alium cepa,*

Andrographis paniculata herb, *Piper betle* leaf , *Zingiber officinalis* rhizome, and *Ficus septica* leaf as an analgesic, antipyretic and anti-inflammatory, and *Lannea coromandelica* bark use to treat inflammatory.

From the identification chemical compound with reagent showed chemical components contained in the sample which affects the central nervous system is a type of alcaloid, flavanoid and volatile oil. Permot herb (*Passiflora foetida* L.) containing a chemical component that is steroids, tannins, saponins, and flavanoid, this plant in etnomedicine been used as a diarrhea medicine, according to research data by Mohanasundari *et al.*, (2007), and Hertina (2006), *in vitro* permot herbs have antimicrobial activity primarily against human pathogenic bacteria. Water spinach (*Ipomea aquatica*) contains a chemical component such as alkaloids, latex. Bitter herb (*Andrographis paniculata*) contains alkaloids and tannins. Chilli fruit (*Capsicum annum*) contains essential oil and alkaloids. Shameful herb (*Mimosa pudica*) contains Mimosin, essential oil. Papaya Leaf (*Carica papaya*) contains alcaloids, glycosides, and saponins. Bitter melon leaf (*Momordica charantia*), contains saponins, alkaloids, starch and resin. Red onion (*Alium cepa*) contains : essential oils, saponin, starch, and glycosides. *Klenhopia hospita*, *Ficus septica* and *Lannea coromandelica* is typical of plants that grow in the area of South Sulawesi, both contain glycoside compounds. *K.hospita* not only used as a fever medicine but also as a hepatitis drug, *F. septica* is also used for spasmodic, and abdominal pain, while *L. coromandelica* often used to treat cataracts and eye pain.

CONCLUSIONS

Etnopharmacology medicinal plant data from three ethnic groups in South Sulawesi used in the treatment of central nervous system obtained :

a. *Passiflora foetida* Herb, *Mimosa pudica* Herb , *Ipomea aquatica* Leaf, and *Citrus limonis* Fruit for sedative hypnotics treatment.
b. *Carica papaya* and *Mamordica caranthia, Klenhopia hospita* to treath of fever and head pain (antipyretic)
c. *Alium cepa, Andrographidis paniculata* Herb, *Piper betle* Leaf , *Zingiber officinalis* Rhizome, and *Ficus septica* Leaf as an analgesic, antipyretic and antiinflammatory
d. Study of Identification chemical compound with reagent showed chemical components contained in the sample which affects the central nervous system is a type of alcaloid, flavanoid and volatile oil, tannin, and saponin.

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MICROSCOPIC IDENTIFICATION AND TLC PROFILE IN JAMU FOR TREATMENT OF URIC ACID

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Abstract: Utilization natural product or herbal medicine , is growing very rapidly, especially in developing countries like Indonesia. Indonesian people have been using herbs for the treatment of hereditary, derived from vegetable material / herbal, animal or mineral. But now many false nostrum on the market, mainly herbal gout. Microscopic identification in Jamu for treatment of uric acid to prove the components of the herbal medicine according to the shown on brosure.

Identification in jamu includes examining microscopic examination and identification of the chemical content of crude drugs by TLC were tested on samples A and B with herbal medicine comparison Nutmeg (*Myristica fragrans*) and Ginger Rhizome (*Zingiber officinalis*). Microscopic identification found Perisperm secondary, bundle tissue, endosperm with starch, the same as in Nutmeg, and the ginger shows Starch, parenchymal with cell secretion, and sclerencym. Identification in jamu for teratmen of uric acid by TLC to UV with wavelengths at 254 nm and 366 nm, and the Iod's spraying showed nutmeg and ginger rhizomes as the composer from both of jamus with the similarity of Rf and color of spot, and use specific reagents vanillin-sulfuric acid showed type terpenoids (essential oil).

Keyword : Pharmacognostic, jamu, uric acid.

INTRODUCTION

Indonesia using Jamu for the treatment has lasted since the beginning of the Majapahit kingdom (and found in the reliefs of Borobudur temple on the use of Kalpataru leaves (leaves lasting) for various diseases). In many regions in Indonesia found many books of medical procedures, there is a long manuscript on palm leaves Husodo (Java), Usada (Bali), Lontarak pabbura (South Sulawesi), documents spell horoscope Fibre, Fibre Wulang nDalem cream concoction and reliefs of Borobudur temple describe people were dispensing medicine (herbal medicine) with plants as the raw material. Herbal Medicine is one type of traditional medicine that originated from a mixing of some fresh bulbs, and dry made for commercial belikan.Biasanya formed in the stocks of durable so can be stored and used without prior processing. Herbal Medicine can be found preformance in the form of powder, chopping, pills, caplets, capsules and liquid medicines (syrup or suspension). Use of herbal medicine is very diverse some of which is to maintain health and prevent and treat various diseases.

Uric acid in The Merck Index, an Encyclopedia of Chemicals and Drugs, 9th edition, expressed as an alkaloid compound derived purine (xanthine). The compound was first discovered by Scheele in 1776 this is the end product of nitrogen metabolism in birds and reptiles. Gout disease is one type of rheumatic disease artikuler, namely that occurred in the rheumatic joint. The disease is also known by the name of rheumatic joints, rheumatism, gout, arthritis piral, or arthritis. Gout comes from Latin gutta, meaning that droplets dipersendian evil. According to ancient beliefs of this disease occurs because of the injury that falls drop by drop into the joints. Someone said to suffer from the disease if levels of uric acid in the blood uric acid increased. uric acid is a purine metabolism in the form of crystals which naturally is in the blood (Purnomo, 2003).

Normal blood uric acid levels were <7 mg / dl, for men and for women <6 mg/dl (Lumenta, 2004). Where, When these compounds accumulate in the number of above normal, will trigger the formation of needle-shaped crystals. The crystals are usually concentrated in areas such as leg joints, knees, elbows, and fingers, resulting in inflammation in the joints. Purine efflorescence also common in capillary blood vessels (Maloedyn, 2006). The product of uric acid occurs in the liver, bone marrow and muscle will metabolize purines into uric acid in these organs because of the role Xantin oxidase enzyme. Uric acid is formed as a result of catabolism (split) which is one of purine nitrogen bases found in DNA. Purine initially decomposed into Hipoxantin, the reaction is then converted into Xantin, Xantin this is what later became the uric acid. On increasing the formation of uric acid resistance of the purine nucleotide purine base guanine is reduced hipoksantin and thus more elaborated into uric acid or negative feedback mechanisms in the synthesis of purine waived, so that more purine formed. (Mutschler, 1999). Joints where the uric acid accumulates usually become swollen, stiff, and feels pain. Stacking Crystals in joints also causes the lymph fluid that serves as a lubricant for joints (Lubricant) loss of function. As a result, the joints can not be moved, in the meantime, if the crystals precipitated in the capillary blood vessels, the pointed tip of Crystal will be poking the blood vessel walls and cause injury and vascular leakage. (Maloedyn, 2006).

The use of jamu to prevent and treat diseases of uric acid has been used Indonesian society, however many herbal medicine currently in circulation contain dangerous chemical compounds were added in herbal preparations, of course, this is very dangerous for health. It is necessary for the identification test sample, which was contained in medicinal herbs in order to prove whether it contains the sample, which in accordance with an indication of the target, so safe to consume for the community.

AIM To Research.

Microscopic identification in jamu for treatment of uric acid to prove the component of herbal acoording to the shown on brosure.

MATERIAL AND METHODS

Material

Sampel of jamu A and B, Nutmeg (*Myristica fragrans*), Ginger rhizoma (*Zingiber officinalis*), Aqua pro analysis, chloride acid, sulphate acid, Ethanol 95 %, Chloroform, Methanol, n-hexan, Chloraldehid

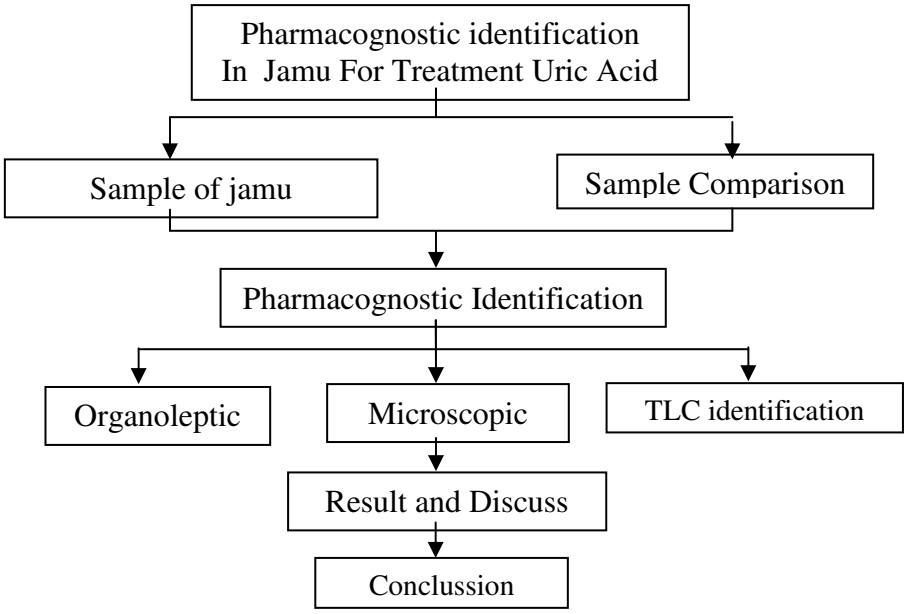
Instrument

TLC instrument, TLC set, UV wavelengths at 254 nm and 366 nm, Microscop (Nikon)

Methods

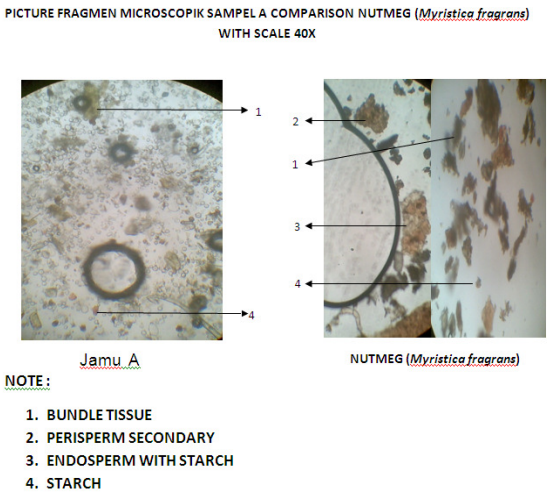
Identification in jamu for treatment of uric acid by microscopic identification and TLC to UV wavelengths at 254 nm and 366 nm, and Iod's spraying.

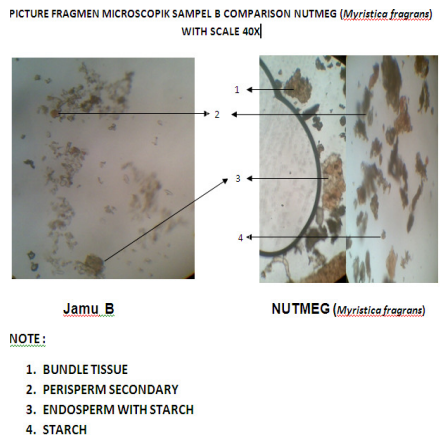
Draft Procedure of Identification Pharmacognostic jamu Uric Acid revolve in Makassar City is following:



RESULT AND DISCUSSION

Identification in jamu includes examining microscopic examination and identification of the chemical content of crude drugs by TLC were tested on samples A and B with herbal medicine comparison Nutmeg (*Myristica fragrans*) and Ginger Rhizome (*Zingiber officinalis*).

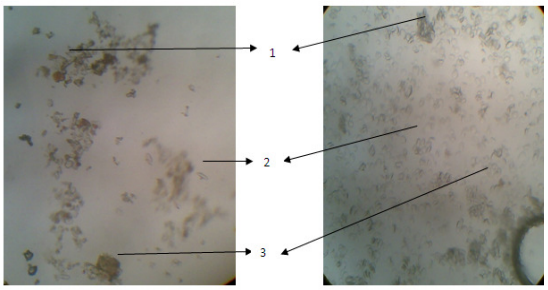




Microscopic identification showed Perisperm secondary, bundle tissue, endosperm with starch, the same as in Nutmeg, and the ginger shows Starch, parenchymal with cell secretion, and sclerencym.

Identification in jamu for treatmen of uric

PICTURE FRAGMEN MICROSCOPIK SAMPEL B COMPARISON GINGER RHIZOMA(*Zingiber Officinalis*) WITH SCALE 40X



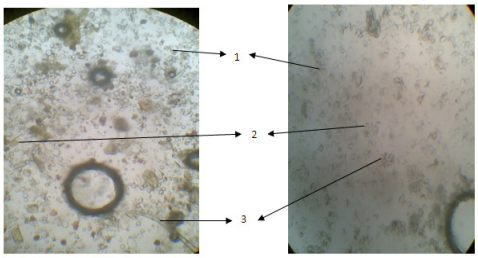
Jamu B

GINGER RHIZOMA(*Zingiber Officinalis*

NOTE

1. FIBER
2. STARCH
3. PARENCYMAL WITH CELL SECRETION

PICTURE FRAGMEN MICROSCOPIK SAMPEL A COMPARISON GINGER RHIZOMA(*Zingiber Officinalis*) WITH SCALE 40X



Jamu A

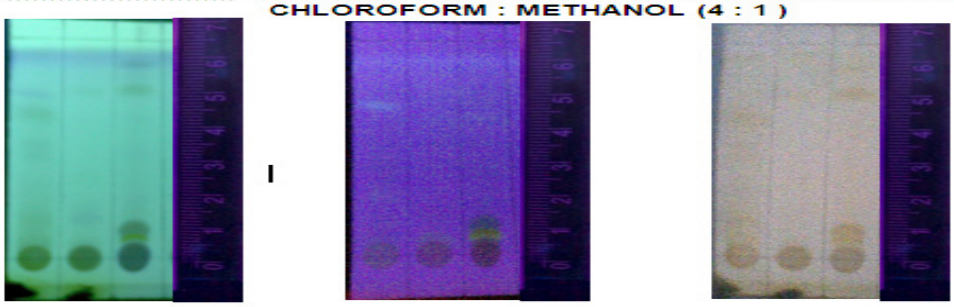
GINGERRHIZOMA(*Zingiber Officinalis*)

NOTE :

1. STARCH
2. CORK TISSUE
3. FIBER

acid by TLC to UV with wavelengths at 254 nm and 366 nm, and the Iod's spraying :

PICTURE TLC METHANOL EXTRACT WITH ELUSIDATION LIQUID CHLOROFORM : METHANOL (4 : 1)



1 2 3 1 2 3 1 2 3

A B C

NOTE :
1. GINGER RHIZOMA (*Zingiber officinalis*)
2. Jamu A
3. Jamu B
A. TLC TO UV 254 nm
B. TLC TO UV 366 nm
C. SPOT WITH Iod

Table 1. TLC Profiling sample of jamu A and B, Ginger and Nutmeg

Sampel	λ 254		λ 366		Iod	
	Rf	Warna	Rf	Warna	Rf	Warna
Ginger	0,90	Blue	0,90	Fluorescent	0,90	Brown
	0,8	Blue	0,8	Fluorescent	0,8	Brown
	0,65	Green			0,65	Brown
	0,54	Blue			0,54	Brown
	0,49	Blue			0,49	Brown
	0,18	Blue			0,18	Brown
	0,10	Blue			0,10	Brown
Nutmeg	0,90	Blue	0,90	Dark	0,90	Brown
	0,83	Blue	0,83	Dark	0,83	Brown
	0,8	Blue	0,8	Dark	0,8	Brown
	0,69	Blue	0,69	Dark	0,69	Brown
	0,49	Blue	0,49	Dark	0,49	Brown
	0,45	Blue	0,45	Dark	0,45	Brown
	0,38	Blue	0,36	Dark	0,36	Brown
	0,32	Blue	0,32	Dark	0,32	Brown
	0,27	Blue	0,27	Dark	0,27	Brown
	0,23	Blue	0,23	Dark	0,23	Brown
	0,12	Blue	0,12	Dark	0,12	Brown
Jamu A	0,90	Blue	0,90	Dark	0,90	Brown
	0,8	Blue			0,8	Brown
	0,38	Blue			0,38	Brown
	0,18	Blue	0,18	Dark	0,18	Brown
Jamu B	0,90	Blue	0,90	Flourecent	0,90	Brown
	0,8	Blue	0,8	Fluorescent	0,8	Brown
	0,49	Blue			0,49	Brown
	0,27	Blue			0,27	Brown
	0,18	Dark	0,18	Dark	0,18	Brown
	0,10	Yellow	0,10	Yellow Fluorescent	0,10	Brown

Utilization of natural materials primarily as a medicinal plant among the community has become part of Indonesian national culture obtained from generation to generation in the form of empirical knowledge (based on experience). The existence of herbal medicine is no longer underestimated in terms of treatment, especially with the many discoveries of new drugs derived from natural materials raises the possibility that the development and clinical use that can be accounted for treatment effects. The rapid development and circulation of medicinal herb industry both produced by the herbal medicine industry in the country and abroad and the increasing public interest in recovering the natural results (Back to nature) are considered to be economically cheaper and have fewer side effects than synthetic drugs, more and raise the prestige of Traditional medicine among the public, including in the field of formal treatment.

Proving the truth of the contents of herbal medicine is done through examination of pharmacognostic organoleptic examination, microscopyc and identify the chemical components of herbal medicine by thin layer chromatography. Microscopic examination of herbal medicine samples A and B by comparison nutmeg seed obtained the same fragment with crude comparison, also the comparison of Ginger.

Microscopic identification found Perisperm secondary, bundle tissue, endosperm with starch, the same as in Nutmeg, and the ginger shows Starch, parenchymal with cell secretion, and sclerencym.

Identification in jamu for teratmen of uric acid by TLC in eluen klroroform-metanol (4:1), yang dideteksi pada UV with wavelengths at 254 nm and 366 nm, and the Iod's spraying showed nutmeg and ginger rhizomes as the composer from both of jamus

with the similarity of Rf and color of spot, and use specific reagents vanillin-sulfuric acid showed type terpenoids (essential oil).

CONCLUSION

Identification in jamu sample A and B for treatment of uric acid showed Nutmeg (Myristica fragrans) and Ginger rhizome (Zingiber officinalis) and chemical compound type terpenoid (esential oil). Identification in jamu for treatment of uric acid by TLC to UV wavelength at 254 nm and 366 nm, and Iod's spraying showed nutmeg and ginger rhizomes as the composer from both of jamus with the similarity of Rf and color spot, and use specific reagent vanillin-sulfuric acid showed type terpenoids (essential oil).

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ETNOPHARMACY OF HERBAL MEDICINE STUDIES FROM WAKATOBI IN SOUTH- EAST SULAWESI

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Abstract: Biological and cultural on Indonesian society contributes to the use of medicinal plants primarily in the discovery of new drugs. Wakatobi Society as a civilized society include with the five ethnic groups, include Wanci, Kaledupa, Tomia, Binongko, and Bajo ethnics have influence treatment system and use of natural ingredients. However, data on the effects of therapy, how to use and the rules of usage of medicinal plants by the community itself is very limited Wakatobi. Therefore conducted a study of ethnicity in the Wakatobi etnopharmacy to explore cultural heritage, especially in developing traditional medicine and medicinal plants as well as inventory purposes.

Data acquisition and sampling conducted on five ethnic groups by using questionnaires and interviews. Herbarium and crude drug samples made, then the use of inventory based on the properties of plants used etnopharmacy Wakatobi community. Results showed statistically etnopharmacy plants with the highest usage of Wanci at 58%, followed by community Tomia at 17%, Kaledupa at 12%, Binongko 10%, and Bajo 3%, with 60 pharmacologic effects of some plants used in a single dosage form, among others, *Lantana camara* for cough medicine, and astrigens, *Loranthus sp* use to cancer, cough, and hemorrhage treatmen, *Sesbania glandiflora* to cure fever, ulcer disease, and stimulant. Wanci ethnic using Mixture Preparations *Loranthus sp, Curcuma domestica, Aglaia odorata, and Cocos oleum* to treat 40 types of disease.

Keywords : wakatobi, etnopharmacy

INTRODUCTION

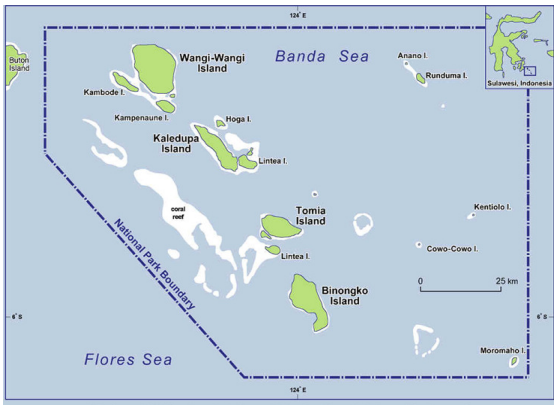
The presence of plants as medicine in Indonesia has been known since thousands of years ago. Evidence of this history is engraved in the palm leaf, the walls of the temple, and the book of the past. Recipes passed down through generations, which was only known to certain circles and then spread to the wider community. The world recorded the booming of east herbal traditions. The value of modernization of the very origins of medicinal plants by the pharmaceutical world is gradually recognized by the scientific community. Although it remains a place for traditional usage. With step and the correct way of processing properties of medicinal plants will not change.

However, the development and use of drugs from these natural materials have been widely experienced difficulty because of the discovery and knowledge about the natural ingredients of this very secret and only passed on by generations by word of mouth based on custom and habit.

Utilization of various medicinal herbs as ingredients in traditional medicines in Indonesia comes from knowledge, traditions and customs of indigenous people (ethnic) who applied were hereditary. Wakatobi Society as a civilized society include with the five ethnic groups, include Wanci, Kaledupa, Tomia, Binongko, and Bajo ethnics have influence treatment system and use of medicinal plant. Ethnic diversity is what will greatly contribute to the development of medicinal plants and of course affect patterns of drug treatment and use of materials, primarily the use of natural substances as drugs.

However, data on the effects of therapy, and the rules of how to use medicinal plants for use by the community itself is very limited Wakatobi. Therefore, research must be done etnofarmasi of ethnic studies at the Wakatobi to explore cultural heritage,

especially in developing traditional medicine and medicinal plants as well as the purpose of inventory.



Wakatobi Marine National Park, South East Sulawesi

Wakatobi is a district that consists of four main islands, namely Wangi-Wangi, Kaledupa, Tomia, and Binongko. So, Wakatobi is an acronym for the name or acronym of the four main islands. Before the division of territory, the islands called the Isles of Iron Tailor / "The Tailor of Iron Island." (Oktora, 2009) and is still part of Buton Regency. (Whina. AY 2009). Tailor the islands called Iron because since the first inhabitants of the archipelago known as a craftsman or a blacksmith, who supply their household needs and tools of war for the kingdom of Buton and surrounding areas. Area of each island is Wangi-Wangi Island (156.5 km2), Kaledupa Island (64.8 km2), Tomia Island (52.4 km2), and Binongko Island (98.7 km2). Therefore, when Wakatobi community we call the people in question are the people who inhabit the four islands. (Anonymous, 2009). In gaografis, Wakatobi district, located in the southern part of the equator, stretching from North to South between 5000 up to 6.250 south latitude, 160 km long and runs from west to east, between 123.340 to 124.640 east longitude, along the 120 km. Bordering north Buton and Muna, bordering the South Flores Sea, West of Buton borders, and borders the eastern Banda Sea (Anonymous, 2009), and including the tropical regions. (Oktora, 2009). The total area of land as a whole is ± 823 km2, an area of 18 377 km2 ± waters, population 106 163 inhabitants. Position adjacent to the equator makes the area a national park Wakatobi tropical. Wakatobi is a fertile area as a source of natural materials which are used as food and as medicine. As the use of turmeric (Curcuma domestica), Loranthus (Loranthus sp) and coconut oil (Cocos oleum) was used as an herb to treat several diseases including cancer, fever, rheumatism, diabetes mellitus, hipertensi and others.

AIM OF RESEARCH

The purpose of this study is as an effort Inventory of medicinal plants that can be utilized as a drug of natural product which include nutritious crops, the effect of therapy, pharmaceutic dosage, and use for treatment.

METHODS

Research design : Observational reasearch with explorer etnopharmacology of medicinal plant in Wakatobi from Southeast Sulawesi

Research Location. This research was conducted in five ethnic in Wakatobi from Southeast Sulawesi: Wanci, Tomia, Kaledupa, Binongko and Bajo.

Procedure Research

Observation

This research was conducted through a series of procedures including; preparation of guidelines for the interview (interview guidelines), the informants, conducting field research (observation and interviews). Collecting data on five ethnic conducted using questionnaires and interview. Determination of Informants with qualitative studies that rely on interviews as a strategy to obtain the data, thus the target of research is the

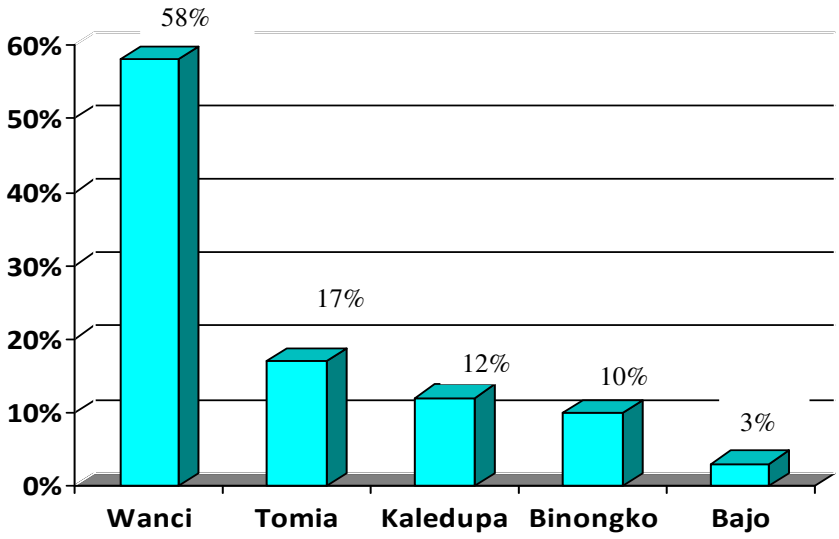
number of informants who were intentionally set (purposive) to know the criteria etnopharmacology (especially directed at traditional healers/dukun).

The population of this research is a native of Wakatobi are knowledgeable about the use of traditional medicine living in the five islands. Sampling in this study used a purposive sampling method. Sampling was conducted on 100 respondents who were taken proportionately spread over four islands of Wakatobi.

RESULT AND DISCUSSION

Results showed statistically etnopharmacy of medicinal plants with the highest usage of Wanci at 58%, followed by community Tomia at 17%, Kaledupa at 12%, Binongko 10%, and Bajo 3%, with 50 pharmacologic effects of some plants used in a single dosage form, among others, *Lantana camara* for cough medicine, and astrigens, *Loranthus sp* use to cancer, cough, and hemorrhage treatamen, *Sesbania glandiflora* to cure fever, ulcer disease, and stimulant.

Picture Prosentation of Utilization plants medicin in 5th ethnic community from Wakatobi Island:



Tabel 1. Etnopharmacy plant medicine in five ethnic from Wakatobi District.

No.	Ethnic	Vern name of Plant	Species	Disease	Plant part	Dosage form
1.	Wanci	Jeruk purut Songgolangit Gambir Kelapa Jarak pagar Turi Benalu Pacar Kunyit Jambu biji Delima Alpokat	*Citrus limonis* *Tridax procumbens* *Uncaria gambir* *Cocos nucifera* *Jatropha curcas* *Sesbania grandiflora* *Loranthus sp* *Aglaia odorata* *Curcuma domestica* *Psidium guajava* *Punica granatum* *Persea americana*	AP HB DM DM,Hipo DM,AP,AI DM,AP,AI DM,AP,AI DM,AP,AI DM,AP,AI Diarrhea Diarhrea Diu,N, Hipo	Fruith Leaf Seed Fruith Oleum Leaf Leaf Leaf Leaf Rhizome Leaf Fruith Leaf	Mixture Single Mixture Mixture Single Mixture Mixture Mixture Mixture Single Single Single
2.	Tomia	Kaca-kaca Sambiloto Jambu Mente Tembakau Mengkudu Delima Cabe	*Piperomia pellucida* *Andrographis paniculata* *Annacardium occidentale* *Nicotiana tabacum* *Morinda citrifolia* *Punica granatum* *Capsicum annum*	HB DM AP AI,ID,L AI, ID Diarhea ID, St,	Leaf Leaf Leaf Leaf Fruith Fruith Fruith	Single Single Single Single Single Single Mixture
5.	Kaledupa	Ciplukan Pinang Kapuk Mengkudu Kecubung gunung	*Physalis peruviana* *Areca catechu* *Ceiba pentandra* *Morinda citrifolia* *Brugmansia suaveolens*	HB DM AP AI, ID Diarhea	Fruith Fruith Leaf Fruith Leaf	Single Mixture Single Single Single
3.	Binongko	Tembelekan	*Lantana camara*	HB,AP,AI	Daun/akar	Single
4.	Bajo	pisang	*Musa paradisiaca*	AP	Buah	Single

Tabel.2. Etnopharmacy plant medicine from Wakatobi.

Vern /Indonesian name	Nama Tumbuhan (Latin)	Pharmaco-logycal activity	Crops drug	Preparation	Dosage
Munte (Jeruk nipis)	*Citrus aurantifolia*	AP, SC,Cancer	Leaf	Mixture	Patch 2-3 x 1
Bafa meha (Bawang merah)	*Allium cepa*	AP	Bulb	Mixture	Patch 2-3 x 1
Kulou (Kelapa)	*Cocos nucifera*	40th diseases	Fruith Oleum	Mixture	Patch 2-3 x 1
Papongke (jarak pagar)	*Jatropha curcas*	AP, D, L	Leaf	Mixture	Patch 1 x 1
Loka (pisang)	*Musa paradisiaca*	AP, AI, L, GID	Leaf Fruith	Single	Patch eaten
Sirkaya (srikaya)	*Annona squamosa*	AP, E,	Leaf	Single	Tea 3 x 1
Kambajafa (turi)	*Sesbania grandiflora*	AP, Aph, UD,St	Leaf	Single	Tea 3 x 1
Malaka (Jambu	*Psidium guajava*	D, Hr,	Leaf	Single	

Biji)		Astrigen			
Jalima (delima)	*Punica granatum*	D, Astrigen	Fruith	Single	
Jambu mente	*Anacardium occidentale*	D	Leaf	Single	Tea 1-3 x 1
Balande (kecubung gunung)	*Brugmansia suaveolens*	D	Leaf	Single	Tea
Kaulalinda (benalu)	*Loranthus sp*	40th diseases	Leaf	Mixture	Tea 3 x 1
Patirangga (Pacar)	*Aglaia odorata*	40th diseases	Leaf	Mixture	Tea 3 x 1
Kuni (kunyit)	*Curcuma domestica*	40th diseases	Rhizo me	Mixture	Tea 3 x 1
Kastelakkau (pepaya)	*Carica papaya*	ID, AP, St L	Leaf Fruith	Single	Tea 2 x 1
Samburoto (sambiloto)	*Andrographis paniculata*	AI, AP, DM, St	Leaf and Herb	Single	Tea 2 x 1
Kafungkafu (kapuk)	*Ceiba pentandra*	AP	Leaf	Single	Tea 3 x 1
Alpokat	*Persea americana*	Diu,N, Hipo	Leaf	Single	Tea 1 x 1
Gandu morunga (Jagung muda)	*Zea mays*	AI, ID, SC, Diu, N,	Seed	Single	Patch
Kaniu-niu panda (Ciplukan)	*Physalis peruviana*	AI, ID,GID	Fruith	Single	Patch
Lindu-lindu (kaca-kaca)	*Peperomis pellucida*	AI, ID, GID	Leaf	Single	Patch
Tabako (Tembakau)	*Nicotiana tabacum*	AI, ID, L	Leaf	Single	Patch 3 x 1
Kunilafa (Temu lawak)	*Curcuma xanthorhiza*	AI, ID, AP	Rhizo me	Mixture	Patch
Fengkudu (Mengkudu)	*Morinda citrifolia*	AI, ID	Leaf	Single	Patch
Kaubaduri (Tembelekan)	*Lantana camara*	AI, ID, Cough, astrigen	Leaf	Single	Patch
Katinti (Anting-anting)	*Acalyptha australis*	AI, ID	Leaf	Mixture	Patch
Fengka (Pinang)	*Areca cathecu*	ID, DM	Fruit	Mixture/ Single	Tea 2 x 1
Soilo (Sirih)	*Piper betle*	ID, AI,AP	Leaf	Mixture/Sin gle	Tea 2 x 1
Kuni mahute (Temu putih)	*Curcuma mangga*	ID	Rhizo me	Mixture	Tea 2 x 1
Ginta matta (Cabe)	*Capsicum annum*	ID, St,	Fruit	Mixture	Tea
Sampalu (Asam jawa)	*Tamarindus indica*	ID, St, CD, L, Hr	Fruit Root	Mixture Single	Tea
Kaubaduri (tembelekan)	*Lantana cemara*	E, GID, CD	Leaf	Single	Tea
Paria (Pare)	*Mamordica charantia*	E	Leaf	Mixture	Tea 3 sdm x 1
Kaudafa (kelor)	*Moringa oleifera*	O	Leaf	Single	Tea
Kopi	*Coffea arabica*	O	Seed	Single	Tea
Bafa kau	*Allium sativum*	Hipo, CD,	Bulbus	Mixture	chewed

(Bawang putih)		Aphro,			
Tangkulela (Belimbing wuluh)	*Averrhoa bilimbi*	Hypotensive	Leaf	Single	Tea 3 x 1
Bae fualo (Ketimun)	*Curcumis sativus*	Hipo	Fruith	Single	Eaten
Beluntas	*Pluchea indica*	Hipo	Flower	Single	Tea
Tagambiri (Gambir)	*Uncaria gambir*	DM, Hr,	Seed	Mixture	Tea 1 x 1
Sungga-sungga (Songgolangit)	*Tridax procumbens*	GI disease	Leaf	Single	Tea
Kaudafa (Kelor)	*Moringa oleifera*	Lactagoge	Leaf	Mixture	Tea/ eaten
Rappo-rappo (Kacang tanah)	*Phaseolus communis*	Lactagoge	Seed	Single	eaten
Karton (Katuk)	*Sauropus androgynus*	Lactagoge	Leaf	Mixture	Tea/dimakan
Kau mohakki (Kayu pahit)	*Quassia indica*	ID, Scin care,	Leaf	Mixture	Patch
Bae (Beras)	*Oryza sativa*	Scin care	Seed	Mixture	Patch
Kamba melati (Melati)	*Jasminum sp*	Scin care	Flower	Mixture	Patch 1 x 1
Munte puru (jeruk purut)	*Citrus limonis*	Cancer, GID,E	Fruith	Single	Tea
Kuwe	Not yet identified	AI, ID	Leaf	Single	Patch 3 x 1
Hao karenga	Not yet identified	AI, ID	Root	Mixture	Patch
Komali	Not yet identified	AI, ID	Leaf	Mixture	Patch
Libo	Not yet identified	E	Leaf	Mixture	Tea 3 x 1
Katolaa (Gambas)	Not yet identified	St, Hipo	Seed	Single	Eaten 2 x 1
Felalo	Not yet identified	AP	Leaf	Single	Chewed 2 x 1
Popasa	Not yet identified	L,AI,AP	Leaf	Single	Tea ½ -1 x 1
Kamanu-manu	Not yet identified	AP	Fruit	Single	Tea
Sangke menihu	Not yet identified	AP	Leaf	Single	Patch
Katolaa (Gambas)	Not yet identified	St, Hipo	Seed	Single	Eaten 2 x 1
Felalo	Not yet identified	AP	Leaf	Single	Chewed 2 x 1
Popasa	Not yet identified	L,AI,AP	Leaf	Single	Tea ½ -1 x 1

Note : AI = antiinflammatory ; ID = Infection Diseases ; AP=Antipyretic; E=Expectorantia; GID=Gastro intenstinal Diseases; St=Stimulant; Hipo=Hypotensive; L=Lacsative : DM=Diabetes mellitus; Hr=Hemorrhage; CD=Cardiovasculer diseases; D=Diarrhea; SC=Scin care; Aphro=Aphrodiasiac; O=Obesity Diseases; Diu=Diuresis ; N=Nefrolysis; UD=ulcer disease.

DISCUSSION

Wanci ethnic used Mixture Preparations *Loranthus sp, Curcuma domestica, Aglaia odorata, and oleum Cocos* to treat 40 types of disease. the types of diseases treated in such: Diuresis, high fever / malaria, bleeding post partum, inflammation, influenza, emanorage, colesterol, aphrodiasiac, tumor, servix and breast cancer, hypertensi, hypnotic, sedative, diabetes mellitus, heart burn, stomach diseases, spasmolytic, stimulant, ashma, bile stone, emesis, infection, nephrosys, liver, cardiovascular diseases, immunosupressive, insomnia, uric acid, rheumatik, artrithis, ulcer disease, hepatitis, etc.

Dosage forms of this herb is made into powder and suspended with water 1-3 times daily. Utilization of various medicinal herbs as ingredients in traditional medicines in Indonesia comes from knowledge, traditions and customs of indigenous people (ethnic) who applied were hereditary. Ethnic diversity is what will greatly contribute to the

development of medicinal plants and of course affect patterns of drug treatment and use of materials, primarily the use of natural substances as drugs. Wakatobi as a regency in Southeast Sulawesi as a combined region-fragrant scent of the dominant tribe inhabited by Wanci, another area is the island Kaledupa, Tomia islands, and island Binongko, while the Bajo tribe is the tribe immigrants but have been merged with the local culture who inhabited the coastal areas .

Based on observations through interviews and questionnaires of the largest tribe is the tribe of using plants etnopharmacy Wanci, followed by interest Kaledupa, Tomia, Binongko, and Bajo. In etnopharmacy, tribes in the Wakatobi used a single dosage or herb plant parts that have been used by a fifth tribe. Although only about 37% of land in the Wakatobi which is used as farmland, where the rest are territorial waters, but the utilization of medicinal plants from each ethnic group who inhabit the Wakatobi remain preserved from ancient times until now evident that traditional treatments conducted by the shaman who is Furthermore, knowledge of hereditary data also showed that about 60 species of medicinal plants with the same plants to treat several diseases such as: Sesbania grandiflora Leaf for antipyretic treatment, Ulcer disease, and Aphrodiasiac, *Andrographis paniculata* leaf and herb for the treatment of Antipyretic, Antiinflammatory, diabetes mellitus, and stimulant, used in a single dosage form. Meanwhile, in the form of a mixture of tribal Wanci use of turmeric (*Curcuma domestica*), Loranthus (*Loranthus sp*) and coconut oil (*oleum Cocos*) was used as an herb to treat several diseases including cancer, fever, rheumatism, diabetes mellitus, hupertensi and others. But a survey found some plants of etnopharmacy that have not been identified the taxonomy, so more research is needed to identify them.

CONCLUSSION

Etnopharmacy medicinal plants in five ethnic Wakatobi were found to be used by Wanci ethnic at 58%, followed by community Tomia at 17%, Kaledupa at 12%, Binongko 10%, and Bajo 3%, with 50 pharmacologic effects of some plants used in a single dosage form.

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PHARMACOGNOSTIC STUDY AND OF CHEMICAL COMPOUND AND CARACTERIZATION OF N-HEXANE EXTRACT OF ASIAN PIGEON WINGS LEAF (*CLITORIA TERNATEA* L.*)*

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Abstract: Indonesia as one of big biodiversity Country is known as warehouse of herbal medicine. Estimated from about 30000 kind of flora at Indonesia tropical forest, about 9600 spesies have been known useful for medicine. From that amount, about 283 species is a important herbal medicine for herbal medicine industry. One of them is Asian pigeon wings (*Clitoria ternatea* L.) herbal medicine for diarrhea, abscess, and expectorant, not seldom as food supplement. There for, it needs to do research of pharmacognosy study and characteristic of chemical compound n-Hexan extract Asian Pigeon Wings Leaf (*Clitoria ternatea* L.*)*. The purpose of this research is study pharmacognostic contain macroscopic and microscopic assay leaf of Asian pigeon wings(*Clitoria ternatea* L.) and known the characteristic of chemical compound of Asian pigeon wings leaf (*Clitoria ternatea* L.) which have been isolated from result of Preparative Thin Layer Chromatography. Sample of Asian pigeon wings leaf (*Clitoria ternatea* L.) is taken from regency of South Halmahera, province of North Maluku, assay gromoded on determination of Asian pigeon wings leaf (*Clitoria ternatea* L.) include family Papilionaceae with spreading stem, on top surface of leaf is slight nigged on botton surface is very nigged, top leaf is acutus, base leaf is obtusus, complex leaf and side leaf is integer. Leaf anatomy showed epidermis type actinositic, parenkim palisade, and spons, glanular trichome, calsium oxalat, and transversal bundle is opening collateral. Characteristic of chemical compound n-heksane fraction according by UV-Vis Spectrofotometry at long wave 256 nm and infrared indicated as group of hydroxyl (OH), alkyle (C-H), amine (-N=). Amine secondary (NH), nitrogen (N), alkene (C=C). Single spot exhibition positive reaction to reagent specifics from group of alkaloid compound.

Keywords **:** Asian pigeon Leaf (*Clitoria ternatea* L.), n-hexane extract, pharmacognosy study and characteristic.

INTRODUCTION

Asian pigeon wings leaf (*Clitoria ternatea* L.) is taken from regency of South Halmahera, province of North Maluku, assay gromoded on determination of Asian pigeon wings leaf (*Clitoria ternatea* L.) include family Papilionaceae. Climbing plants, reptiles or irregularly shaped with woody rhizomes. With slender stems 0.5-3 m long, hairy or bald, sometimes somewhat erect stem. Leaves pinnate, with 5-7 strands, menjorong shaped, oval, oval-melanset or almost rounded, bare upper leaf surface, while the lower surface hairy. Flowers in axillary, single or in pairs, white or greenish white suburbs often with blue or blue all the basic areas are often the center is yellow or greenish, thick hairy edge. Ribbon-shaped oval pod, bald or with a mixture of long hair attached and very short hair. Seeds numbered 8-10, oval, olive green, brown, young or old, reddish brown with dark stripes or almost dark.

Asian pigeon (*Clitoria ternatea* L.) is a plant of lowland tropical moist and slightly humid, but tolerant of dry season in tropical areas (with rainfall 500-900 mm). These types of nuts grow on the prairie, open woodland, scrub, river vegetation and disturbed places. The best growth under full sunlight. Annual rainfall needs to be able to survive may be as low as 400 mm, but requires about 1500 mm (or with additional

irrigation) to get the best production. Altitude ranges between 0-1600 m and scope of the annual average temperature of 19-28 °C. Blue flower has the ability to adapt to the vast land (pH 5.5-8.9), but like the land is fertile and will grow poorly in the sandy barren land if not given fertilizer. This is one of a small pea with a good herb that can adjust themselves to high clay in areas of moderate to semi-humid tropics and grows on dry meadows with a blend of irrigation, but it can not survive with high flooding or drought.
Asian pigeon wings (*Clitoria ternatea* L.) herbal medicine for diarrhea, abscess, and expectorant, not seldom as food supplement. There for, it needs to do research of pharmacognosy study and characteristic of chemical compound n-Hexan extract Asian Pigeon Wings Leaf (*Clitoria ternatea* L.*)*. The purpose of this research is study pharmacognostic contain macroscopic and microscopic assay leaf of Asian pigeon wings (*Clitoria ternatea, L*) and known the characteristic of chemical compound of Asian pigeon wings leaf (*Clitoria ternatea* L.) which have been isolated from result of Preparative Thin Layer Chromatography.

MATERIAL AND METHODS

Material

Distilled water, hydrochloric acid, sulphuric acid 10%, leaf of a Asian pigeon (*Clitoria ternatea.* L), reagent of Dragendorf, ethyl acetate, chloroform, cobalt (II) thiocyanate, TLC plates (E.merck), preparative TLC plate (E.Merck), methanol, n-hexane, Lieberman-Burchard and Mayer reagent, Silica Gel G60 F254. Spray bottle spray reagent, chamber (Camag), separating funnel (pyrex), exicator, a set of UV lamps, ovens (Memert), Rotavapor, a set of tools maceration, IR spectroscopy (FTIR-5400S), UV-Vis spectroscopy (UV-1601Shimadzu), and a set of tools TLC

Methods

a. Pharmacognostic Assay

Blue flower leaf (*Clitoria ternatea* L.) in slices crosswise and lengthwise placed on the object glass and covered deck on the glass and then observe in the microscope and observed them and the network provider of specific fragments.

b. Liquid-liquid extraction with n-hexane solvent

Condensed methanol extracts obtained as many as 10 grams suspended with 25 ml of water and put into separating funnel, was added n-hexane of 40 ml, was closed and shaken. After shaking, settling a few moments until the separation between the water layers and layers of n-hexane. N-hexane layer is collected in a container and the water layer re-extracted with n-hexane to n-hexane layer of clear (done as much as seven times). N-hexane extraction results obtained and then evaporated to obtain dry extract of n-hexane.

Isolation by preparative chromatography

Hexane Extract *C. ternatea* L. leaf is placed on TLC plates size 20 x 20 cm, then eluted with n-hexane: ethyl acetate eluent (8: 2).After that, the plates are removed and dried in the air and then observed under UV wavelength 254 nm and 366 nm. Plates that have been observed given the limits of dirt and dredged, then housed in the vial. The result is then added dredged from methanol and filtered. The filtrate was then evaporated followed by purification.

c. The purity test isolates

i. Two-dimensional thin layer chromatography

Isolates obtained was dissolved in chloroform: methanol (1: 1), then place on TLC plates size 10 x 10 cm. Then eluted with the eluent n-hexane: ethyl acetate (8: 2) direction of the first and benzene: ethyl acetate (4: 1) to the second direction. The second elution process is done by turning the plate opposite the clockwise direction so that the results of the first elution be a starting point for the second time

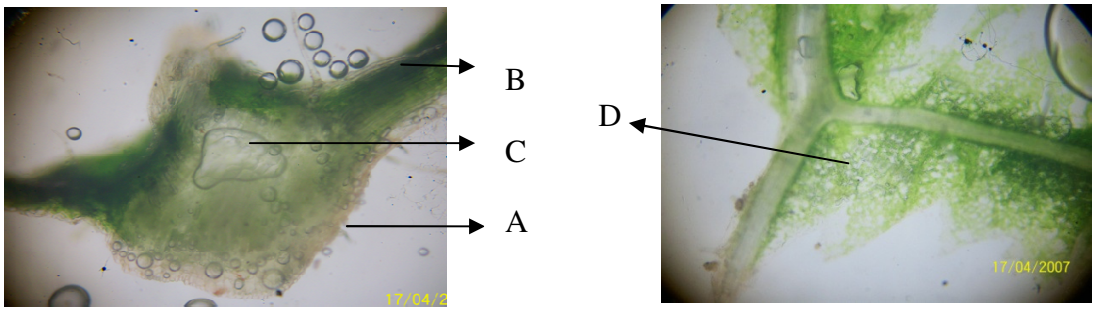
pengelusian elution process is done, there is one single spot, we can conclude that the crystal is a single chemical component.

ii. Multi eluent system

Test the purity of isolates was also done using some variation of the n-hexane eluent: ethyl acetate (8: 2), benzene: chloroform (1: 2) and benzene: ethyl acetate (5: 1). The appearance of single spots indicating that the compound obtained from isolate a single chemical component.

RESULTS AND DISCUSSION

a. Pharmagconostic Assay



Picture of microscopic which transversal section *C.ternatea* leaf showed (A) epidermis with glandular trichoma type, (B) palisade parencym tissue, and (C) bundle tissue, (D) Stomata is actinositic type.

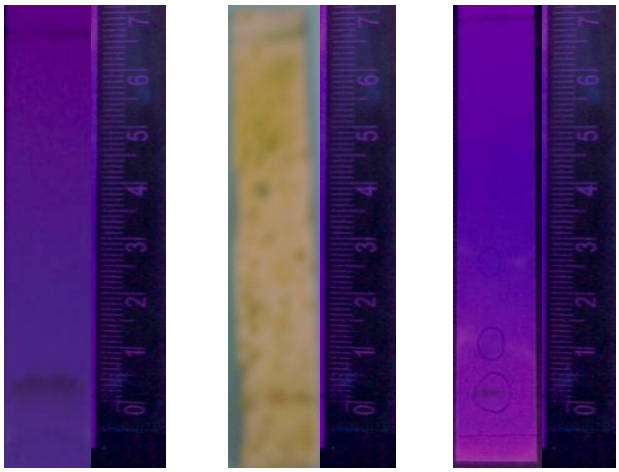
Sample of Asian pigeon wings leaf (*Clitoria ternatea, L*) is taken from regency of South Halmahera, province of North Maluku, assay gromoded on determination of Asian pigeon wings leaf (*Clitoria ternatea, L*) include family Papilionaceae with spreading stem, on top surface of leaf is slight nigged on botton surface is very nigged, end leaf is acutus, base leaf is obtusus, complex leaf and side leaf is integer. Microscopic of leaf indicate epidermis with actinositic,stomata type, trichoma is glandular type , and transversal bundle is opening collateral type, and parencym palisade.

b. Identification and Characterization with Chemical Reagent

Single Isolate followed by the identification of chemical that is by spraying Dragendrof, Mayer's, Cobalt (II) thiocyanate, Bauchardad, Vanillin-sulfuric acid, Lieberman-Burchard reagent, $FeCl_3$ and several other classes of reagents.

c. Identification of UV-Visible spectroscopy

Isolates obtained were identified by UV-Visible spectroscopy. Compounds dissolved in methanol pro analysist and then the sample is placed between the monochromator and detector. The resulting spectrum is recorded on recording devices. Identification of infrared (IR).spectroscopy. isolates obtained from the identification fraction followed by infrared spectroscopy by placing the samples as thin films between two transparent layers of sodium chloride, then placed on the slit infrared light. The results recorded on the recording devices.



Description:
Plate 1 = dragendrof f reagent in UV 366 nm
Plate 2 = mayer reagent in UV 254 nm
Plate 3 = kalium (II) tiosianat reagent
Stationary phase silica gel GF = 254 nm
Mobile phase = n-Hexane: Ethyl acetate (8:2)

1 2 3

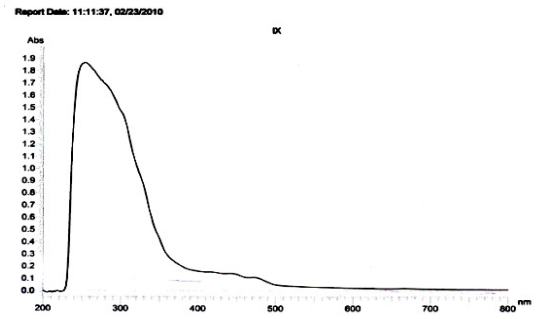
Picture TLC profiling n-Hexan fraction eluted with mobile phase of n-Hexan-ethylacetate

Table TLC profiling that identificated with chemical reagent

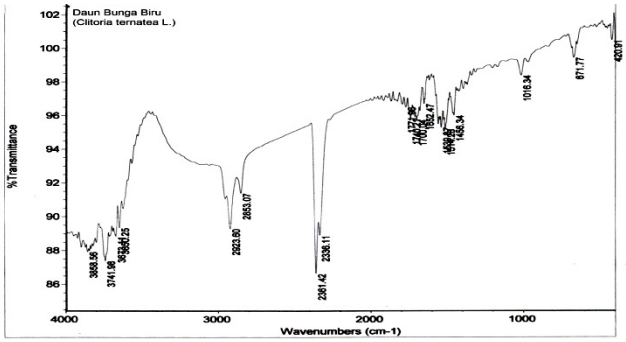
Sample	Reagent identification	Number of spoth	Rf	Color spots
1	Dragendorf reagent	1	0,48	Orange
2	Mayer reagent	1	0,5	Brownish orange
3	Kalium (II) tiosianat	1	0,57	Red

Identification of reagent in the reagent showed orange Dragendorf enduring, red to brown color on the reagent red in Mayer and potassium (II) thiocyanate. Results reactions that occur in these reagents with a single spot on the fraction 7 showed that the single spot into the group of alkaloids, with a wavelength of 255 nm.

Characterization results :



Data of UV-visible Spectrophotometer



Spectra data infrared(IR)

Data IR Spectrophotometer

Identification of an ultraviolet-visible spectroscopy showed a maximum absorption at a wavelength of 255 nm while the IR spectrum showed absorption at wave numbers385.56 cm^{-1}, 3741.98 cm^{-1}, 3673.11 cm^{-1}, 3650.25 cm^{-1}, 2923.60 cm^{-1}, 2853.07 cm^{-1}, 2361.42 cm^{-1}, 2336.11 cm^{-1}, 1771.96 cm^{-1}, 1740.21 cm^{-1}, 1700.04 cm^{-1}, 1652.47 cm^{-1} 1539.87 cm^{-1}, 1514.28 cm^{-1}, 1456.34 cm^{-1}, 1016.34 cm^{-1}, 671.77 cm^{-1}. spectral images can be seen above.

From the research showed that the fraction of to-7 belong to this class of alkaloids with a maximum wavelength of 255 nm and the infrared spectrum shows interprentasi functional groups (OH), alkyl (CH), amen group (-N =), secondary amen group (NH), clusters of nitrogen (N), Alken group (C = C) aromatic and supported from the results of the chemical components of reagent groups showed alkaloid class of compounds.

Characteristic of chemical compound one of n-heksane fraction according by UV-Vis Spectrofotometry at long wave 256 nm and infrared indicated as group of hydroxyl (OH), alkyle (C-H), amine (-N=). Amine secondary (NH), nitrogene (N), alkene (C=C). single spot exhibited positive reaction to reagent specifics from group of alkaloid compound.

CONCLUSION

Asian pigeon wings leaf (*Clitoria ternatea* L.) is included in family Papilionaceae. Microscopic of leaf indicated epidermis type actinositic, trichoma glanular, and transversal bundle is opening collateral. Characteristic of chemical compound of n-hexane fraction showed group of alkaloid compounds.

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A REVIEW: FEASIBILITY STUDY OF EUGENOL HERBAL EXTRACT AS POST GINGIVAL CURETTAGE TREATMENT.

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Abstract: Most all medicamentosa used in dental practices are imported. This condition may increase the cost of dental health services. Gingival pack is one of that medicamentosa, which widely used in post gingival curettage. Technically, gingival pack is made by mixing ChKM (Chlorophenol Camphor) and calsium hydroxide. In this case, ChKM may act as antiseptic and antiinflammatoric agent, and calcium hydroxide as a granule forming agent. Several herbal extract consisting some phenol have bqcteriocidal effectReferent by denaturing the protein membrane of microorganism and increasing its permeability. Some of this phenol are allylkatekol, cardinene, charvacol, charyophyllene, chavibetol, chavichol, cineol, eugenol, eugenol methyl ether and pirochetechin. We concluded that several herbal extracts could substitute ChKM as a gingival pack. We also known that this extract is more cost efficient than ChKM.

Keywords : herbal extracts, Chorophenol camphor, gingival pack, cost efficient, gingival curettage.

INTRODUCTION

Most of medicamentosa used in dental treatment are imported materials. This condition was a significant factor which taken role of costly dental health services in Indonesia. One of that medicamentosa was the eugenol, which mostly formulated as liquid. There were many application in dental health services. One of this application was the gingival pack in gingival curettage treatment. The COE pack was the brands name of gingival pack, that familiar in dentistry.

Gingival curettage was the treatment of periodontal disease. Technically, this treatment done by scrab off the gingival walls of periodontal pocket. The objective of the treatment were discharging necrotic tissues and inflammation products, and made it's refirmed to cemento-enamel junction of the affected teeth. In this case, gingival pack act as fixation agent to assist refirmed of the gingival tissue.

Refferent study stated that several Indonesian herbs, such as piper betle, pomegranate rind, bay leaf (Eugenia polyantha wight), morinda citrifolia linn, containing many kind of phenol campound, including eugenol (Sukanto, 2003; Agustin, 2005; Sumono and Wulan, 2008; Khoswanto, 2010). Phenol compounds were find also in basil, betle, merica, guava, apple, cinnamon. Some data described that phenol compounds were bacteriocide as well as anti-fungal effect, and be able to extracted from it's herb, and formulated similarly to the gingival pack. According to appropriate technology, futher study probably give some reason to formulate this phenol, so this formula could directly used. As suggested by Sumono and Wulan (2008), bay leaf (Eugenia polyantha wight) extract could be used as basic of therapie, or basic ingredients of treatment. directlyViewed from the sources of eugenol, it was assumpt that eugenol derived from Indonesian herbs could cut off the cost of dental health services in Indonesia.

The aim of the present review was to address following problems:

1. Are the herbal extracts containing some kind of compounds were medically and farmacologically suitable used as the gingival pack in the treatment of gingival curettage ?.

2. Related with the suitability of herbal extracts, the second question was the efficiencies of the cost of the gingival curettage treatment. Were the implementation of herbal extracts means cost efficient of gingival curettage treatment. Thus it would be decrease the cost of dental health services?

DISCUSSION.

Gingival curettage treatment was the treatment of periodontal disease. It was already known that periodontal disease was the most prevalent disease of the teeth and oral mucosa in Indonesia. Recent data stated almost 70% of Indonesian people affected by periodontal disease, ranged from mild to severe (Rubianto and Chiquita, 1996).

Gingival curettage treatment done by scrabing off the inner wall of the infected gingiva till reached the healtier layer of the gingival wall, and root planning of the affected teeth. By this way, the dentist could discharge necrotic tissue (periodontophatic bacteria) and inflammation products, and made it's area clean and delicate. This was the preferable condition of epithelial attachment development. The epithelial attachment was the epithel layer that tight in firmly to the cemento-enamel junction of the teeth, and taken role as the barrier from many stimulate to the periodontal ligament. Thus the epithel attachment was the indicator of healthy gingival. (Kurihara, 1996; Witjaksono, 2006). In the healthy gingiva, there was about 1,5 to 2 mm depth of the gingival sulcus. This sulcus was measured from the existence of epithelial attachment to the gingival crest. It was mean that the existencies of epithelial attachment were the important point of the healty gingiva.

In case of developed and refirmed of epithelial attachment, instead of healthy area, immobilized gingival walls was the favorable condition in fixing and refirming the epithelial attachment. Immobilization would be increasing adhesivity of blood cloth to the surfaces of the root (Rubianto ang Chiquita, 1996). According this condition, gingival pack after gingival curettage treatment was the important role. Gingival pack would immobilized the gingiva, and give some changes to develope their epithelial attachment. Based on this concept, the ability to immobilized was the fullfiled ability of the gingival pack. While on the other side, eugenol in the gingival pack was taken role as bacteriocide, anti-inflamation and analgetics.

Many dentist used fabricated gingival pack such as COE Pack. While others used gingival pack, prepared by mixing eugenol or ChKM liquid with calcium hydroxide in powder formula. Sometime dentist also used zinc oxide powder to replace calcium hydroxide especially for better plasticity. This formulation aim to be plastic mass, and become harder for several times. So this pack could immobilized the gingiva. This plasticity aim to be easier to insert this mass on the interdental area (Ardanari, 2005; Zubaidah, 2006).

The active ingredient of ChKM were para-monochlorophenol, camphora and menthol. According the phenol's effect, ChKM act as bacteriocide, anti-fungus and anti-inflammation, through denaturation of protein membrane of the cells, and increase it's permeabilty. Futher data explained that some phenols, especially terpenoids and flavonoids could promote the proliferation process of fibroblast and collagen production (Sumono and Wulan, 2008; Khoswanto, 2010). It was mean that terpenoids and flavonoids were the ingredient significant to the new epithelial attachment.

The active ingredient of ChKM was para-mono chlorphenol camphora and menthol. Other preparations were also often used ZOE (Zinc Oksida and Eugenol). Some properties of compounds functioning as anti-inflammatory, analgesic , bacteriocide, and gingival tissue fixation in order to immobilized the gingival tissue to build anew ephithelial attachment. Herbal extracts characterized a sticky nature and still contain resin. The extract containing phenol compounds and their derivatives are betel, cloves, cashew, cinnamon, piper, apples and some plants that contain these

compounds in the extract. The extracts are generally extraction by common solvent, using percolation method.

The extracts using on the treatment, the active ingredients was not on the form of essential oil, but extracted by ethanol, than evaporated. These extracts will be a viscous extract form. Although this condensed have shortcoming, but the linkage characteristic would give some advantage to increase the power of cohesion. On the other hand total extract would also be an adventage in the use of simple formula whereby the exsistence of resin that usually interfere in formula would increase setting time and improves cohesion. In these compounds are still created as separate dosage form of active ingrediants with the addition of vegetables extracts and olive oil, CaOH or ZnO in powder/granules form which will be mixed as gingival pack.

On the side of formulation, the extraction of eugenol performed as the total extraction. Theoritically, total extract have some advantages especially on the fisico-chemical aspect of gingival pack. The existance of resin on the total extract would increasing setting time, and improves cohesion. These two factors were the fullfiled factors for it's function of the gingival pack.

CONCLUSIONS

1. Herbal extract derive from Indonesian plants, were medically and farmacologically suitable for gingival pack in the gingival curettage treatment.
2. Based on the source and preparation, the application of herbal extracts on dental treatment in Indonesia, especially gingival curettage would be decrease significantly to the cost its treatment.

RECOMMANDATION

The active ingredient was formulated from herbal extract, and performed as paste or liquid, combining with calcium hydroxide or zinc oxide for the gingival pack.

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FORMULATION OF COLA (*COLA NITIDA* A.CHEV) EFFERVESCENT TABLET

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Abstract: Cola (*Cola nitida* A.Chev) have an effect of central nervous stimulant, usually formulated as soft drink. To lowering production and shipping cost, cola was formulated as effervescent tablet. The aim of this research was to know concentration of PVP K-30 as binder in tablet effervescent formulation. Cola extract was obtained from percolation by alcohol 95% of cola seed powder. Effervescent tablet of cola extract was made by wet granulation with concentration of PVP K-30 as binder was 1%, 3%, and 5%. Tablet weight was 1 gram contain of cola extract 31,25%. Evaluation of granule quality consist of moisture content, flow rate, repose angle, and compressibility, whereas tablet quality was hardness, disintegration time, friabilitas and uniformity of weight. Based on the data, formula with 1%concentration of PVP K-30 give the better result.

Keywords: cola (*Cola nitida A.Chev*) seed, effervescent tablet, PVP K-30

INTRODUCTION

Cola (*Cola nitida* A. Chev) was first consumed by the people of Africa by way of chewed as stimulantia, resisting hunger and to reduce fatigue (Ettarh *et al.*, 2000). Traditionally, a cola is used as a medicine and tonic headache with approximately five grams of cola nut powder 0.5 cups brewed with hot water, cooled and filtered, the filter taken at the same time (The Ministry of Health of the Republic of Indonesia, 1991). Pharmacological behavioral tests on rats showed cola nut stimulantia effect on the central nervous system (Ettarh *et al*., 2000).

Cola nut contains the alkaloid xantin (kofein 1 to 2.5%, theobromine, theophylline), glucoside (kolantin, kolatein), carbohydrates, fats, tannins, red pigments (antocyanin) and a lipase (Tjitrosoepomo, 1994).

Cola is generally formulated as a non-alcoholic beverages are very popular around the world. One drawback perfomed liquid (drink) on the market are relatively expensive cost of transportation is therefore necessary to find another alternative dosage form that is more compact so that it can reduce production costs. One alternative is made with consideration of effervescent tablets is quite easy and practical use by adding water and then crushed tablets will release CO_2 gas that will provide fresh taste when drunk. CO_2 gas bubbles will also accelerate the dissolution of the ingredients in cool water. Transportation costs relative tablet cheaper than liquid preparations.

Effervescent tablet is a tablet that can release gas after contact with water. Gas bubbles that are a result of chemical reaction between the acid and alkaline. Acid is often used is citric acid, and sodium bicarbonate as base. Citric acid is easily soluble in water, high-strength acid, can form good granules and have the easy flow properties. Sodium bicarbonate can dissolve completely in water, is inexpensive, commercially available and are also easy to flow (Lieberman *et al*., 1989).

The selection of the binder type and concentration is critical for the formulation of effervescent tablets. Binder that can be used must be soluble in water so that the resulting solution should be clear then in this study used PVP K-30. Concentrations commonly used are 1-5%, low concentrations causes fragile tablets whereas high

concentrations cause the tablet difficult destroyed. Therefore in this study used concentrations of 1, 3dan 5%. Concentration ratio was carried out to determine the concentration of PVP K-30 is good enough as a binder for the effervescent tablets in terms of physical quality test tablets.

MATERIAL AND METHOD

Equipment: Percolator, oven (WTB Binder Germany), TLC plates (Merck, Germany), porcelain crucible, a water bath scales, Moisture Balance, thermometer, UV light 254 nm and 366 nm (Camag TLC Scanner, Germany), chamber (Merck , Germany), microscope, single punch tablet machine (RRC), Schleuniger Hardness Tester, Tester Friabilator Erweka, Brookfield viscometer and other supporting equipment.

Material: Beans cola or *Cola nitida A. Chev,* rubra varieties obtained from the Medicinal Plant Research Institute (BPTO) Tawangmangu Solo. Additional materials unless otherwise stated have a pharmaceutical grade which include citric acid (Brataco), sodium bicarbonate (Brataco), PVP K-30 (BASF, Germany), lactose (DMV International, Netherlands), sodium benzoate (Wuhan Organic Chemicals, China), polyethylene glycol 6000 (Japan Sino Chemical, Japan), alcohol 96% (PT Aneka Kimia Nusantara, Indonesia).

Standardization of crude drug quality

Crude drugs used before the standardization of research first conducted including: test appearance, qualitative, ash content, water content, loss on drying.

Making Extracts

To determine the concentration of alcohol used as solvent for the extraction of orientation first performed using a concentration of 30, 50, 70 and 96%. Results indicate the orientation of 96% alcohol could increase the number and clarity of stains or stains the best thickness so that the subsequent extraction process using 96% alcohol solvent. Crude drug that has been milled into powder weighed as much as 2 kg, moistened with alcohol solvent 96% during the three hours. Moist powder which is inserted percolator and add 96% alcohol until the residue is dripping clear or residues have not left their mark on the paper if evaporated. Perkolat generated evaporated on a water bath at a temperature not exceeding 50^{o}C to obtain viscous extract, then added lactose as filler and dried in an oven at a temperature of 50^{o}C to obtain dry extract. The resulting powder must be crushed and sieved with 100 mesh sieve. The resulting dry extract was weighed and used as active ingredients for effervescent tablet formulations. Dry extract produced prior to use standardized test done first.

Extracts Weight Determination for Each Tablet

Cola nut powder required for a headache remedy and tonic: 5 g / day (Dep Kes RI, 1991). Cola nut powder that is extracted is 2000 g, equivalent to 500 g of dry extract. Dried extract of cola nut 500g/2000g X 5g = 1.25g.

To be not too large tablets, made four tablets taken at once so that the content of dry extract per tablet = 312.5 mg. Each formula is made three batches and each batch of 200 tablets.

Table 1. Formula effervescent per tablet

No	Component	Formula A(g)	Formula B(g)	Formula C (g)
1.	Dry extract	0.3125	0.3125	0.3125
2.	Citric acid	0.25	0.25	0.25
3.	Na bicarbonate	0.325	0.325	0.325
4.	PVP K-30	0.01	0.03	0.05
5.	Sucrose	0.0515	0.0315	0.0115
6.	PEG 6000	0.05	0.05	0.05
7.	Na Benzoate	0.001	0.001	0.001

Production Method

Granulation method used in the study were wet granulation method with non-reactive liquids (alcohol). Drying of granules made in the oven at a temperature of not more than 50 ° C within 18 hours to 24 hours. Granulation process is divided into two parts: the part of acid and alkaline. Section consists of a mixture of acid half of the dry extract with citric acid which has been finely crushed, plus half of the sodium benzoate and a half parts of sucrose, plus half of the PVP K-30, then 96% alcohol added to the mass formed granules, sieved with a sieve 18 mesh, is inserted in the oven at 500C until dry, sieved again with a 20 mesh sieve. Base part consists of a mixture of half of the dry extract with sodium bicarbonate, plus half of the sodium benzoate and a half parts of sucrose, plus half of the PVP K-30, then 96% alcohol added until a granular mass, sieved with 18 mesh sieve, was added to in the oven at 500C until dry, sieved again with a 20 mesh sieve. Part of acids and bases are mixed until homogeneous parts in a hot mortar, placed in 500C oven until dry, then test the physical quality of granules which include: Determination of moisture content, flow time, dwell angle and compressibility of granules. Granule eligible added PEG 6000 which was 100 mesh sieved and then mixed homogeneous done tabletasi using a single punch tablet machine. Tablet was tested and the physical quality of the data analysis using anova followed, when there are significant differences continued LSD (Least Significant Difference Procedure).

RESULTS

Standardization of crude drug test results as shown in Table 2 and the dried extract in Table 3.

Table 2. Standardization of Crude and Kola Seed Extract (Dep Kes RI, 1980)

No	Test	Standard	Result	Note
1	Descriptions of Form: Color: Taste: Odor:	 Powder Chocolate Slightly bitter Typical colas	 Powder Chocolate Slightly bitter Typical colas	 + + + +
2	Identification + 5 drops of H2SO4 + 5 drops of concentrated HCl + 5 drops of 5% NaOH + 5 drops of 25% NH4OH + 5 drops of FeCl3	 Light brown Light brown Blue Yellow brown Blue	 Light brown Light brown Blue Yellow brown Blue	 + + + + +
3	Ash content	Not more than 4%	3,6%	+
4	Loss on Drying	Not more than 10%	8,7%	+
5	Moisture Content	Not more than 10%	8,0%	+

Table 3. Standardization of Dry Extract and viscosity of viscous extract.

No	Observation	Result
1	Descriptions of Form: Color: Taste: Odor:	 Powder Brownish white Slightly bitter Typical colas
2	Viscosity	9.83 ± 0.153 Poise

The plant material unwound before use research conducted at the Research Institute of Medicinal Plants (BPTO) Tawangmangu Solo, to know that the bulbs used in this study according to the sample, which was intended. Results showed all the bulbs standardized examination that meets the requirements of Materia Medika feasible to use for further research. The product quality is largely determined the quality of crude drugs used. Tests for moisture content specified in relation to the possibility of mildew and mold growth, degradation by the enzyme reaction of the active substance in the bulbs. Microorganism growth does not occur if the water content in the bulbs is less than or equal to 10% (Soetarno & Soediro, 1994).

Extraction process used in this study with percolation as cola nut and contains glucoside kolatin, kolatein unstable on heating. Granulation method used is wet granulation with a non-reactive alcohol solvent with a consideration to avoid the occurrence of effervescent reaction and avoid high temperatures during drying granules thus the possibility of decomposition of active ingredients can be avoided.

Table 4. Granule Quality Test Results

No.	Test	Standard	Formula A	Formula B	Formula C
1	Moisture Content	≤10 %	7.04 ± 0.129	7.13±0.085	7.72±0.183
2	Flow rate	≤ 10 detik/100g	9.46±0.338	9.63±0.165	9.79±0.150
3	Repose Angle	25 – 40^{o}	33.47±0.692	36.53±0.930	39.20±0.055
4	Compresibility	5 – 15%	12.52±0.554	11.65±0.353	10.57±0.351

Granule quality test results generated as shown in table 4 shows the formulas A, B and C have the flow properties and compressibility good so hopefully the problem does not occur in the process of granule compression. Tablet was tested quality as shown in Table 5.

Table 5. Tablet Physical Quality Test Results

No.	Test	Standard	Formula A	Formula B	Formula C
1.	Weight	F. Ind. III	1002.86±0.525	1003.98±4,268	1006.00±3.486
2.	Uniformity	≥ 8 Kgf	8.85±0.095	15.60±0.163	22.90±0.089
3.	Hardness	1 – 2	1.50±0.061	2.01±0.172	2.11±0.135
4.	Disintegration	menit	0.80±0.055	0.63±0.050	0.48±0.020
5.	Time	< 1.0%	6.97±0.153	7.04±0.147	7.26±0.197
	Friability	<10%			
	Moisture Content				

Formula weight uniformity test results A, B and C were 20 tablets no weight deviations greater than 5% according to the requirements of Pharmacopoeia Indonesia III, the results of the above because all the formulas have a good flow properties so that the granules into the hole filling the die is relatively uniform.

Tablet hardness test results of all formulas have a tablet hardness over 8 kgf (Pharos, 1992). The harder the better an effervescent tablet disintegration time and the vulnerability of origin requirements. Anova test results with $\alpha = 0.05$ showed no significant difference because the calculated F> F table (11399.23> 5.14). Least significant difference test showed violence tablets Formula A <B Formula <Formula C.

Test results of disintegration time qualifying while the formula A Formula B and C are not eligible (Lieberman et al., 1989). Tablet friability test results showed all eligible formula is less than 1.0% Banker & Anderson, 1994). Anova test results with $\alpha = 0.05$ showed no significant difference because the calculated F> F table (47.90> 5.14). Least significant difference test showed the fragility of Formula A> Formula B> Formula C. The water content test effervescent tablet formulation meets all the requirements of less than 10% (Soetarno & Soediro, 1994). Viewed from the physical quality tests show the resulting effervescent tablets Formula A with the concentration of PVP K-30 as much as 1% is the best formula.

CONCLUSION

Based on the results cola effervescent tablet formulations with different concentrations (1, 3 and 5%), PVP K-30 as a binder, it can be concluded that the formula that using PVP K-30 with a concentration of 1% is the best formula.

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ANTI-INFLAMMATORY AND ANALGESIC EFFECT OF ETHANOL EXTRACT OF GEDI LEAF*(ABELMOSCHUS MANIHOT* L.MEDIK*)* COMPARED TO DICLOFENAC SODIUM IN PAW EDEMA OF RAT

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Abstract : Inflammation is a life tissue reaction to all kinds of injury and a protective tissue response that acts locally. Gedi leaves (*Abelmoschus manihot* L. Medik) is a plant which is used as an anti-inflammation. This research was conducted in order to know whether ethanol extract of gedi leaves has anti-inflammation and analgesic effects and to compare them with diclofenac sodium. This experimental study employed 30 Wistar female rats, meeting the inclusion criteria. They were divided into 6 groups (n=5). All groups were treated by oral administration, one hour after lambda carrageenan. Groups I and IV, and negative control, only received 1 % of CMC solution. Groups II and V, and positive control, took Diclofenac Sodium with a dose of 4.5 mg/kgBW. Groups III and VI, and testing group, received ethanol extract of gedi leaves 36 mg/kgBW. The parameter observed in group I,II, III, was the percentage of increase of edema volume. This was measured every hour, commencing from the first until the 6th hour from the rat paw tissue. MDA level was measured only at the 6th hour after lambda carragenan.. The parameter observed in groups IV, V, VI, was hyperalgesia prevention. Data were analyzed by Oneway Anova and Duncan test. This research showed that ethanol extract of gedi leaves with a dose of 36 mg/kgBW, could significantly reduce the increasing percentage of edema volume, prevent the hyperalgesia, and decrease the MDA level ($p<0,05$) compared to negative control, and has effectivity as good as natrium diclofenac. The study concluded that ethanol extract of gedi leaves has anti-inflammation effect, analgetic effect, and has effectivity as good as diclofenac sodium.

Keywords : gedi leaves, diclofenac sodium, lambda carrageenan, inflammation, analgesic, edema volume of rat paw, MDA, hyperalgesia

INTRODUCTION

Inflammation is a living tissue reaction against all forms of injuries. In the process of inflammation there are active production of inflammatory mediators, which causes infiltration of leucocytes to areas of inflammation, and then releases a number of free radicals. Reaction of free radicals with cell membrane phospholipids produces various products, such as malondialdehyde (MDA) (Mitchell and Cotran, 2003; Marks *et al.*, 2000). Inflammation is a kind of body defense, but this process can trigger or exacerbate variety of diseases, thus requiring anti-inflammation medicine (Gupta *et al.*, 2003; Mycek *et al.*, 2001).

Patients treatment with inflammation based on two main objectives, namely to overcome the pain and slow or stop the process of tissue damage. The use of NSAIDs often causes serious side effects, so that the experts continue to seek the new alternative of anti-inflammatory drugs which is effective and secure. One alternative therapy that mostly is tried and developed is the use of various medicinal plants.

Among many medicinal plants, gedi leaves (*Abelmoschus manihot* L. Medic.) is one of the medicinal plants have been used widely in society. The chemical composition of gedi leaves which is supposed to have anti-inflammatory effects are flavonoid and tannin compounds. Some researches represent that gedi leaves contain flavonoid

compounds which are isoflavones, chlorogenic acid, steroid compounds and triterpenoid (Rao *et al.*, 1990; Utari and Toto, 2007; Trisnabudi, 1996; Mitchell and Cotran, 2003). This herb is supposed to have anti-inflammation effect because of containing flavonoid and tannin compounds.

In pre elementary study, using ethanol extract of gedi leaves 36 mg/kg, 72 mg/kg, and 144 mg/kg bw, proved that the doses which have anti-inflammatory effect is the first one. Analgesic and anti-inflammatory effects of gedi leaves research has not been done before. This research is purposed to determine the effect of ethanol extract of gedi leaves to the prevention of edema, hyperalgesia, and increasing MDA levels, and compare it with diclofenac sodium.

METHOD

The subject of this research is 30 wistar rats, meeting the inclusion and exclusion criteria. The first one includes: (1) Female rats, (2) healthy and active movement rats, which are characterized by clean and no loss hair, no injuries, active, having run away and easily started response, (3) Weight about 180-200 grams, (4) Age approximately 12 weeks. The second one includes: (1) Weight loss during adaptation is more than 10% of early weight, (2) Looks sick during the period of adaptation.

Approximate 18 hours before experiment, the rats fasted but given water to drink. Thirty white rats are divided into six groups. All groups were given the test and control substances by oral and 1 hour later were induced inflammation using karagenin-λ. Group I and IV (negative control) are only received carboxyl methyl cellulose (CMC) about 1%. Group II and V (positive control) are received diclofenac sodium 4.5 mg/kg. Group III and VI (test group) are received gedi leaves ethanol extract of 36 mg/kg. In group I, II, III, the parameter measured is increasing of edema volume (measured every hour starting at first until sixth hour) and MDA levels of paw rats tissue (at sixth hour). In group IV, V, VI, the parameter is time of paw pulling rats to rate hyperalgesia.

This research was carried out in several places, extracts was made at the Laboratory School of Pharmacy ITB Bandung, maintenance of rats, anti-inflammatory testing with pletismometer and hyperalgesia at the Laboratory of Pharmacology RSHS/Medical faculty of Padjadjaran University Jl. Pasirkaliki Bandung, MDA examination at the Laboratory of Medical Research Unit-UNPAD RSHS Jl. Pasirkaliki Bandung. The research was held from May until August 2009.

Statistical analysis used was statistical test of one-way ANOVA, followed by Duncan's multiple range guard with 95% confidence level ($p < 0.05$). The analysis was done using SPSS statistical computer program 16:00.

RESULT

The average results of the percentage of increase of edema volume from the rats model of inflammation per hour in group I to III can be seen in Table 1.

Table 1 Average Percentage of Increasing Edema Volume Each Group

Group	The Average of Edema Volume Increasing Percentage (%)±SD					
	1 hour	**2 hours**	**3 hours**	**4 hours**	**5 hours**	**6 hours**
I	0	28,00±3,29	46,08±9,59	53,94±3,66	53,94±3,65	53,94±3,65
II	2,20±4,92	12,56±1,17	17,56±6,89	19,76±5,97	24,80±7,62	34,58±7,78
III	2,50±5,59	21,9±5,41	34,40±11,23	34,40±11,23	34,40±11,23	48,88±9,19

Notes : I : Negative Control (CMC 1%)
II : Positive Control (Na Diclofenac 4,5 mg/KgBB)
III : Testing Group (gedi leaves ethanol extract 36 mg/KgBB)

Increasing edema volume appeared since first hour and reached a peak at third hour, and edema volume continued to increase until sixth hours after the induction of carragenin-λ.

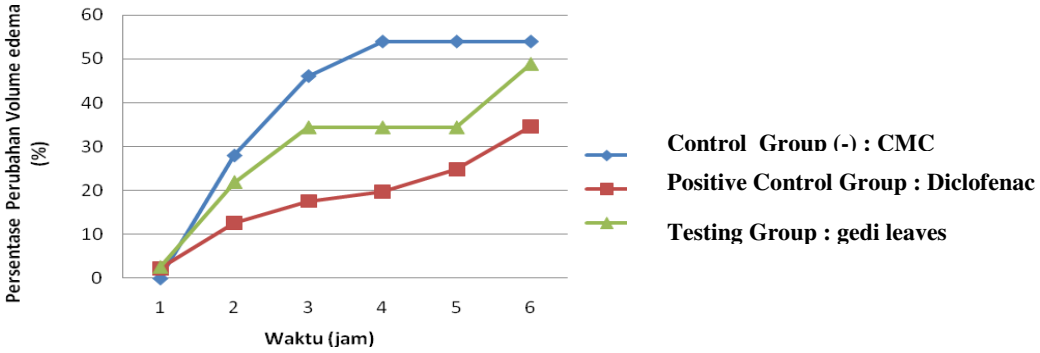

Figure 1 Average Percentage of Increasing Edema Volume Each Group

Based on Figure 1, it can be seen that there are differences in the average percentage increase in edema volume between the groups. The highest percentage was found in group I, that is negative controls, whereas the lowest was found in group II, that is a positive control. Group III: the group that got gedi leaves ethanol extract in between negative and positive controls.

Differences between groups were analyzed further to determine whether the difference was significant statistically. Increasing edema volume was tested by *One Way* Anova test. Kolmogorov-Smirnov normality test and homogeneity with Levene test showed that the data are normal and homogeneous. Through the One Way Anova test found significant differences ($p < 0.05$) between the groups, then followed by Duncan mulitple advanced test.

At first hour, group I, II, and III are in one subset, that is *subset 1*. This happens because in the first hour the changing of edema volume has not occurred in most samples.

At second hour and fourth hour, showed that ethanol extract of gedi leaves 36 mg/kg has significant difference with positive control and negative control group. This extract was able to decrease the edema significantly better than negative control, but its ability is lower than diclofenac.

At fifth hour, showed that there were no significant differences between ethanol extract of gedi leaves group of 36 mg / Kg BW with positive control group, and significantly different from negative control group.

At third hour and sixth hours, showed that there was no significant difference between ethanol extract of gedi leaves group of 36 mg / KgBW with negative control, and significantly different from positive control.

Hyperalgesia

Measurement result of rats' left paw and expressed as paw pulling time in seconds.

Table 2. Paw Pulling Time (seconds)

Groups	Average of Paw Pulling Time (in seconds)±SD					
	1 hour	2 hours	3 hours	4 hours	5 hours	6 hours
IV	2.52±0.23	2.46±0.34	2.14±0.36	2.46±0.36	2.14±0.36	2.46±0.39
V	6.24±3.02	5.94±2.56	6.42±3.02	6.34±3.09	6.34±3.08	6.10±2.36
VI	6.68±4.18	5.72±2.82	6.68±4.18	6.82±4.11	6.68±4.18	5.72±2.82

Notes : IV : Negative Control (CMC 1%)
V : Positive Control (Na Diclofenac 4,5 mg/kg bw)
VI : Test Group (gedi leaves ethanol extract of 36 mg/kg bw)

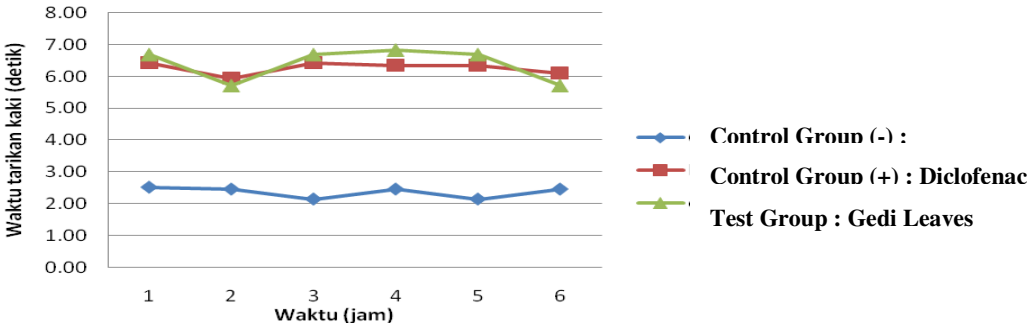

Figure 2. The Graph of Average Time of Paw Pulling (in seconds)

From the table and graph above, it can be seen that average score of fastest time of paw pulling occurred in group II (positive control group) and longest time occurred in group I (negative control group).

Paw pulling time becoming hyperalgesia assessment indicators was tested by One Way Anova test. Kolmogorov-Smirnov normality test and homogeneity with Levene test showed that the data are normal and homogeneous. Through one way Anova test, data showed significantly different among three groups ($p < 0.05$) at second hour and sixth hour.

Through Duncan's further multiple test, known at second and sixth hour, gedi leaves ethanol extract does not have significant differences with positive control group, but differed significantly with negative control group.

MDA Levels

The examination of MDA levels from paw edema of rats tissue was done at 6 hours after induction of carragenin-λ. Examination with *Thiobarbituric Acid Reactive Substance* (TBARS) method, which is then measured using spectrophotometry at wave length of 532 nmol/ml.

Tabel 3. Average of MDA Level (nmol/ml)

Groups	Average of MDA Level (nmol/ml)±SD
IV (CMC)	304.29±58.59
V (Diclofenac)	167.00±29.37
VI (Gedi Extract)	194.28±18.29

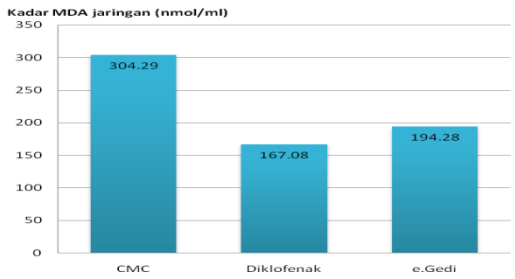

Figure 3. Average of MDA Level (nmol/ml)

According to the table and Figure 3 can be seen that there are differences in MDA levels between the groups. The highest MDA level was found in group I (negative control group), while the lowest was found in group II (positive control group).

The differences were then analyzed statistically using One Way Anova test. Previous by Kolmogorov-Smirnov normality test and homogeneity test with Levene note that the data are normal and have homogeneous variance.

In One Way Anova test found significant differences ($p < 0.05$) between the groups. Duncan's multiple further test was then done and showed that there was no significant difference between MDA levels of group II (positive control group = diclofenac) and III (gedi leaves ethanol extract). MDA level difference significantly found between groups II and III with group I (negative control group = CMC).

DISCUSSION

Edema Volume

Clinical manifestation of inflammation occurs through three main processes, namely local vein vasodilatation, increased capillary permeability with leakage of fluid into the interstitial space, and the migration of leucocytes from capillaries into surrounding tissues (Mitchell and Cotran, 2003).

Edema (swelling) caused by exudates (fluid and inflammation cells) that accumulate in areas of inflammation due to the influence of inflammatory mediators. This is caused by increased permeability of vein due to the influence of inflammatory mediators such as vasoactive amines, complement (C3a, C5a), Leukotrienes C4, D4, E4, and *Platelet Activating Factor* (PAF) (Mitchell and Cotran, 2003).

Important inflammatory mediator is prostaglandins. Prostaglandin synthesis is initiated by the stimulation of cell injury that causes the release of arachidonic acid which is at the cell membrane phospholipids. This process is aided by Phospholipase A, Phospholipase C and lipase diglycerides. Arachidonic acid would oxygenated through 4 different routes, namely cyclooxygenase, lipoxygenase, P450 epoxygenase, and isoprostane. Cyclooxygenase and lipoxygenase is the main route that play a role in the inflammation process (Kidd, 2001).

Ethanol extract of Gedi leaves has anti-inflammatory effect in suppressing the occurrence of edema because of active substances contained in gedi leaves, including flavonoid and tannin.

Flavonoid effects as anti-inflammation is through the inhibition mechanism of cyclooxygenase and lipoxygenase pathway, which in turn decrease arachidonic acid metabolism and biosynthesis of eicosanoid, such as prostaglandin. Against leucocyte activity, flavonoid has ability to inhibit neutrophil degranulation, decrease the number of leucocytes, which is associated with total decreased of complement level in serum (Suralkar, 2008). Flavonoid also has ability to reduce vascular permeability (Alan and Miller, 1996).

Beside flavonoid, the other content of gedi leaves is tannin. It is shown to have anti-inflammation effect partly derived from its antioxidant activity, that is ability to destroy free radicals better than the phenolic compounds with low molecular weight (Rimando and Chavez, 2008; Middleton *et al.,* 2000). Flavonoid and tannin contained in ethanol extract of gedi leaves cause them have effect in depressing edema volume induced by carragenin-λ.

Edema suppression effect of gedi leaves ethanol extract at second and fourth hour is not better that diclofenac sodium, but this effect is significantly better than negative control group. This can be caused by inhibition of prostaglandin biosynthesis induced by flavonoids was lower than the inhibitory effect induced by diclofenac. At fifth hour edema suppression effect by ethanol extract of gedi leaves equivalent to diclofenac. This can be caused by the levels of flavonoid and tannin in ethanol extract of gedi leaves, culminating in the blood and giving maximum effect in ederma prevention. At sixth hour, ability of ethanol extract of gedi leaves in inhibiting edema increased equivalent to negative control, and lower than diclofenac effect. This can be caused by flavonoids and tannins level in the blood decreased.

Hyperalgesia

Inflammation pain is caused by the mediators released during tissue damage. These are bradykinin, histamine, serotonin, nitric oxide (NO), and acid products such as prostaglandins and leukotrien (Kidd, 2001)

Prostaglandin causes increasing nosiseptor sensitization, by lowering the activation threshold for sodium channel. Leukotrienes B4 which is lipoxygenase product works indirectly by stimulating the release of 8R, 15S-diHETE of polymorphonuclear leukocytes. 8R, 15S-diHETE causes hyperalgesia directly by lowering threshold of C fibers to heat and mechanical stimulus (Hagerman, 2002).

Flavonoid in ethanol extract of gedi leaves has effect in reducing hyperalgesia through mechanism of cyclooxygenase and lipoxygenase point inhibition in arachidonic acid metabolism. This leads to inhibition of biosynthesis of prostaglandins and leukotrienes B4 which is responsible in pathophysiology of pain and hyperalgesia.

From One Way Anova Test, it's known that the data having significant average difference ($p < 0.05$) are the data at second and sixth hour. Ethanol extract of Gedi leaves has hyperalgesia prevention effect comparable significantly with diclofenac, and differs significantly from negative control group.

This effect is obtained through active substances effect, especially flavonoid in inhibiting cyclooxygenase and lipoksigenase path leading to the inhibition of prostaglandins and leukotrienes biosynthesis. This effect is comparable with the effect of diclofenac. It inhibits hyperalgesia through inhibition of cyclooxygenase path.

MDA Levels

Clinical manifestation of inflammation occurs through three main processes, namely local vein vasodilatation, increasing capillary permeability with leakage of fluid into the interstitial space, and migration of leucocytes from capillaries into surrounding tissues (Mitchell and Cotran, 2003).

Leucocytes that have migrate to the tissue and then perform a series of processes, such as phagocytosis. During phagocytosis occurred release of reactive oxygen species by macrophages, neutrophils, and eosinophils are activated. Activation of NADPH oxidase, which is expected to occur on the outer side of plasma membrane, triggering an explosion accompanied by the formation of superoxide respiratory generating other reactive species. In small amounts, this ROS can increase chemokine, cytokine, and adhesion molecules resulting in increased production of inflammation mediators. In large quantities, it cause endothelial damage resulting in increased vein permeability and thrombosis, activation of protease and antiprotease enzymes inactivation, causing excessive solution from the extracellular matrix, and cell damage directly (Mitchell and Cotran, 2003).

MDA is lipid peroxidation indicator of the most frequently used, and is a potential biomarker of oxidative stress and cell damage (Dray, 1995). Malondialdehyde can also be generated from the metabolism of arachidonic acid into prostaglandin. It is derived from the breakdown of PGH2, an unstable compound on PG biosynthesis. In addition, some enzymes such as thromboxane synthase would convert PGH2 to thromboxane A2, hidroksiheptadekatrienoik acid (HHT), and MDA in the ratio 1:1:1, while prostacyclin synthase will produce MDA from PGH1 (Nielsena, 1997; Plastaras *et al.*, 2000).

Diclofenac can inhibit the formation of MDA, through its effect as an anti-inflammation that inhibits prostaglandin synthesis through inhibition of cyclooxygenase pathway, so MDA as a byproduct of prostaglandin biosynthesis is also inhibited (Plastaras *et al.,* 2000)

Gedi leaves ethanol extract has active substances, they are flavonoid and tannin that have antioxidant effects. As body protector of SOR, flavone and catechin seems to be the most powerfull antioxidants (Wilmana, 1995; Nijveldt *et al.*, 2001)

Body's antioxidant defense mechanism is mediated enzymes such as superoxide dismutase, catalase, and glutathione peroxidase, while also Co-enzymes such as glutathione, ascorbic acid and-tocopherol. Increased production of ROS in the injury caused the consumption and depletion of endogenous binding compounds. Flavonoids may have addictive effects on the binding of endogenous compounds (Wilmana, 1995; Chlubeka, 2003).

Flavonoid can prevent injury caused by free radicals in several ways. It is to clean up free radicals directly. Flavonoid is oxidized by the radicals, making them more stable and less reactive. In other words, flavonoid stabilizes ROS by reacting with reactive radical compound. Because of high reactivity of hydroxyl groups in flavonoid, free radicals become inactive. Certain flavonoid can directly clean superoxide, whereas the others can clean oxygen free radicals are highly reactive, called peroxynitrite (Halliwel, 1994).

The main mechanism of flavonoid as antioxidants possibly due to its effect as a binder radicals or interact with a variety of enzyme systems, may also through two mechanisms tersebut (Halliwel, 1994). Certain flavonoid may reduce complement activation resulting in lower inflammation of endothelial cell adhesion, and generally cause the failure of inflammation response. Another possibility is to reduce the release of peroxidase. It was inhibited by neutrophil SOR production by affecting activation α_1-antitripsin (Halliwel, 1994).

Flavonoid may inhibit the formation of MDA, through its effect of inhibiting prostaglandin synthesis through inhibition of cyclooxygenase path, so MDA as a byproduct of prostaglandin biosynthesis, is also inhibited. Prostaglandins will increase the amount of blood flow in inflammation area through vasodilation process. Decreasing the amount of prostaglandin by itself will reduce the number of leukocytes that infiltrated inflammation area. This reduces phagocytosis process and free radicals formation, so that increased in MDA can be prevented (Plastaras *et al.,* 2000).

Beside flavonoid, the other content of gedi leaves is tannin. It's shown to have anti-inflammation effects partly derived from its antioxidant activity, that is ability to destroy free radicals better than phenolic compounds with low molecular weight (Rimando and Chavez, 2008; Suralkar, 2008)

The ability of flavonoids and tannins as antioxidants appears from the results of research showing that MDA levels in the group that get gedi leaves ethanol extract comparable significantly ($p < 0.05$) with the group that get diclofenac, and significantly different with negative control group.

CONCLUSION

Gedi leaves ethanol extract has the effect of decreasing edema, reducing the hyperalgesia and reducing tissue MDA level in paw edema of rats model. The effects owned by ethanol extract of gedi leaves is comparable with Diclofenac Sodium.

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BIOLOGICAL ACTIVITIES AND DEVELOPMENT OF HERBAL PRODUCTS FROM *SONNERATIA CASEOLARIS* L. ENGL.

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Abstract: Since the phytochemical analysis of the ethanolic extracts showed the presence of alkaloids, flavonoids, triterpenoids, steroids, saponins, and tannins, that further investigation were carried out covering antibacterial- and antifungal assay, antioxidant and toxicity of the extracts of fruits, leaves and twigs of Rambai sungai (*Sonneratia caseolaris*). Antioxidant activity was determined by using the DPPH (*1,1-diphenyl-2-picrylhydrazyl*) free radical scavenging method, antibacterial and antifungal assays were conducted by agar diffusion method against several strains of pathogenic bacteria and fungi. Toxicity of the extracts was evaluated by using *brine shrimp lethality test* against *Artemia salina* Leach. Rambai sungai water extracts showed neither antifungal activity nor antibacterial activity. On the other hand, Rambai sungai water extracts possessed antioxidant potency and comparable to the antioxidant of ascorbic acid with 96% activity. Based on the toxicity test, *S. caseolaris* has possibility to be developed as a safe product for human; and the higher antioxidant potency lead the plant to be developed as herbal products sources.

Keywords **:** *Sonneratia caseolaris*, antioxidant, toxicity

INTRODUCTION

Indonesian tropical forest area include of 110 mill. Ha, are riched on medicinal plants. In so far, there are about 1.000 species have been used world-wide as traditional medicines (Pramono 2002).

In the last two decades, the utilization of natural resources medicine are tremendous grown up, which is indicated by higher variety in herbal medicine and higher number of herbal medicine producers. In contras, there are changed in deseases pattern, donated by degenerative and cronical deseaseas, i.e chollesterol, hypertency, diabetic, cancer, and new infection deseases, more resistance against deseases caused by microbial agents. Those cases lead to the people tendency not to choose chemical medicine, but the more safely natural medicine. Increasing of herbal products, supported by more investigation of natural medicines. Based on traditional wisdom of indigenous people, plants were determined as herbal raw materials. Rambai sungai (*Sonneratia caseolaris)* is one of potential mangrove plants as herbal medicine based on traditional utilization information by indigenous people. Mangrove plants potency as medicinal plant was reported by Purnobasuki, (2004).

In Bangladesh, Shaddu *et al*., (2006) isolated flavonoid as antioxidant from *S. caseolaris*. Leaf and fruits of this plant traditionally used as herbal medicine for stomach, blooding, antiseptic. Based on this information, it is very urgent to investigate the potency of Rambai sungai for herbal products. This research aimed to study the potency for antimicrobial agent from Rambai sungai plant.

MATERIALS AND METHODS

Materials

Sonneratia caseolaris plant from the Mahakam river side Samarinda East-Kalimantan.Tested fungi: *Aspergillus níger, Candida albicans,* and *Trichopyton mentagrophytes*.

Tested bacteria culture: *Salmonella thypi, Bacillus cereus* from BPOM Samarinda and *Staphylococcus aureus* from Healthy Laboratory Samarinda. *Artemia salina* Leach for *Brine Shrimp Lethality Test*.

Extraction

Samples were extracted twice by diluted powdered samples with hot water (ratio of 1:10) put in a watterbath at 100 ^{0}C for 3 h. After filtration, the aqueous solution was evaporated by rotary vacuum evaporator at 40ºC to yield gummy water extracts.

Toxicity test

Toxicity of extracts were tested against brine shrimp (*Artemia Salina* Leach). Extracts were decided as toxic, when the LC_{50} < 1000 mg/ml.

Phytochemical analysis

Extract was subjected to phytochemical analysis (Harborne, 1987). Phytochemical analysis were conducted by coloring test, included testing for alkaloids, steroids and triterpenoids, saponin, flavonoids, carbohydrates, and tannin (Kokate, 2001).

Antimicrobial activities assay

Extracts were tested for antifungal activities assay against *Aspergillus níger, Candida albicans,* and *Trichopyton mentagrophytes* in a *paper disk diffusion agar* method. For anti bacterial activities assay, extracts were subjected to *Salmonella thypi, Bacillus cereus* and *Staphylococcus aureus.* Antimicrobial assay by diffusion method with extracts concentrations of 40 µg, 60 µg, 80 µg. Antimicrobial activity was determined based on the *Minimum Inhibitory Concentration* (MIC). Myconazole and terramycine were used as positive control for antifungal and antibacterial assay respectively, Acetone was used as negative control. The mean values of inhibition were obtained from triplicate experiments.

Testing for Antioxidant activity against DPPH (*1,1-diphenyl-2-picrylhydrazyl*) free radical

Testing for antioxidant activity of extracts against DPPH (*1,1-diphenyl-2-picrylhydrazyl*) free radical solution were conducted by spectrophotometer at ambient temperature (25^{0}C) with wave length of 514 nm. For this test, ascorbic acid was used as positive control.

Making of herbal products

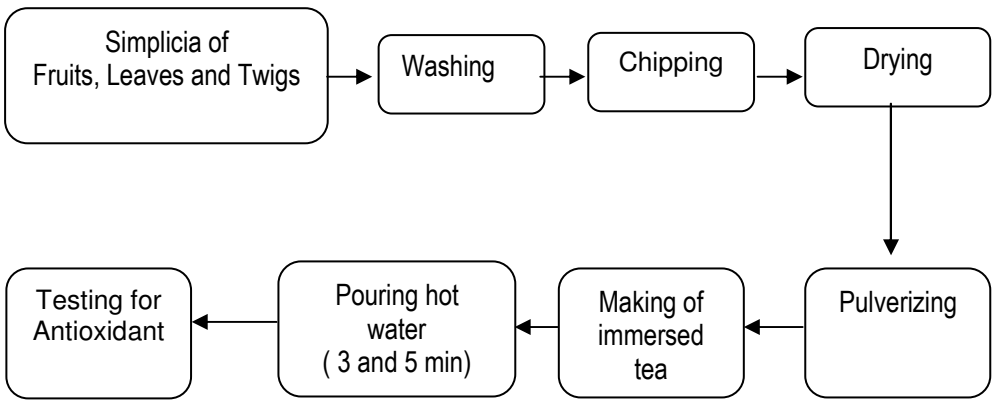


Figure 1. Scheme of Herbal Production from *S. caseolaris*

RESULTS AND DISCUSSION

Extracts Yield

The total extracts from the *S. caseolaris* by aqueous solution at 100^{O}C for 3 h, after filtering and drying (based on oven-dried) are given in Table 1.

Table 1. Water Extracts yields of *S. caseolaris*

Extracts	Part of Plants	Sample (g)	Extracts (g)	Yield (%)
Water extracts	Fruit	44.08	20.71	46.98
	Leafs	43.12	4.96	11.50
	Twig	44.50	6.95	15.62

Pulverizing the samples before extraction has a large influence on the extractable amount of extractives. Grinding can increase the leaching of water soluble organic compounds ten times more than that without grinding. The higher extracts yield may also be caused by higher polarity of aqueous solution, which dissolved some chemical compounds.

Phyitochemichal Analysis

Phytochemical analysis of *S. caseolaris* ethanolic extracts showed the main active compounds as presented in Table 2.

Table 2. Water Extracts yields of *S. caseolaris*

Extracts Compound	Part of Plant		
	Fruit	Leaf	Twig
Alkaloid	+	+	+
Flavonoid	+	+	+
Saponin	-	+	+
Tannin	+	+	+
Triterpenoid and Steroid	(-/-)	(-/+)	(-/-)

(+) = active, (-) = not active

Antimicrobial Activity Assay

In particular, aqueous solution are able to dissolve simple sugars, i.e. carbohydrates and starch, which are foodstuffs for microorganism. The heating while the extraction processing could be able to damage the thermolabile-active compounds of extracts and may degrade the chemical structures of active compounds (Fengel dan Wegener,1984; Harborne, 1987). Those should be the reason why, antimicrobial activity assay of *S.caseolaris* extracts shown neither antifungal- nor antibacterial activities. Antimicrobial secondary metabolites-compounds of *S. caseolaris* supposedly did not dissolve in aqueous solution.

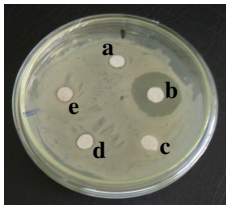
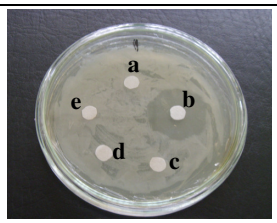
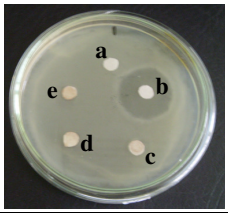
Fruit	Leafs	Twig
		

Figure 2. Antibacterial activity assay against *Salmonella thypi* of rambai sungai water extract

(a)= Negative control, (b)= Terramycine, (c)= Extracts 40µg, (d)= Extracts 60µg, (e)= Extracts 80µg

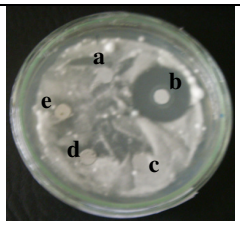
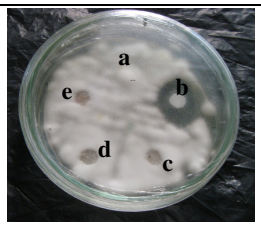
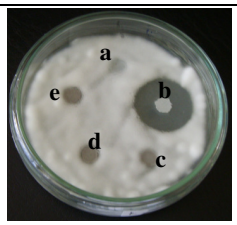
Fruit	Leafs	Twig
		

Figure 3. Antifungal activity assay against *Tricophyton mentagrophytes* of rambai sungai water extract

(a)= negative control, (b)= Myconazole, (c)= Extracts 40µg, (d)= Extracts 60µg, (e)= Extracts 80µg

Testing of antioxidant Activity

In order to explore the mechanism of the free radical scavenging effect of *S. caseolaris* extracts, the antioxidant activity was examined. It was hypothesized that the antioxidant activity of *S. caseolaris* extracts may protect from oxygen free radicals. The study was designed to measured how well *S. caseolaris* extracts scavenged DPPH free radicals.

The data from this study indicate that *S. caseolaris* extracts is strong antioxidant which can scavenge DPPH free radicals. The higher inhibition are in water extracts of leaves and twigs (94%), nearly that of ascorbic acid activity (96%) as shown in Fig. 4.

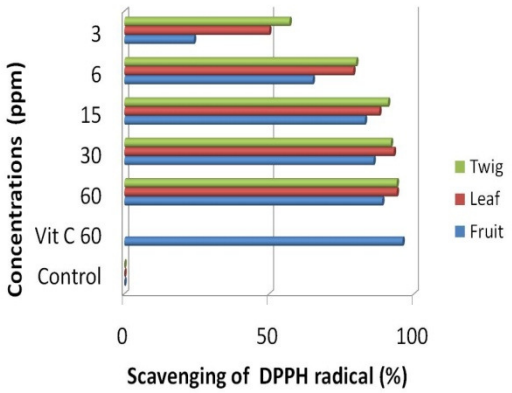


Figure 4. DPPH free radical scavenging activities of water extracts

The testing of Rambai sungai extracts against DPPH free radical showed a positive reaction. These results are supported by the presence of flavonoid in overall part of the plant. The flavonoid compounds are able to be scavenging of free radicals. The higher activity of fruit part was caused not only by presence of flavonoid compounds, but also by presence of glycosides from carbohydrates and supposing of vitamine C containing.

Testing of antioxidant Activity of Rambai Sungai Herbals

Figure 5 showed the scavenging of DPPH free radical of herbal samples higher than 50% . The higher scavenging activities are given by fruits herbal, 85% and 89% for pouring time of 3 and 5 min respectively.

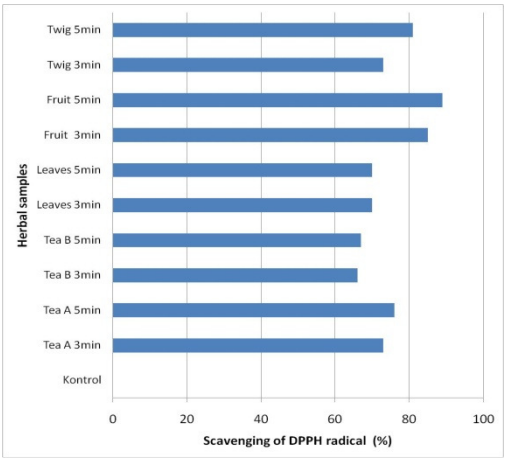


Figure 5. DPPH free radical scavenging activities of herbal products from Rambai sungai

Those scavenging values are higher than that of commercial tea A and tea B as positive control. The higher DPPH free radicals scavenging of fruits herbals in hot water caused by dissolved flavonoid, tannin, and other secondary metabolite compounds, which have antioxidant properties. The sour fruits-tasted are supposed containing of ascorbic acid, well-known as antioxidant.

Toxicity Test (*Brine Shrimp Lethality Test*)

Toxicity test was conducted based on the *brine shrimp lethality test* by using *Artemia salina* Leach (Meyer et al., 1982). The testing results are given in Tabel 3.

Tabel 3. Toxicity test of Rambai Sungai (*S. caseolaris*) extracts

Extracts	Part of Plant	Mortality (%)				
		25 ppm	50 ppm	100 ppm	250 ppm	500 ppm
Water extracts	Fruits	0	0	0	0	2
	Leaves	0	0	0	0	0
	Twigs	0	0	0	0	0
Gallic acid		0	20	50	90	90
Negative control		0	0	0	0	0

Based on the results of investigation with different extracts concentrations, Rambai sungai water extracts showed the mortality of 2 %. The tested extracts overall possessed LC_{50}>1000 ppm. This showed that Rambai sungai extracts have the quality of non toxic. Meyer *et al.,* (1982) stated that a compound identified as a toxic, if it possesses LC_{50} < 1000 ppm.

The toxicity of a plant extracts affected to the utilisation of plants as oral medicine. Rambai sungai showed the lower mortality against *Artemia salina,* that the plant is safe to be produced as herbal products. Preparation and processing of this plant lead to the utilisation of Rambai sungai, either as medicines or as foods products.

CONCLUSION

Rambai sungai (*S. caseolaris*) aqueous extracts plays role as antioxidant and can be developed as herbal products. The aqueous extracts showed neither antifungal activities nor antibacterial activities.

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VITEX PUBESCENS AND *TERMINALIA CATAPPA* PLANT SPECIES FROM KALIMANTAN AS AN ANTI DENTAL CARIES AGENT

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Abstract: Methanol and 50% ethanol aqueous extract of two plants from East Kalimantan forest, *Vitex pubescens* and *Terminalia catappa* were tested for dental caries-inducing properties of *Streptococcus sobrinus* bacteria as well as for brine shrimp lethality. The stem wood, stem bark, and leaves were separately collected and investigated for their activities. The result showed that the extracts of these plant exhibited potency as an anti dental caries agents.

Keywords : *Vitex pubescens, Terminalia catappa, Streptococcus sobrinus,* Brine shrimp lethality

INTRODUCTION

Plant produce a wide variety of secondary metabolites which are used either directly as precursors or as lead compounds in the pharmaceutical industry and it is expected that plant extracts showing target sites other than those used by antibiotics will be active against drug resistant microbial pathogens. However very little information is available on such activity of medicinal plant and out of the 4000000 plant species on Earth only a small number has been systematically investigated for their antimicrobial activities. Additionally, there is a local ethnobothanical knowledge and bibliography describing the species most frequently used by population to cure diseases (Shokeen, 2009). Over 80% of the world's population, especially in the developing country, using plant extracts as traditional medicine to provide their health (WHO, 2002)

Vitex pubescens species belong to the family of Verbenaceae and some of species in this family has activity to treat the diseases. The genus Vitex consist of over 270 species, predominantly trees and shrubs, and is restricted to tropical and sub tropical regions. There are many species of Vitex with have medicinal and phytochemical importance. Genus *Vitex* is used as a traditional medicine in China, Indo-China, Indonesia and the Philippines. *V. pubescens* has been introduced to tropical Africa and South America. It is planted in Southeast Asia (Kalimantan) on trial for charcoal production. Tannin is extracted from the bark, leaves, roots, and fruit shell. A black dye is obtained from the bark, fruit, and foliage. The leaves and bark have a wide range of medicinal uses (Lex *et al.*, 2006). In traditional medicine, a decoction of the bark of *V. pubescens* is used to treat stomachache, and a poultice of its leaves is used to treat fevers and wounds.

Terminalia catappa L. is a Combretaceous plant whose leaves are widely used as a folk medicine in Southeast Asia. In traditional medicine, *T. catappa* leaf, bark and fruit are used in treating dysentery, rheumatism, cough and asthma. Some studies on medicinal properties of *T. catappa* roots and fruits have been also reported. The activity of five *Terminalia* species were reported as antibacterial against human pathogens *E. coli*, *P. aeruginosa*, *B. subtilis*, *S.aureus* and *S. epidermidis. Terminalia catappa* found to possess the compounds which are more antibacterial (Shinde *et al*., 2009)

Even there were some studies about their potency as medicine, there is a little information about the potency of these plants as toothache medicine. Some investigation

about the activity for treat the tooth dieases have been reported in different species of *Vitex* and *Terminalia* (Padmalatha, 2009 and Kamal, 2009), but the anti caries potential of *T. catappa* and *V. pubescens* had not been mentioned in previous report.

Herbal remedies have long history of use for gum and tooth problems. The use of herbal chewing stick for relieving dental problems is common in many traditional cultures. Oral pain occurs as a result of bacterial activity in the pulp of a carious tooth. One of the bacteria types have been implicated in caries formation is *Streptococcus sobrinus.* There are several strategies to preventing the formation and development of dental caries, such as controlling the growth and cell adhesion of mutans streptococci.

The objective of this research is to determine the effect of methanolic and 50% ethanolic of *Terminalia catappa* and *Vitex pubescens* in different plant material (stem wood, stem bark, and leaves) on the growth and cell adhesion of *S. sobrinus*. The brine shrimp lethality bioassay was also carried out to investigate the cytotoxicity of extracts of medicinal plants.

MATERIAL AND METHOD

Plant material and preparation of extracts

Terminalia catappa and *Vitex pubescence* were collected from East Kalimantan. The plant materials were air dried to constant weight, grinded to powder and stored in container for further use. Two different solvent, methanol and 50% ethanol were used for extraction. Exactly 100 g of the plant material powder were extracted in methanol and 50% ethanol. The separated extracted were then filterded using filter paper (Whatman no 1). Furthermore the methanol and 50% ethanol filtrate were separately concentrated to dryness using a rotary evaporator to remove the solvent.

Antimicrobial activities against *S. sobrinus* by agar well diffusion

The *S. sobrinus* 6715 bacteria were first grown in a Nutrient broth for 18 h before use and standarized to 0.5 Mc Farland standard. The antimicrobial activities of the various plant extracts were evaluated by means of agar-well diffusion assay. Forthy five milliliters of Nutrient Agar media were poured into 100 x 100 mm sterile petri dishes. Standarized cell suspensions of *S. sobrinus* that prepared before then spread onto the surface using sterile swab sticks. Once the plates had been aseptically dried, seven 6 mm-diameter wells were bored in each dish by removal of the agar using a sterile cork borer, then immediately filled with the test and control materials (one well for each substance). Extracts and control (40µl) of different concentrations (3.0 – 8.0 mg/ml) were placed into the wells, left for one hour at room temperature for diffusion and the plates were incubated at 37^0C for 24 h. Chloramfenicol (0.5 mg/ml) and mouthwash containing hexetidine 1% were used as positive control. After incubation, the diameter of the zones of bacterial growth inhibition formed around the wells was measured in millimeters with a ruler under reflected light. The antimicrobial activity indicated by an inhibition zone surounding the well greater than 6 mm.

Determination of the cell adhesion of *Streptococcus sobrinus*

S. sobrinus culture was grown in a test tube containing 3 ml of Nutrient broth with 1% (w/v) glucose at 37˚C and held at an angle of 30˚ for 18 h. After 18 h incubation, the adhering cells were collected by sonicating and washing the tube with saline (0.9% NaCl), and then determined turbidimetrically at OD 550 nm. This gave the total cell number and represented 100% adhesion. The experiment was repeated by incubating the *S. sobrinus* culture in 3 ml Nutrient broth with 1% (w/v) glucose and different concentrations (0.25 – 1 mg/ml) of plant extracts. This gave the total cell number adhering in the presence of different concentrations of the extract,and this was expressed as a percentage of the total cell number in the absence of the extract.

Brine Shrimp Lethality Assay

This assay was carried out to investigate the cytotoxity of *Terminalia catappa* and *Vitex pubescens* extracts. Brine shrimp eggs (*Artemia salina*) were hatched in artificial sea water. After 48 hours incubation at room temperature (25-29°C), nauplii (larvae) were collected by pipette and used for the assay.The plant extracts were tested at concentration 10, 100, 1000 ppm in sterile vials containing 3 ml of sea water and 10 shrimps. Survivors were counted after 24 hours, and the percentages of lethality at each concentration were recorded according to
Abbot's formula (McLaughlin, 1991) :

$$\%M = [(m_e - m_b) / (10 - m_b)]*100$$

Where m_e = dead shrimp in the sample and mb = shrimp dead in the blank
LC_{50} values were obtained from the best-fit line plotted concentration versus percentage lethality.

RESULTS AND DISCUSSION

The uses of plant extracts with medicinal properties represent a concrete alternative for the treatment of different pathological stages. The antimicrobial properties have been reported in a wide range of plant extracts and natural products attempting to contribute with the development of new drug, which can generate a significant improvement in managing several kinds of health disorders (Alviano *et al.*, 2004). Herbal preparations have been used by local practitioners to treat various ailment. Stems and leaves of certains herbs are commonly used in cleaning and treatment of dental diseases (Okafor, 2001).

S. sobrinus was choosen as test microorganism because it have been implicated in dental caries. The antibacterial assays against *S. sobrinus* in this study were performed by the agar well-difussion methods. The *in vitro* antimicrobial activities of the crude plant extracts of *Vitex pubescens* and *Terminalia catappa* are shown in Table 1. The susceptibility of the bacteria to the crude extracts on the basis of zones of growth inhibition varied according to plant material and extracting solvent. In this investigation most of the sample in this study showed inhibition of the growth of *S. sobrinus*, the sizes of zones of growth inhibitions vary from one plant material to another.

According to the findings of this study, the crude extracts from *V. pubescens* and *T. catappa* plants showed varying degree of antibacterial activities against *S. sobrinus*. Regarding inhibition, the most potent extract was the *T. catappa*-leaves-methanol extract with 10.6 mm zone inhibition at different concentration. The methanol extracts (inhibition zone 8 – 10 mm) was found to be more effective than the 50% ethanol extracts (inhibition zone 7 – 9 mm) against *S. sobrinus* bacteria. The antibacterial activities of the methanol and 50% ethanol extracts compared favourably with that of two positive control (chloramfenicol and mouthwash). This finding implies that the active compound have polar characteristics. Chloramfenicol and mouthwash containing 1% hexetidine which served as positive control produce zone inhibition measuring 13 mm and 9.5 mm, respectively. Highest activity was demonstrated by the control standard antibiotic (chloramfenicol), while the negative control (acetone) produced no observable zone. The high activity of chloramfenicol is because the antibiotic is in pure state and has refined processes that have established it as a standard antibiotic (Abubakar, 2009). Their effects on the growth of *S. sobrinus* were most likely due to the release of chemicals from the crude extracts into the medium. The different reactions of *S. sobrinus* to the different extracts indicated that each solvent extracted different chemical components of plants

The phytochemicals of *Terminalia catappa* include tannins), flavanoids and triterpinoids. Probably, the bioactivite flavonoids and other phenol compound and its derivates present in *T. catappa* are involved in its biological activity. Tanin and flavonoids have been reported to be responsible for the antibacterial activities of the extracts (Heminway and Karchesy, 1991). The inhibitory effect of the extracts in this

study as antimicrobial against *S. sobrinus* can introduce these plants as a potential candidate for antimicrobial agent development for the treatment of dental caries.

Table 1. Antibacterial activities profile of *Terminalia catappa* and *Vitex pubescens* extracts against *S. sobrinus*

Plant species	Part	Control	Zone of inhibition (mm)					
			3 mg/ml		6 mg/ml		8 mg/ml	
			MeOH	50% EtOH	MeOH	50% EtOH	MeOH	50% EtOH
Vitex pubescense	Stem		8.2 ± 0.1	-	8.6 ± 0.3	7.2 ± 0.3	9.2 ± 0.3	8.2 ± 0.1
	Bark		-	8.2 ± 0.2	8.2 ± 0.3	8.2 ± 0.1	8.2 ± 0.3	8.2 ± 0.3
	Leaves		-	9.2 ± 0.3	9.2 ± 0	9 ± 0.4	10.0 ± 0.2	9.6 ± 0.3
Terminalia catappa	Stem		9 ± 0.1	8.4 ± 0	9 ± 0.2	8.8 ± 0.1	10 ± 0.0	9.6 ± 0.2
	Bark		-	-	8.6 ± 0.5	9 ± 0.3	9 ± 0.1	9.2 ± 0.2
	Leaves		9.6 ± 0.5	8.6 ± 0.1	9.8 ± 0.2	9 ± 0.4	10.6 ± 0.1	9.4 ± 0.2
Mouthwash		9.5 ± 0.5						
Chloramfenicol (0.5 mg/ml)		13.0 ± 0.2						

Figure 1a and 1b show the effect of plant extracts on the adhesion of *S. sobrinus* to a glass surface. It was found that the *T. catappa* and *V. pubescence* extracts could reduce the cell adhesion of *S. sobrinus.* The *T. catappa* extracts exhibiting a stronger activity than the *V. pubescence* extracts. The inhibitory effect increased with increasing the concentration.

It is clearly shown that all the extracts tested in this study affected the cell adhesion of *S. sobrinus*. The extract from *T. catappa* can reduce the cell adhesion at range 17 – 85.5 %, while V. pubescens show lower activity to reduce the cell adhesion (12 – 84.3%). It was observed that the ability of *S. sobrinus* to adhere to a glass surface was affected by the presence of crude extracts. The receptors on the cell *S.sobrinus* may be modified by components in the crude *T. catappa* and *V. pubescence* extracts, leading to reduction of adhesion ability. The adhesion was reduced to 69.7% at 0.25 mg/ml *T. catappa*-bark-methanol extract, and a higher concentration of the other extracts were required for a similar reduction.

The highest activity for reducing the cell adhesion of *S. sobrinus* come from *T. catappa* – stem – 50% ethanol extracts at 1 mg/ml concentration, followed by *V. pubescens* – bark – methanol extracts in the same concentration. The reduced adhesion of *S. sobrinus* cells to the tooth surface would disrupt the colonization of the tooth surface by the microorganisms, and this could affect plaque accumulation.

Brine shrimp larvae have been used as a bioassay for a variety of toxic substances. The method has also been applied to plant extracts in order to facilitate the isolation of biologically active compounds. Cytotoxixity activity of extracts was tested in order to evaluate a limited toxicity. Brine shrimp nauplii have been used in a number of bioassay systems (McLaughlin *et al.*,1990) and the crude extracts of the plant extracts of *T. catappa* and *V. pubescence* were subjected to this evaluation.

The extracts were tested at concentrations of 10, 100 and 1000 ppm (data not shown) and data LC_{50} value shown in Table 2. The result obtained the variety of toxicity value on each extracts.

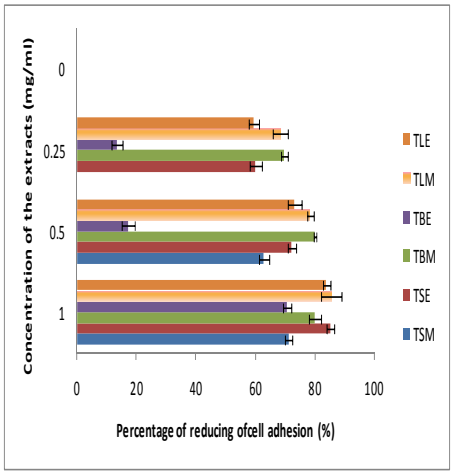

Figure 1a Effect of crude *T. catappa* extracts on the reducing cell adhesion of *S. sobrinus*

T : Terminalia catappa, L : Leaves, S : Stem, B : Bark, M : MeOH, E : 50% EtOH

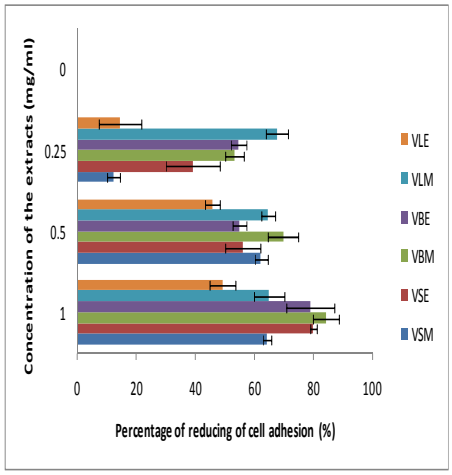

Figure 1b Effect of crude *V. pubescence* extracts on the reducing cell adhesion of *S. sobrinus*

V : Vitex pubescence, L : Leaves, S : Stem, B : Bark, M : MeOH, E : 50% EtOH

The toxicity showed that there were differences in LC_{50} values for type of plant extracts and plant species.

Table 2. Brine shrimp toxicity value (LC_{50}) of plant extracts of *Vitex pubescens* and *Terminalia catappa*

		***Vitex pubescens* (ppm)**	***Terminalia catappa* (ppm)**
Stem wood	MeOH	305.5 ± 0.27	17.78 ± 1.1
	50% EtOH	543.3 ± 0.21	0.44 ± 0.2
Stem bark	MeOH	298.5 ± 0.15	59.2 ± 0.2
	50% EtOH	10 ± 0.2	317.7 ± 1.2
Leaves	MeOH	2.9 ± 0.1	0.01 ± 1.4
	50% EtOH	40 ± 0.9	0.43 ± 0.75

In this study, the methanol extracts from *T. catappa* leaves showed very toxic activity (LC_{50} value = 0.01 ppm), followed by 50% ethanol extract of *T. catappa* leaves, 50% ethanol extracts of *T. catappa* stem wood, methanol extracts of *V. pubescense* leaves, 50% ethanol extracts of *V. pubescense* stem bark, and methanol extracts from *T. catappa* stem wood with LC_{50} value 0.43 ppm, 0.44 ppm, 2.9 ppm, 10 ppm and 17.78 ppm respectively. Other plant extracts in this study, however, showed moderate brine shrimp lethality or toxic, where the LC_{50} were found to be at lower than 1000 ppm. Toxicity of extract has classified by the value of $LC_{50} \leq 30$ ppm = very toxic; $31 \leq LC_{50} \leq 1000$ ppm = toxic; $LC_{50} > 1000$ ppm = practically no toxic (Meyer et, al, 1982).

The variation in BSLA results (Table 2) may be due to the difference in the amount and kind of cytotoxic substances (e.g. tannins, flavonoids, triterpenoids, or coumarins) present in the crude extracts.

Moreover, this significant lethality of the crude plant extracts (LC_{50} values less than 100 ppm or µg/mL) to brine shrimp is indicative of the presence of potent cytotoxic which warrants further investigation. BSLA results may be used to guide the researchers on which crude plant extracts/fractions to prioritize for further fractionation and isolation of these bioactive compounds. (Nonita, 2010). From this study the bioactivity of *T. catappa* and *V. pubescens* indicating the presence of compounds with possible biological activity.

CONCLUSION

The extracts of both species of *Terminalia catappa* and *Vitex pubescens* from different plant material proved to exert in vitro antibacterial and cell adhesion reduction against *S. sobrinus.* The ability of the crude extracts to inhibit the growth and reduce the cell adhesion of *S. sobrinus* used in this study is an indication that *T. catappa* and *V. pubescens* are a medicinal plant and can be used as an agent for developing broad spectrum antibiotics, which further validates its use in traditional herbal medicine to treat dental caries. The phytochemicals can further be isolated and undergo further pharmacological evaluation

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IMPROVEMENT CHARACTERISTICS OF CURCUMA TAMARIND TRADITIONAL JAMU BY ADDITION OF THICKENING AGENT

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Abstract : We have recently improved characteristics of Curcuma Tamarind Traditional Jamu (*Jamu Gendong Kunyit Asam*), CTTJ, as functional food by addition of CMC and Pectin as thickening agent as well as emulsifier to improve the homogenous of the CTTJ. Factorial experiment of two factors (addition of CMC and Pectin) arranged in Completely Randomized Design with three replications for each treatments was applied in this study. Addition of CMC of about 8 g in 1 litre CTTJ (0.8 %) showed a completely homogenous CTTJ, however CMC in this concentration reduce taste acceptance. On the other hand, the completely homogenous CTTJ could be achieved by addition of CMC and pectin of 4 g (0.4 %) and 10 g (1 %) in 1 litre CTTJ, respectively. At this level of CMC and pectin concentration, the acceptance of sensory characteristics of CTTJ was like control (CTTJ without thickening agent).

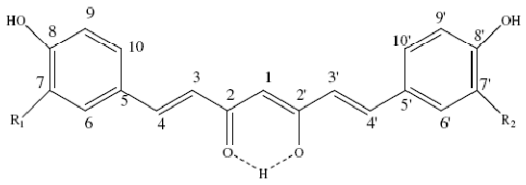
Keywords **:** Curcuma, tamarind, CMC, pectin

INTRODUCTION

Curcuma Tamarind Traditional Jamu (CTTJ) is become populer functional food (Winarti and Nurdjanah, 2005; Chooi, 1997) because of bioactive content of the raw material used, turmeric (*Curcuma longa* Linn.) and tamarind (*Tamarindus indica* Linn.). Turmeric consist of 3-5% curcuminoids, which are divided into three components, curcumin (75%), demethoxycurcumin (15-20%), and bisdemethoxycurcumin (3%). Curcumin is the most important fraction, which is responsible forbiological activity (Çıkrıkçı *et al*., 2008; Chapman and Hall, 1992). Curcuminoid has pharmaceutical effect to diabetes melitus, thypus, disentry, morbili (Winarto, 2003), as weel as antimicrobial and antioxidant activity (Çıkrıkçı *et al*., 2008), while tamarind is containing tartaric acid and used as natural preservatives agent (BPPT, 2005).

In home industry scale (*jamu gendong*), production of CTTJ is as follows. One liter of CTTJ is prepared from 150 g of curcuma, 80 g of tamarind, 130 g of brown sugar, 80 g of sugar, and some salts. Tamarind flesh is crushed with some water, while curcuma is shredded and extracted by swueezing. The extract is then let some time to precipitate non soluble particle. The liquid part of turmeric extract is then mixed with tamarind-water as well as brown sugar, sugar, and some salt. This mixture is boiled until the thickness of the mixture increase.

In the last decade, CTTJ has already been produced in industrial scale, however it is still constrained by physical characteristics of sedimentation because of non dissolve particle from curcuma and tamarind extract, which cause CTTJ become non homogenous, and also some other characteristics still need to be improved. Curcumin is soluble in and acetone, but insoluble in water (Çıkrıkçı *et al*., 2008).



Compound	R_1	R_2
Curcumin	OMe	OMe
Demethoxycurcumin	H	OMe
Bisdemethoxycurcumin	H	H

Figure 1. Chemical structures of curcuminoids (Çıkrıkçı *et al*., 2008)

Emulsifiers is predicted to be able to overcome this problem. Yolk, gelatin, pectin, carboxy methyl cellulose (CMC), amilose, casein, and albumin are some of emulsifier used in food (Hartomo and Widiatmoko, 1992; Winarno, 1991). In this report we described study of addition of CMC, pectin, and their combination in CTTJ. CMC and pectin were selected in this study because they can also being thickening agent, which is very important to improve the physical characteristic of CTTJ to be slightly more thick. Manoi (2006) has demonstrated the use of CMC to improve physical characteristics of cashew juice, as well as Ulfa and Budiyanto (2008) for CTTJ.

MATERIALS AND METHODS

Rhizomes of turmeric was obtained from middleman to get a homogen of size and age, tamarind, brown sugar and sugar were obtained from traditional market. Commercial CMC was purchased from food and pastry shop, while pectin was obtained from chemicals store.

Factorial of 4x4 in completely randomized design was applied in this experiment. CMC (C) concentration was the first factor and Pectin (P) concentration was the second factor, and the consentration were about 0-1.2% and 0-1.5%, respectively. Physical characteristics (viscosity and homogenous level) as well as sensory characteristics (taste, aroma, color, viscosity) were observed. Data were analyzed by ANOVA and continued by *Duncan Multiple Range Test* (DMRT) at α=0.05.

Physical characteristic of viscosity was stated as flow time, which is needed to run out the CTTJ from 10 mL Mohr pippete in straight position (Markham and Charles, 1954), while homogenous level was determined by calculating percentation of height level of suspension of CTTJ in test tube (11 cm) following over night (24 h) incubation at room temperature. Sensory characteristics was determined by hedonic (1 = dislike very much, 2 = dislike moderately, 3 = like slightly, 4 = like moderately, 5 = like very much) and hedonic quality test for taste, aroma, color, and viscosity (Soekarto, 1985). Scale for hedonic quality were:

Taste: [1] unvicidity moderate, [2] unvicidity slight, [3] vicidity slight, [4] vicidity moderate, [5] vicidity strong

Aroma: [1] very slight, [2] slight, [3] moderate, [4] strong, [5] very strong

Color: [1] very unclear, [2] unclear slight, [3] clear slight, [4] clear moderate, [5] very clear

Kekentalan: [1] very slight, [2] slight, [3] moderate, [4] strong, [5] very strong

Procedure

CTTJ was produced by methode described at Figure 1. CMC and pectin were added following mixing the both with white sugar (sucrose).

RESULT AND DISCUSSION

In the pre-research step, it was determined that the optimum concentration of CMC and pectin in 1 liter CTTJ was 12.0 g and 15.0 g, respectively. The both thickening agents could be added in the form of powder following mixing it completely with white sugar. The using of CMC/pectin powder in this research was to make the production of CTTJ more simple, so that it can be applied in small scale industry (home industry). In some reports, CMC/pectin were added as concentrate because of the difficulties to dissolve them (in the form of powder) completely in the solution (Manoy, 2006; Ulfa and Budiyanto, 2008). In this research, it was found that the problem was overcome by mixing the CMC/pectin with white sugar prior adding to CTTJ.

Table 1, 2, and 3 showed the influence of CMC, pectin, and their combination on physical and sensory characteristics of CTTJ, respectively.

Viscosity

The addition of CMC and pectin gave significant influence on physical and sensory characteristics of CTTJA. CMC, pectin, and their combination gave significant influence on physical characteristics (viscousity and percent homogenity). The more CMC/pectin added, more viscous the CTTJ produced (Table 1 and 2). However the addition of pectin alone did not increase the viscousity significantly, but it will be significant if the pectin was given in the combination with CMC. On the other hand, CMC will increase the viscousity of CTTJ significantly without combination with pectin.

Sensory test showed the same data, CMC/pectin gave influence on the viscousity of CTTJ significantly, and CMC gave stronger taste of viscous compare to pectin. Based on hedonic quality test data, viscosity of 1 liter CTTJ will be significantly influenced by each more addition of CMC of 4.0 g, while the same influence was determined for pectin by more addition of pectin of 10.0 g.

Hedonic test data of sensory characteristics on viscousity of CTTJ showed that acceptance level on viscosity of CTTJ decreased by more addition of CMC. This respond was unsuitable for pectin, the addition of pectin until 15.0 g per liter CTTJ did not give significant effect on acceptance level of CTTJ viscosity.

CMC and pectin combination in CTTJ did not influence significantly on acceptance level of viscosity of CTTJ, which was around 2.42 – 3.48. The lowest acceptance level (2.42) on viscosity of CTTJ, which is between “dislike moderately” and “like slightly”, was determined at addition of CMC and pectin combination of 6.0 g and 7.5 g, respectively, while CTTJ without any addition of thickening agents (control) showed acceptance level on viscosity of 3.48, which is between “like moderately” and like “very much”.

Percent Homogeneity

Single addition of CMC of 8,0 g per 1 liter of CTTJ gave complete homogeneity (100 %), while single addition of pectin of 15.0 g per liter of CTTJ (optimal concentration advised in CTTJ) gave only 15% homogeneity (Table 3). When addition of CMC and pectin combination was applied in CTTJ, complete homogeneity was determined at CMC dan pectin addition of 4.0 g and 10.0 g, respectively. From this result, it is recommended that 8.0 g of CMC per liter CTTJ (0.8 %) could be used to get complete homogeneity of CTTJ, and to reduce the vicidity cause of CMC, combination of CMC and pectin could be applied with concentration of CMC and pectin of 0,4 % and 1 %, respectively, which is for 1 liter CTTJ the CMC and pectin used are 4.0 g and 10.0 g, respectively. Manoi (2006) showed that CMC concentration of 1.5 % could increase stability (homogeneity) of fruit cashew concentrate significantly, which was 82.95 % for control and became 89.55 % following addition of CMC.

Taste, Color, and Aroma

Single addition of CMC in CTTJ gave significant influence on sensory characteristics of taste, color, and aroma. At addition of 4.0 g CMC per liter CTTJ, acceptance level started to shift to "like slightly", and more addition of CMC until maximum concentration recommended for CTTJ gave a respond between "like slightly" and "dislike moderately". On the other hand, single addition of pectin did not give significant influence on hedonic sensory characteristic of taste, color, and aroma of CTTJ. Addition of CMC and pectin combination in CTTJ gave signicant influence on hedonic sensory characteristic of taste, but not aroma.

Table. 1. Influence of CMC and pectin on physical and sensory characteristics of CTTJ

CMC (g)	Pectin (g)	Physical Characteristics		Sensory Characteristics		
				Hedonic		Hedonic Quality
		Flow time (second)	Homogeneity (%)	Taste	Color	Color
0.0	0.0	19.86 a	8.79 a	3.61 cd	3.67 e	3.30
	5.0	23.30 a	8.03 a	3.76 d	3.24 cde	3.36
	10.0	24.09 a	8.18 a	3.21 bcde	3.39 de	3.15
	15.0	27.58 a	14.71 b	3.06 abcd	3.27 cde	3.09
4.0	0.0	26.77 a	7.58 a	3.00 abc	3.03 bcd	2.82
	5.0	27.52 a	58.48 c	3.15 abcd	3.09 bcd	2.70
	10.0	33.56 ab	96.97 de	3.24 cde	3.06 bcd	2.70
	15.0	37.42 ab	100.00 e	2.91 abc	3.12 bcde	2.73
8.0	0.0	48.92 bc	100.00 e	2.73 abc	2.42 a	2.18
	5.0	39.44 ab	100.00 e	2.64 a	3.33 cde	3.55
	10.0	60.48 c	98.18 de	2.97 abc	2.85 abcd	2.45
	15.0	53.91 bc	95.45 d	3.00 abc	3.30 cde	3.00
12.0	0.0	84.11 d	100.00 e	2.82 abc	2.82 abc	2.52
	5.0	99.80 d	99.70 e	2.67 ab	2.91 abcd	2.64
	10.0	167.49 f	100.00 e	2.91 abc	2.88 abcd	2.67
	15.0	121.54 e	100.00 e	2.91 abc	2.67 ab	2.55

Notes: Addition of CMCis for 1 liter CTTJ; Flow time was used to determined physical characteristic of viscosity; Sensory characteristics was conducted by 11 respondens (total of 33replication for each sample)

CONCLUSION

Addition of CMC and pectin combination at minimum concentration to get complete homogeneity (100 %) for 1 liter CTTJ was 4.0 g CMC and 10.0 g pectin. Acceptance level on hedonic sensory characteristic of taste of this CTTJ was not significantly different compare to control. It means that the addition of CMC and pectin combination did not shift hedonic sensory characteristics of taste and color of CTTJ. Hedonic acceptance level on taste of this CTTJ was around 3.24, which is between "like slightly" and "like moderately", while the acceptance level for control was 3.61. On the other hand, this CTTJ showed significant difference on hedonic and quality hedonic sensory characteristic of color. The acceptance level of hedonic and quality hedonic sensory characteristic of color of this CTTJ were 3.06 ("light slightly") and 2.70 (between "unclear moderately" and "unclear slightly"). The acceptance level of control for hedonic sensory and quality hedonic sensory characteristics were 3.67 (between "like moderately" and "like very much") and 3.30 (between "clear slightly" and "clear moderately"), respectively.

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ANTI-ATHEROSCLEROSIS EFFECT OF *PHYSALIS ANGULATA* L. HERB EXTRACT ON: INHIBITORY LDL OXIDATION AND ITS RELATION BETWEEN *IN VITRO* AND *IN VIVO*

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Abstract: Oxidative modification of low density lipoprotein (LDL) in artery wall is now widely regarded to play an important role in the development of atherosclerosis. We have carried out an experiment to measure the anti-atherosclerotic effect of *Physalis angulata* L. herb extracts. In this study, the extract prepared fromliquid-solidextraction to found ethyl-acetate , non-dissolved ethyl-acetate and ethanolic 96% extracts. In *in vitro* studies, the formation of conjugated dienes and thiobarbituric acid (TBA) reactive substances and increase in electrophoretic mobility of LDL were monitored as markers of the oxidation of LDL and in *in vivo* studies, we investigated lipid accumulation in aorta and monitoring oxidation of LDL using electrophoresis with hypercholesterolemic mice model. These results suggest that *Physalis angulata* ethyl-acetate Herb Extract mayhave a role to play in preventing the LDL oxidation involved in atherogenesis.

Keywords : Oxidation, LDL, TBA, and *Physalis angulata*

INTRODUCTION

Numerous studies have shown that elevated serum low-density lipoprotein (LDL) cholesterol is the crucial factor for the initiation and progression of atherosclerosis, and lowering LDL cholesterol can largely reduce the incidence and mortality of cardio- and cerebrovasculardiseases (Mamdani and Tu, 1994; Anonim, 1998; Anonim, 2002). Oxidative modification of low density lipoprotein (LDL) in artery wall is now widely regarded to play an important role in the development of atherosclerosis (Witztum and Steinberg. 2001; Steinberg *et al.,* 1989).

The oxidation of LDL gives rise to atherogenic changes including the formation of oxidized lipids which act as chemotactic and mitogenic agents and the modification of the charge on the apolipoprotein B (apo-B) moiety of LDL creating a ligand for the scavenger receptors on macrophages (Oparil and Oberman, 1999; Kullo *et al.*, 2000). With increasing evidence that LDL is oxidized *in vivo*, and that oxidized LDL is involved in atherogenesis, it is thought that antioxidants which could slow or inhibit the oxidative process may be an important therapeutic strategy to prevent and possibly to treat athrosclerosis (Steinberg *et al.,* 1989; Austin *et al*., 1988).

The use of natural products in the treatment of a variety of diseases has increased due to the considerable number of medicinalplants with proven biological activity applicable to the treatmentof some diseases. *Physalis angulata* L. is an annual herb distributed in many countries located in tropical and subtropical regionsof the world. This plant is widely used in popular medicine as atreatment for a variety of diseases and antitumour activity hasbeen demonstrated (Lin *et al.*, 1992; Chiang *et al.*, 1992).

MATERIAL

Plant Material

The herb of *P angulata* were collected at Makassar, Indonesia in April, 2009. The specimen was identified by Prof. Gemini Alam and deposited in the herbarium of the Pharmacy Faculty, Hasanuddin University, Indonesia

Reagents

Disodium ethylene diamine, butylatedhydroxytoluene, polyacrylamide, copper (II) sulfate, disodium phosphate, sodium dodecyl sulfate,sodium phosphate, sodium chloride, Human LDL, were purchased from Sigma Chemical Co. (St. Louis,MO, U.S.A.).Ethanol, Ethyl-acetate, agarose, SDS, glycine, trichloro-acetate, thiobarbituric acid from Merck.

METHODS

Extraction

The dried herb of *P. Angulata* were blended to a fine powder and one kilogram ofpowders was extracted with ethanol 96% at room temperature for one week and filtered throughfilter. The filtrates were pooled and dried in rotary evaporator. The EtOH extract (623 g) was partitioned with EtOAc with solid extraction method and dried in rotary evaporator. We were found EtOAc (153 g) and non-disolved EtOAc extract (470 g).

In vitro Oxidation Studies

Continuous Monitoring of Formation of Conjugated Dienes in LDL

LDL was adjusted to 50 mg/mL of LDL protein with 10mM PBS pH 7.4 and incubated at 30°C for 1h with one concentration of EtOH, EtOAc and non-disolvedextract of *P. angulata* L. (250 µg/mL). Oxidative modification of LDL was initiated by addition of freshly prepared 10mM $CuSO_4$solution at 30°C in water bath for 200 minutes. Conjugated dienes (Esterbauer *et al*., 1989) formation during oxidation of LDL was continuously monitored by the spectrophotometric based on changes in absorbance at 234 nm using Spectrophotometer (Agilent 8453). LDL not treated with test substances was used as a control. The results were recorded as the level of conjugated dienes at 200 minutes and the rate of dienes formation (Sattler, 1998).

Assay of the Formation of Thiobarbituric Acid Reactive Substances (TBARs)

Various concentrations (100, 200, 300, 400 and 500 µg/mL) of EtOH, EtOAc and non-disolvedextract of *P. angulata* were employed in this study. After initiating the oxidation process with 10 mM $CuSO_4$, the sample mixtures were incubated at 30°C for 5 h in a water bath and the reaction terminated by adding 50 mL of 100 mM butylatedhydroxytoluene (BHT). The following reagents, in the order of 1 mL of 10% trichloroacetic acid (TCA), 0.5 mL of 5mM disodium ethylenediamine (Na_2EDTA), and 1.5 mL of 0.61% thiobarbituric acid (TBA), were added into 1 mL of the aliquots taken from the incubation mixture and heated at 65°C for 1 h. TBARs formation was measured in a Spectrophotometer (Agilent 8453).wavelength of 515 nm. The results were recorded as malondialdehyde (MDA) equivalent content (nmol/mg LDL-protein) (Ohkawa *et al.*, 1979).

Electrophoresis of Apolipoprotein B-100 (apoB-100) Fragmentation

The fragmentation of apoB-100 following $CuSO_4$-induced oxidation was determined using SDS-polyacrylamide gel electrophoresis (PAGE) system. The oxidized LDL solution was denatured with 3% SDS, 10 % glycerol and 5% bromophenol at 95°C for 10 min. The electrophoresis was run on SDS-PAGE (4%) at 100 V for 80 min, and then stained with Coomassie Brilliant Blue R250.10 (Jeong *et al.,* 2004).

In vivo Oxidation Studies

Animal Preparation

Male mice 6 week-old.In the nonatherogenic mice, animals were fed with a normal diet and in the atherogenicmice, animals were fed with an atherogenic diet consisting of the normal diet plus 15% fatty acid, 1.5% cholesterol and 0.5% cholic acid (Takako *et al.*, 2006). Male mice were divided into five groups: Group I-negative control; Group II-EtOH 100 mg/mL; Group III-EtAOc 100 mg/mL Group IV-non-dissolved EtOAc 100 mg/mL and Group V-healthy mice. The mice were given water ad libitum.

After 8 weeks, the mice anesthetized with chloroform with use closed method. The blood sample was obtained via a heart puncture and collected into microfuge tube. The samples were centrifuged at 1500 x g for 15 minutes at room temperature and plasma was stored at -20^{0}C.

Mice aortic tissue isolation with Mika Kobayashi procedure with modification. The midline of the abdomen is incised, the thorax opened to expore the heart and lungs. The abdominal aorta is cut at the middle to release the blood and then perfused in PBS. The aorta is dissected out from the aortic arch to the abdominal aorta. A cannula is inserted into the paroxysmal portion aorta and placed them in sucrose-paraformaldehyde formal solution pH 7.4 were protected from light (Kobayashi *et al.*, 2004).

Lipid Accumulation in Aorta

After overnight in sucrose-paraformaldehyde (4 g paraformaldehyde, 5 g sucrose, 0.01 mg butylated hydroxytoluen and 0.039 g EDTA in 100mL double-distilled H_2O), samples were rised in PBS and then ethanol 7% for 1 minute. Aortic tissue was immersed in a solution of hematoxylin and eosin (HE) following a standard procedure. Intimal thickening of each aorta was estimated as an index of the extent of atherosclerosis by measuring the cross-sectional area of intima and media of five serial sections using microscope (Olympus Optical, Tokyo, Japan) (Olszanecki *et al.,*2005).

LDL oxidation

LDL isolation. Lipoproteins were isolatedfrom serum using density-gradient ultracentrifugation, as described by Havel et al.Lipoprotein fractions were isolatedfrom 1 mL of serum using a. Serumwas transferred to a tube and density was adjusted to 1.006,1.019 and 1.063 g/ml with the same volume of KBr solution.Serum was divided into three lipoprotein classes by density:very low density lipoprotein (VLDL, d 1.006); intermediatedensity lipoprotein (IDL, 1.006<d<1.019); LDL (1.019<d<1.603). The appropriate times were calculated to be 16 hfor VLDL, 18 h for IDL, and 20 h for LDL isolation at 4°C (Havel *et al*., 1955).

Electrophoresis of LDL.Electrophoresis of LDL using SDS-polyacrylamide gel electrophoresis (PAGE) system. The LDL solution was denatured with 3% SDS, 10 % glycerol and 5% bromophenol at 95°C for 10 min. The electrophoresis was run on SDS-PAGE (4%) at 100 V for 80 min, and then stained with Coomassie Brilliant Blue R250.10 (Jeong *et al.,* 2004). Lipoprotein profiles were analyzed using densitometer at 630 nm with WinCATSCamag.

RESULTS AND DISCUSSION

P. angulata L. is considered to be a valuable natural substance,the herb has been reported to contain many steroid such aswithangulatinand physalin (Brustolin *et al.*, 2010). Thus, it has been reported to have a variety of biological effects including antioxidant, antimicrobial, antitumor, anti-inflammation and anti-protozoa (Fang *et al.*, 2010; Maldonado *et al.*, 2010)

Recently, it has been reported that the oxidative modification of LDL (Ox-LDL) is the major factor that stimulates thedevelopment of atherosclerosis. Therefore, the major

objective of this study was to determine the antioxidant effects ofthe various extracts from *P. angulata* L. herb using the invitro and in vivo models.

In in vitro model we used $CuSO_4$-induced LDL oxidation.To investigate the potential effect of *P. angulata* L. in the inhibition of $CuSO_4$-induced LDL oxidation, the formation of conjugated dienes and TBARs was determined. Currently, formation of conjugated dienes, measured at 234 nm, is considered the most appropriate marker of LDL oxidation.Theformation of conjugated dienes represents an early stage inthe oxidation process whilst TBARs formation represents alate stage in the oxidation process.

Continuous Monitoring of Formation of Conjugated Dienes in LDL

In this study, we examined the inhibitory activity of various extract of *P. angulata* L. against the LDL oxidation mediated by$CuSO_4$. The oxidation of LDL was assessed by the formation of conjugated dienes. As presentedin Fig. 1, the reaction kinetics of diene formation consists ofa lag phase characterized by a low oxidation rate due to theconsumption of endogenous antioxidants, a second phase ofmaximal rate of oxidation that starts when the antioxidantsare consumed, and a third terminal phase with a plateau indiene formation.But in the Fig 1. We doesn't found the lag fase but we can see that EtAOcextact has a stronger activity than non-dissolved EtAOc and EtOH extract.

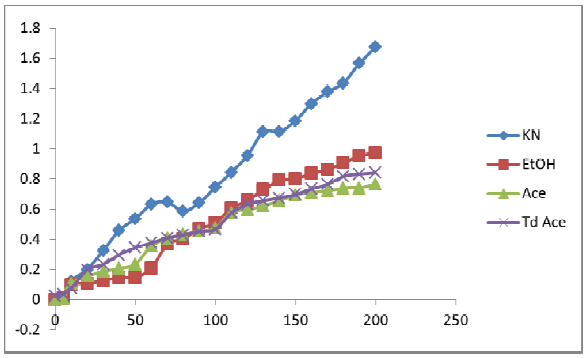


Figure 1. Effects of various extract of *P. angulata* L. on the formation of conjugated dienes of $CuSO_4$-induced LDL oxidation. Continuous monitoring of formation of conjugated dienes in LDL.

Assay of the Formation of Thiobarbituric Acid Reactive Substances (TBARs)

Subsequently, the oxidation of LDL initiated by $CuSO_4$andthe azo compound was measured by the formation of malondialdehyde (MDA) using the TBARS assay. As shown inTable 1, comparable results were obtained. EtAOc extract exhibited the strongest inhibitory activity against $CuSO_4$ andAAPH-mediated LDL oxidation with IC50 values of 143.8 ppm, respectively, followed by EtOH (IC_{50}334.5 ppm). Non-dissolved EtAOc extract did not show inhibitory activity in LDL oxidation.

Table 1. Effects of various extract of *P. angulta*L. on LDLoxidation mediated by $CuSO_4$

Extract	IC_{50} (ppm)
EtOH	334.5
AtOAc	143.8
Non-dissolved AtOAc	>500

Electrophoresis of Apolipoprotein B-100 (apoB-100) Fragmentation

It has been reported that products of lipid peroxidationsuch as oxidixedphosphatidylcholine or MDA could causefragmentation of apoB-100, a major component of LDL, we examined the effect of these extract on the fragmentation of apoB-100 using electrophoretic analysis. As shown in Fig. 3, SDS-PAGE analysisrevealed that incubation of LDL with $CuSO_4$led to a loss ofapoB-100. When the

LDL (50 mg/ml in PBS) was incubatedwith $CuSO_4$ alone, the apoB-100 band completely disappeared, whereas in the presence of extract, the fragmentationof apoB-100 was inhibited in a dose-dependent manner.Under the same condition used, EtAOc extract. Itis known that transition metal ions and free radicals are involved in LDL oxidation in vivo. We subjected LDL tooxidation by copper ions and free radicals directly generatedby AAPH. The mechanism inhibit LDL oxidation is unknown. Since they could inhibitboth $CuSO_4$ and AAPH-mediated LDL oxidation, they may actas antioxidants by chelating metal ions and scavenging freeradicals. Furthermore, we found that EtAOc extract had the capacityto protect the fragmentation of apoB-100.

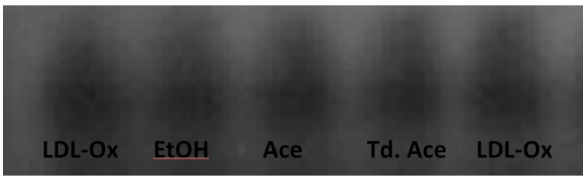
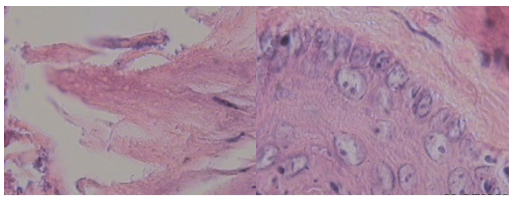


Figure 2. **Inhibition of $CuSO_4$mediated ApoB-100 fragmentation in LDL**

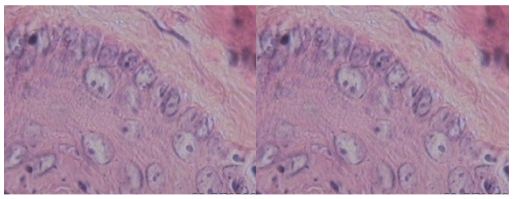
In vivo Oxidation Studies

Table 2. Histological analysis of mice aorta

Group	Hyperplasia			Lipid Acumulation
	T. Adventitia	T. Media	T. Intima	
Normal Control	0	0	0	1
Negatif Control	2	2	2	4
EtOH 100 mg/mL	2	1	1	2
EtAc 100 mg/mL	1	1	1	2
No-EtAc 100 mg/mL	2	2	2	3



Negatif Control EtOH 100 mg/mL



EtAc 100 mg/mL No-EtAc 100 mg/mL

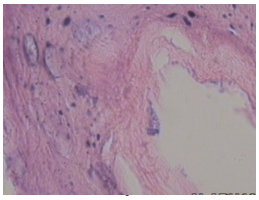


Figure 2. Comparative hematoxylin and eosins staining of atherosclerotic aorta wall.

LDL oxidation

Table 3. shows the levels of LDL isolated from the blood of normal and cholesterol fed mice. Thestronger inhibition to 24.084% level of LDL was observedwith the 100 mg/mL dose of EtAc.

Table 3. Inhibitory of LDL oxidation

Sampel	**AUC**	**% inhibition**
Normal Control	7396.077	43.64769
Negatif Control	13124.710	
EtOH 100 mg/mL	9963.777	24.084
EtAc 100 mg/mL	7767.527	40.818
No-EtAc 100 mg/mL	9296.050	29.171

Figure 3. LDL isolated from mice plasma, stained with Coomassie Brilliant Blue R250.10. (A : Control, B-D : EtOH 50, 100, 150 mg/ml, E-G : EtOAc 50, 100, 150 mg/mL, H-J : No-EtAc 50, 100, 150 mg/L, K : Control

CONCLUSION

In conclusion, our results showed that the EtAOc extractsfrom herb of *P. angulata* L. posses as potent, to protection ofLDL oxidation induced by $CuSO_4$ in vitro, and posses as potent to protection mice from atherosclerosis.

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ANTIDIARRHEAL ACTIVITY OF ETHANOL EXTRACT OF SARANG SEMUT *(MYRMECODIA TUBEROSA)* IN EXPERIMENTAL ANIMALS

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ABSTRACT: *Myrmecodia tuberosa* is used as remedies for many ailments such as treatment of hyperuricemia, anticancer, and anti hemorrhoid, but it has not been evaluated for its pharmacological activity as antidiarrhea. This study was therefore aimed to evaluate scientifically the antidiarrheal activity of ethanol extract of *Myrmecodia tuberosa*, using castor oil induced diarrheal model in mice. Mice were divided into five groups of five mice each. Group one served as control (CMC Na 0.5 %, 1 ml/20 g bw p.o), groups two, three, and four received ethanol extracts (15, 25, 35 mg/ 20 g bw p.o respectively) while group five received standard drug loperamide 0.21 mg/20 g body weight p.o. These doses were taken 1 hour and 30 minutes after castor oil administration (0.715 ml/20 g bw p.o). The results showed that there were no significant differences (P>0.005) in the frequency of defecation, consistency of faecal matter and total weight of faecal output in mice treated by ethanol extracts (15, 25, 35 mg/20 g bw) in comparison to those of standard drug loperamide group at sampling time of 180-360 minute. On the other hand, there were significant differences in the above parameters of ethanol extracts compared to control group. These result indicated that the ethanol extract of *Myrmecodia sp* possesses antidiarrheal activity.

Keywords : *Myrmecodia tuberosa*, sarang semut, antidiarrheal activity, castor oil induced diarrhea.

INTRODUCTION

Indonesia is well known as a country which has many medicinal plants. One of them is *Myrmecodia tuberosa* and variously known as sarang semut (Indonesia). It is found in Ambon, West Sumatra, North Sulawesi and Kalimantan and used as remedies for many ailments such as treatment of hyperuricemia, anticancer, and anti hemorrhoid (Srikandi and Syamsir, 2006)

Diarrhea is an increased frequency and decreased consistency of fecal discharge as compared to an individual's normal bowel pattern (Dipiro et al, 2005). It results from an imbalance between the absortive and secretory mechanism in the intestinal tract (Gandhimathi et al, 2009) and recognized as an important health problem in developing countries especially in Indonesia. People in Kalimantan, Indonesia use *Myrmecodia tuberosa* traditionally for treating diarrhea infections but it has not been evaluated for its pharmacological activity as antidiarrhea. Therefore, the purpose of the present study was to evaluate the antidiarrheal activity of ethanol extract of *Myrmecodia tuberosa* in experimentally induced diarrhea in mice.

MATERIAL AND METHODS

Collection of Plant Materials

Myrmecodia tuberosa sample was collected at 10 am in Loksado forest, Hulu Sungai Selatan, South Kalimantan. It was authenticated by Laboratorium Dasar of Biology Department FMIPA Universitas Lambung Mangkurat.

Preparation of Plant Extract.

The fresh herb of *Myrmecodia tuberosa* was washed finely, chopped into small pieces, air dried under shade and ground into powder form and stored in an airtight container at room temperature (Innayatullah et al, 2010). 200 g powder was macerated in 2 L 96 % ethanol for 48 hours at room temperature with occasional shaking. The extract was concentrated under reduced pressure below 50ºC through rotary evaporator. The filtrate was then transferred to an evaporating dish and was evaporated using a water bath. The concentrated extract was kept in refrigerator at 4ºC and prepared freshly by each study (Mohammed et al, 2009 and Innayatullah et al, 2010)

Phytochemical screening

Ethanol extract of *Myrmecodia tuberosa* was subjected to phytochemical screening testing for the presence of alkaloid and tannins. These were identified by charasteristic colour changes using the standard methods (Trease and Evans, 1983).`

Antidiarrheal Activity

Experimental animals

Male mice (25-30g) obtained from the animal unit, Pharmacology laboratorium, PS Farmasi FMIPA Universitas Lambung Mangkurat. The animals were maintained on standard animal feed and water. They were in the laboratory for a minimum period of 10 days prior to experimentation. All experiments were performed after an overnight fast (Owalabi et al, 2007).

Castor Oil Induced Diarrhea

Mice were randomly divided into five groups of five animals each. Group one served as control (CMC Na 0.5 %, 1 ml/20 g bw p.o), groups two, three, and four received ethanol extracts (15, 25, 35 mg/ 20 g bw p.o respectively) while group five received standard drug loperamide 0.21 mg/20 g body weight p.o. These doses were taken 1 hour and 30 minutes after castor oil administration (0.715 ml/20 g bw p.o). the animals were placed on individual cages over clean filter paper. The following parameters were observed every 30 minutes in a period of 6 hours the frequency of defecation, consistency of faecal matter and total weight of faecal output in mice. (Adnyana et al, 2004 and Sugiarto, 2008).

Statistical Analysis

The results were analysed using Tukey HSD test and were regarded as significant when $P < 0.05$.

RESULTS AND DISCUSSIONS

Castor oil is a well known induction of diarrhea which attributed to its active ingredient ricinoleic activity. It irritates the intestinal mucosa causing inflammation and stimulates the production of chemical mediator substance such as prostaglandin. Release prostaglandin is a major cause of arachidonic acid induced diarrhea (Owalabi et al, 2007). It stimulates gastrointestinal secretion, motility, epithelial permeability and edema of the intestinal mucosa (Ezenwali et al 2010). Therefore this model is used to establish the antidiarheal activity of ethanol extract of *Myrmecodia tuberosa* in mice.

The consistency of faecal matte, total weight of faecal output and frequency of defecation of all groups in a period of 6 hours as shown in fig 1, 2 and 3. The effects of both extract 35 mg/20 g bw and loperamide in a periode of 300-360 minute can decrease concistency of faecal matter as shown in fig 1. It means that there is no diarrhea in 360 minute.

Fig 2 shows that both extract 35 mg/20 g bw and loperamide can decrease total weight of faecal output in a periode of 300-360 minute. It means that plant extract in dose 35 mg/20g bw gave antidiarrheal activity.

The effect of ethanol extract of *Myrmecodia tuberosa* 25, and 35 mg/20 g bw in a period 300-360 minute can decrease frequency of defecation that there is as shown in fig 3. They have similiarly effect to standard drug, loperamide.

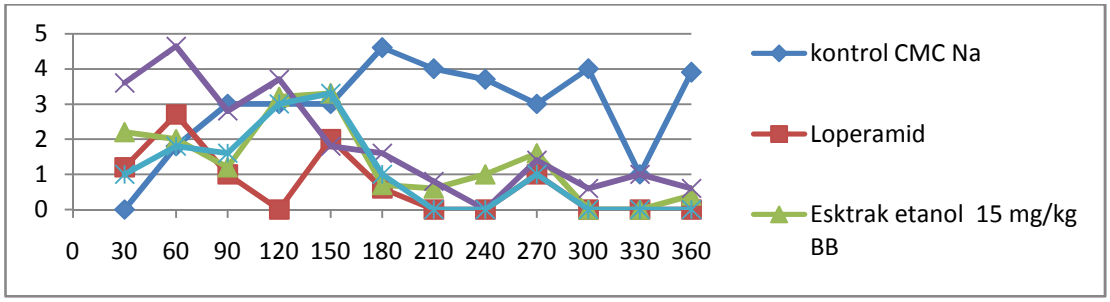

Figure 1. Graphic of consistency of faecal matter in a periode 360 minute

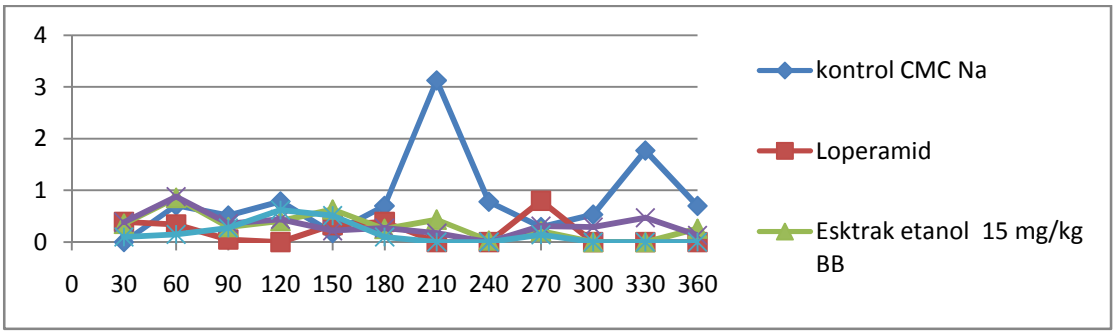

Figure 2. Graphic of total weight of faecal output in a periode 360 minute

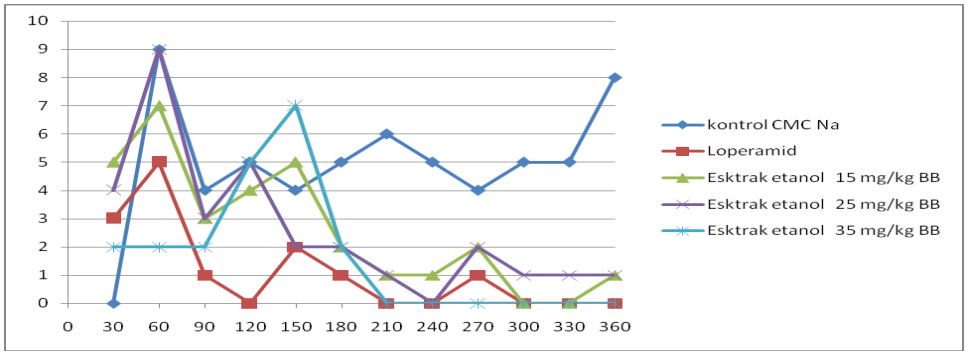

Figure 3. Graphic of frequency of defecation in a periode 360 minute

On its antidiarrheal effect, the extract produced an inhibition of castor oil induced diarrhea with the 35 mg/ 20 g bw dose giving the highest effect from any other extracts on the number, weight and consistency of stool produced. The result of analytical statistic is shown in table 1.

All doses of ethanol extract of *Myrmecodia tuberosa* significantly ($p<0.05$) decreased the number, weight and consistency of stool produced as compared to the castor oil treated control groups. Loperamide as standard drug at a dose 0.21 mg/20 g bw

po also produced a marked antidiarrheal effect and there is no significant difference ($p>0.05$) between ethanol extract of *Myrmecodia tuberosa* and Loperamide.

Table 1. Antidiarrheal activity of the extract and loperamide on castor oil induced diarrhea in mice

Action	Weight	Consistency	Frequency
Loperamid	0.160±0.03578	0.1160±0.37119	0.2000±0.44721
Dosis 15 mg	0.2100±0.19455	0.7640±0.19034	1.2000±0.44721
Dosis 25 mg	0.3320±0.36969	0.7340±0.67136	1.2000±1.09545
Dosis 35 mg	0.0280±0.06261	0	0.2000±0.44721
Na-CMC	1.4560±1.01458	3.2160±0.61358	6.6000±1.67332

The phytochemical screening of ethanol extract of *Myrmecodia tuberosa* revealed presence of tannins but flavonoid and saponin were absent. Tannins may be responsible for antidiarrheal activity of *Myrmecodia tuberosa* by stimulating the normalization of the derayed water transport accross he mucosal cells (Oben *et al.*, 2006). Therefore the extract of this plant can reduce gastric contents and watery texture of diarrheal stools also gastrointestinal motility (Ezenwali *et al.*, 2010).

CONCLUSION

The results of this study can produce scientific basis for the traditional use of Myrmecodia tuberosa as an antidiarrheal activity. The ethanol extract of this plant has a potential effect as a remedy for diarrhea. Further studies are necessary to improve the safety of this traditional herbal and isolate the antidiarrheal constituents of this plant.

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PESTS OF *STELECHOCARPUS BURAHOL* (BLUME) HOOK. F.&THOMSON IN PURWODADI BOTANICAL GARDEN

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ABSTRACT: *Stelechocarpus burahol* is local fruit that has use as medicinal plant. The species is important to be conserved and cultivated because the plant population is rare and tend to threat in the wild, specially in East Java. Inventory of pests attacking the plants was needed to manage the plants in otder to grow well in cultivation. Reseach to invent the pests of *Stelechocarpus burahol* was conducted in Purwodadi Botanical Garden in 2009 by direct observation in the fields.The pests obtained attacking the plants were identified. The results showed that there were 6 species of the pests attacking the plants those were 5 species attacked flowers/and young fruits ; and 1 species attacked leaves. Aphid and white fly was serious pests that attacked the flowers and the fruits.

Keywords **:** Pest, *Stelechocarpus burahol*

INTRODUCTION

Stelechocarpus burahol is a local fruits that grows and distributes in Java, from West to East Java. It is sometimes planted by people in home garden or other places for ornamental plant. However, it is rare to be obtained in the wild, specially in Java. The species needs to conserve and to develop for plant genetics and species conservation because it has potencies as traditional medicinal and fruit plant. It was reported that the species was included one of developed prominent plants to become phytopharmaca and Standardized Herbal Medicine (Technology Indonesia, 2009). The ripe fruit which was eaten fresh can produce aroma like flower of violtjes in urine, sweat and breath. Its kernel also as diuretic, preventing to chafe kidney and good for preventing pregnancy (Sanarto, 1992) ; its leaf has use to degrade kholesterol and heal blood-vessel acid (Siswono, 2008).

Pests are important to control for seccesfully plants cultivation because they will influence the plants growth and development. The pests will destruct on the plants organs such as leaves, roots, stems, flowers and fruits. Their attacking will decrease quality and quantity of the plants products such as fruits, leaves and flowers. The pest attacking of cocoa pod borer (*Conomorpha cramerella* Snell) on fruits reached 90.35 % (Sulistyowati, Wardani and Mufrihati, 2005); on forest trees to 60% with the total cost reach 21.6 billion (Tambunan, 2008). The pest attacking of *Orgyla postica* in nursery of *Acacia* reached 80-100% (Dendang and Sudomo, 2008).

The objective of this research is to invent the animal pests on *Stelechocarpus burahol* in Purwodadi Botanical Gardens

MATERIALS AND METHODS

Inventory of the pests attacking *Stelechocarpus burahol* was conducted in Purwodadi Botanical Garden in 2009 on plants specimen collected in the gardens in plots XII.G.D.4; XIV.G.I.13; XVIII.C. 10-10a-10b that were collected from Java.

Purwodadi botanical Garden is located at 300 m above sea level; rainfall 1763.9 mm/year; air relative humidity 76.73 %; average minimum temperature 20.4 ^{o}C; and everage maximum temperature 31.46 ^{o}C (Anonymous, 2010).

The pests infesting the plants were catched, identified (Borror, Delong and Triplehorn, 1979) and documented. The presentage of the pests attacking the plants was calculated by formula:

Attacking percentage = Plants infested / Total number of plants specimen x 100

The seriousness of the pests attacking was scaled according to Likert scales as follows:

5 : very serious
4: serious
3: rather serious
2: less serious
1: not serious

RESULTS AND DISCUSSIONS

There were four pests that founded attacking all of the plants specimen (100 %) that were white fly (*Dialeurodes citr),* phids(*Myzus persicae),* leaf roller (*Archips absoletania* Walker), and ant (Table 1). Two of them, (white fly (*Dialeurodes citri*) and aphids(*Myzus persicae)* attacked leaves, flowers, and young fruits. The pests that attacked specific organs were the leaf roller that attacked the young leaves; and squirrel that attacked the mature fruits.

Table 1. Attacking percentage of the pests on *Stelechocarpus burahol*

No.	Species	Attacking percentage	Explanations
1	White fly (*Dialeurodes citri)*	100	Leaf, flower,fruit
2.	Mealy bug (*Pseudococcus citri* Risso)	20	Leaf, flower,fruit
3.	Aphids (*Myzus persicae)*	100	Leaf, flower,fruit
4.	Squirrel (*Callosciurus notatus)*	20	Fruit
5.	Leaf roller (*Archips absoletania* Walker)	100	Leaf
6.	Ant (*Monomorium minimum* Buckley)	100	Flower,young fruit

The seriousness of the pests attacking can be showed by the total value of seriousness according to Likert scales on the Table 2. The Table showed that there were two pests causing serious damage on the plant organs that were, (white fly (*Dialeurodes citri*) and aphids(*Myzus persicae)* that the total value pest attacking reached 8. White fly attacked the leaves, flowers (especially female flowers) and young fruits. The young flowers or the young fruits was the most organs attacked by the white fly. The attacking value of this pest on the young flowers reached to 4.

Aphid was also a serious pest on *Stelechocarpus burahol* in Purwodadi Botanical Gardens. It attacked and destructed the young leaves, the flowers (male and female flowers) and the young fruits (Table 2). The Table showed that the pest attacking on the flowers was serious (4) and the attacking on the young fruits was rather serious (3).

Table 2. The seriousness of the pests attacking on *Stelechocarpus burahol*

Pests	Organ			total
	Leaf	Flower	fruit	
White fly (*Dialeurodes citri)*	1	4	3	8
Mealy bug (*Pseudococcus citri* Risso)	1	1	1	3
Aphids (*Myzus persicae)*	1	4	3	8
Squirrel (*Callosciurus notatus)*	0	0	2	2
Leaf roller (*Archips absoletania* Walker)	2	0	0	2
Ant (*Monomorium minimum* Buckley)	0	2	1	3

1. Aphids (*Myzus persicae*)

Figure 1. Aphids (*Myzus persicae*)

Aphid is relative small insect, 0.5-2 mm long, softy body, light green or yellowish green or blackish, winged or not; on horny on the tip of abdomen. The females have hight reproductivity that can produce thousands of nimphas in 4-6 week. Most of the aphids are wingless and walk slowly from place to place about 5 cm in a minute. The Aphids have already been described as pests on many crops such as *Cucumis melo, Solanum melongea, Andrographis paniculata, Citrus spp.* and *Sauropus androgenus*. They feed by sucking the sap from the tender plants, often causing the plants to become deformed on the leaves (curling), distortion, stunting and retarding plants organs growth.

The insects produce sweetish honeydew which is attractive to ants and on wich certain fungi grow. The aphids are also the carriers or vector of certain plants diseases plants such as Potato Virus A (PVA), Potato Virus Y (PVY) and Potato Virus Mosaic (PVM) (Solikin, 2009). The pest is attracted to yellow colour so the border plants that have the same colour can reduce attacking flowers of kepel.

Figure 2. Lady beetle (*Coccinella trasversoguttata*) eated the aphid

Natural enemies can be very important in the control of aphid, especially in the garden not sprayed with broad spectrum pesticide that kill natural enemy species. Many predators feed on aphids such as lady beetle and lacewing. Weather can also impacts aphids.

High levels of nitrogen fertilizer favor to aphid reproduction. Never use more nitrogen than necessary. Use less soluble forms of nitrogen and apply it in small portions througt the season rather than all at once. Biopestiside such as garlic can be used to control the aphids

2.Whitefly (*Dialeurodes citri*) Order Homoptera, Family Aleyrodidae

This insect attact fruits and leaves of fruits plants such as *Syzygium cumini, Syzygium javanium* as *Citrus* spp by sucking saps on young organs such as the flowers or the fruits. The flowers or the young fruits dried and fall because of saps loss. Infested trees have a blackened appearance due to fungi which grows in the honeydew given off by the whitefly nymphs. This sweetish, sticky honeydew is discharged in large quantities from alimentary tact. It is a serious pests of *Stelechocarpus burahol* in dry season.

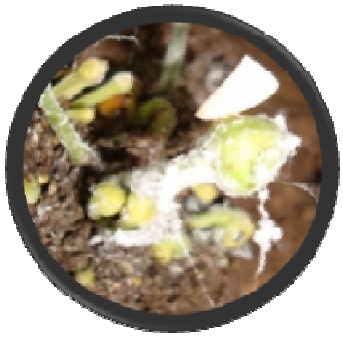

Figure 3 Whitefly (*Dialeurodes citri*)

All stages of the insects may be found throughout the year. The eggs are pale yellow; those of the cloudy-winged white fly are black, and those of the woolly whitefly are brown and curved like miniature, fat sages. They hatch in from 4-12 days into active pale yellow flattened six-legged'crawlers' or nymphs. They move about for a short time, mainly on the lower sides of the leaves, as they avoid strong light. These crawlers soon insert their beaks into the leaves ad begin sucking the sap, they soon

molt, losing their legs in the process, and then have the appearance of minute, flattened, oval bodies. After two more molts, the adults emerge. They are four-winged insects about 1/12 inch long. They have a white appearance because of the fine white powder which completely covers the wings and body. Controlling the pest can be conducted by biopestiside such as neem and garlic.

3. Leaf roller (*Archips absoletania* Walker). Order Lepidoptera; Family Tortricidae

Figure 4. Leaf roller (*Archips absoletania* Walker).

Small greenish or bronze caterpillars, ½ inch long, fold a roll leaves, fastening them together and feeding within. This pest attack young leaves of trees, vegetables, and ornamental shrubs and flowers. In the garden the pest also attack *Manilkara microsperma*. The moths lay the eggs 20-120 on undersides of the leaves. These eggs hatch in about 1 week and the caterpillars coming from them feed at first on the under surface of the leaves, under a silken cover, migrating when about half grown to the upper side of the leaves, where they fold a leaf about themselves in the characteristics manner, holding it with fine silken threads. They feed for 35 – 50 days and then transform into brown pupae inside the folded leaves. Biological control can be conducted by ants because they can kill and eat the pest.

4. Ants

Figure 5. Ants : a. Brown (right); b.Black (left)

Ants are not primer pest on *Stelechocarpus burahol,* however they make mutualism simbioses with aphids, white fly and mealybug. The aphids produce sweet honey dew that attract the ants to suck this material as the ants food. The ants keep the aphids from attacking other animal. There are two species of ants ; brown and black ants. The black ants are often founded with black ants. Ants have extremely varied diet. They eat seeds, fruits, insect and almost all organic matter except plants stems and leaves. Ants often pick up the aphids and carry them to new shoots in less than two minutes (Albert Burchted, May, 3, 2009).

Nguyen van chung (2010) reported that to control the ants can be conducted by giving trap such rotting fish sowing 0.5 m^2. The ants will come ant eating the fish then the fish was burned (in dry season). In rainy season the ants make nest in dry place so by hunging the dry of leaves or grasses they will assemble and concentrate in this place than the leaves or the grasses were burned,

5. Squirrel (*Callosciurus notatus*)

Figure 6. Squirrel (*Callosciurus*

Squirrel usually attack plants by eating the fruits. In the garden there are many squirrels that attacking the kinds fruits such as *Cocos nucifera, Adansonia digitata, Mangifera indica* and others. The mature fruits of *Stelechocarpus burahol* was also eated by the squirrel. To control the Squirrel the fruits be wrapped by paranets .

6. Mealy bug

Mealy bug attack leaves or fruits of many cultivated plants species in the field such as *Citrus* spp; *Annona muricata* and *Anthurium spp*. It attacked fruit of *Stelechocarpus burahol* by sucking fruit sap,

CONCLUSION

There were six pests infesting and attacking *Stelechocarpus burahol* in Purwodadi Botanical Garden. They attacked leaves, flowers and fruits, Aphid (*Myzus persicae*) and white fly (*Dialeurodes citri*) were serious pests attacking the plant.

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PREPARING AND IMPLEMENTING MODULE ON HERBAL MEDICINE FOR MEDICAL STUDENT

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Abstract : The concept and practice of herbal medicine among medical doctors, who were trained according to the western medical education, are not yet fully accepted. Nevertheless, since 1995, the Ministry of Health of Indonesia instructed through a decree (SK Menkes No. 0584/Menkes/SK/VI/1995) that every public health services should have a Center of Development and Application of Traditional Medicine. In medical schools, students are taught exclusively on modern-medicine approach for treating the diseases. Though Indonesia is the second nation after Brazil on biodiversity, but, unfortunately, the development of herbal medicine is lacking. Although most of the people still using "jamu" (traditional herbal preparation) as it is, some producers have been repackaging the jamus into capsules, tablets or powder to give them more convenient to be used. Some of the jamus have been through pre-clinical or toxicological studies, but only few have been clinically studied. Since 2006, the Faculty of Medicine of Universitas Kristen Indonesia (FM-UKI) has been implementing the new curriculum, named Competence-based Curriculum, of which problem-based learning is the main method of learning. After 3 years of implementation, the Medical Education Unit (MEU) asked the Dept. of Pharmacology to set up a special module on Herbal Medicine that should be offered for the students at the end of their pre-clinical years. The module consists of theoretical and practical aspects of herbal medicine. The students learn the basis of herbal medicine and have an experience on how the herbal are grown, harvested, selected, tested and prepared. At the end of the course students gave their comments on their experience.
This paper will discuss the module and the experience on implementing the module for medical students

Keywords : herbal medicine, conventional medicine, competence-based curriculum, medical school

INTRODUCTION

Traditional medicine in Indonesia has been practiced since hundred years ago, and known as indigenous knowledge, the practice of traditional medicine are orally transcribed from generations to generations. Though, some were carefully recorded in traditional writings like lontar and pustaha. As we observe in traditional chine medicine (TCM) and Ayurveda, they build their traditional healing ways based on their philosophical paradigm on health and sickness which has inter-related between micro and macro-cosmos, between body and spiritual balance (Schwarz, 2004; Wong, 2006; and Maiers, 2009) On the other hand, the philosophical background of Indonesian traditional medicine (ITM) has not been fully explored, although some practitioners in ITM recently published books which reveal the paradigm of ITM.

Jamu, a specific name given to indonesian herbal preparation, has been officially branded as the national herbal medicine and since then, Jamu is extensively researched by private and governmental research agencies. Indonesia considers jamu now as one of the Indonesia's national excellences which should be propagated into world like TCM. At the moment, the herbal medicine in Indonesia is categorised into 1. Jamu (a traditionally prepared herbal medicine), 2. Standardised herbal medicine (herbal medicined which has passed at least, toxicity test) and 3. Phytopharmaca (herbal medicine that has passed clinical trials).

The main concern is now, from the perspective of conventional/modern medicine, how to increase the awareness and usage of ITM by the medical doctors, who are trained by modern (west) medical system. A quick glance on medical education in Indonesia is briefly discussed in the following passages.

Medical Education in Indonesia

In the 14th century, the westerners (Portuguese & the Netherlands) came to Indonesia looking for spices, of which, at the end brought to colonialism to Indonesia. On 2nd Januari 1849, Medical education in Indonesia was introduced, with the main objective is to produce local "barefooted" doctors to help the Dutch doctors in vaccination against variola outbreaks, also they teached local people on primary health care issues, such as sanitation, which reduced the diarrhea outbreaks significantly. In 1875 the curriculum and objective of the school was revised and aimed not only to produce vaccination's assistant but medical doctor named "Dokter Jawa" or Java's doctor. In 1899 in Jakarta, **STOVIA (*School tot Opleiding voor Indische Artsen*)** was established and the school was changed many times into ***School tot Opleiding van Inlandsche Geneeskundigen***, and ***School tot van Inlandsche Artsen*** respectively.

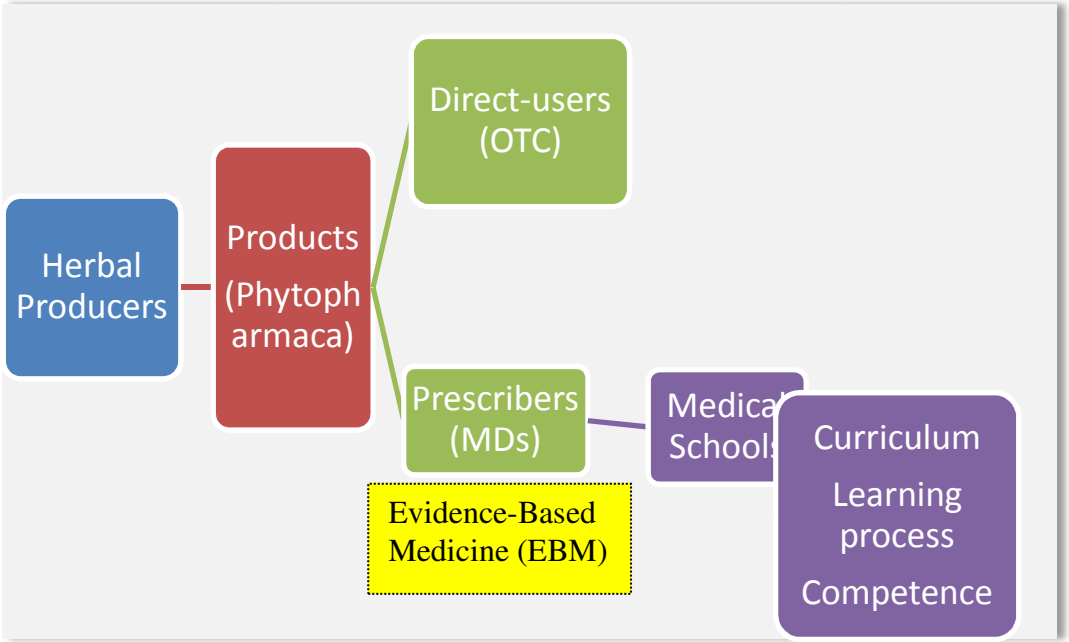

Figure 1. Diagram of the interconnections between herbal (medicine) producers and conventional medicine

Although in 17 August 1945 Indonesia declared its independence, but up to 1950s – the medical education system was still greatly influenced by the Dutch (colonial) system. From 1950s to the present time there are some minor and major changes of the medical education system. One of the major changes was in 2003 as the Directorate General of Higher Education through its decree anounced that all medical schools should use the emphasize competence in accordance to the new paradigm of medical doctors instilled by World Federation of Medical Education (WFME), that leaded to the establishment of competence-based curriculum or Kurikulum-berbasis kompetensi (KBK).

Meanwhile, in 1995 the Ministry of Health through its decree announced that every governmental health services should have a Center of Development and Application of Traditional Medicine. Unfortunately, only few of governmental hospitals follow this decree, among others are Sutomo General Hospital in Surabaya-East Java, followed by General Hospital in Malang-East Java and Cipto Mangunkusomo Hospital in

Jakarta. Thus, in nutshell, the medical education in Indonesia is greatly influenced by the western/conventional medicine.

Medical doctors are accustomed to use (chemical) medicine which has been proved by clinical trials. Therefore, when they are willing to use herbal medicine, they demand also (clinical) evidence. As we all know, clinical trial is a painstaking scientific methods of proving the efficacy and safety of drugs applied to the real patients. Moreover, the studied-drugs have to be evaluated and compared with the available medicines not only to placebo (head-to-head comparison). Therefore, only phytopharmacas would have been prescribed by the doctors.

Evidence-based Medicine

Evidence-based Medicine (EBM) is the conscientious, explicit and judicius use of current best evidence in making decisions about the care of the individual patient (Sackett, 1996). The practice of EBM means integrating *individual clinical expertise* with the best available *external clinical evidence* from systematic research (Balatbat, 2008). The "least evidence" lies at the bottom of the hierarchical pyramid of the evidence (see Fig. 2) such as laboratory studies and animal research while the "best evidence" in clinical context is systematic reviews.

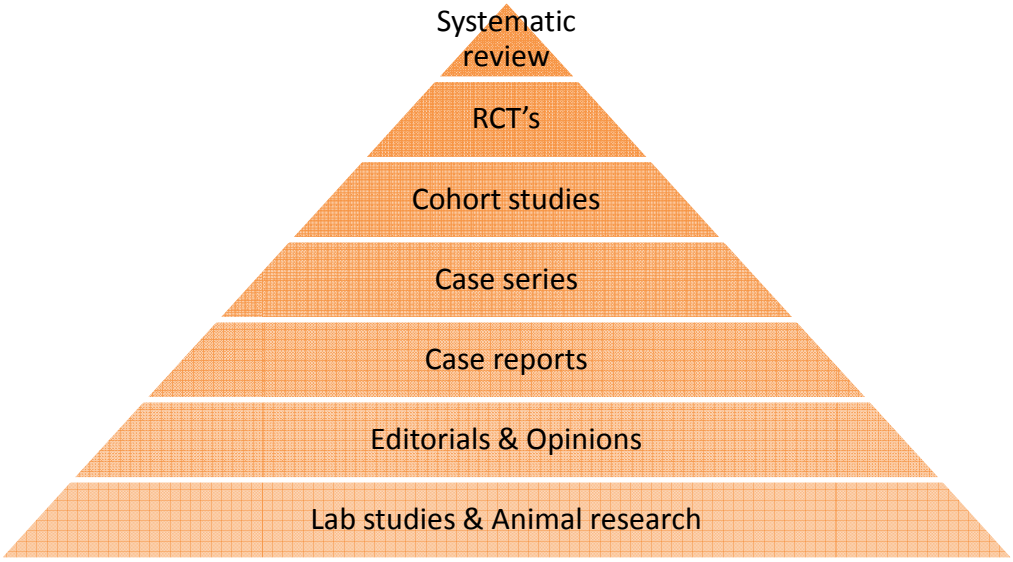

Figure 2. The pyramid of Evidence-Based Medicine

By far, most of the herbal studies done in Indonesia are animal studies, and only a small numbers studies go directly to clinical trial. This can be seen, that we only have 5 phytopharmacas registered although there are more than 100 herbal with various active substances available. While most of the people is still using jamu and standardised herbal medicine, then people think, there is no need to invest for clinical trials to produce phytopharmaca. Therefore, it is a big challenge to those who are interested to develop extended clinical evidence or efficacy and safety profile of any given herbal medicine (Verma, 2008; Anonymous, 2009). However, the debates between modern and traditional medicine is never end. Most people who practice modern medicine calls the traditional, phyto- or herbal medicine as "alternative" thought the modern medicine actually is a "deviation" or the true "alternative" medicine according to traditional healers, while traditional medicine have been practiced thousand of years throught the world (Kheel, 1989).

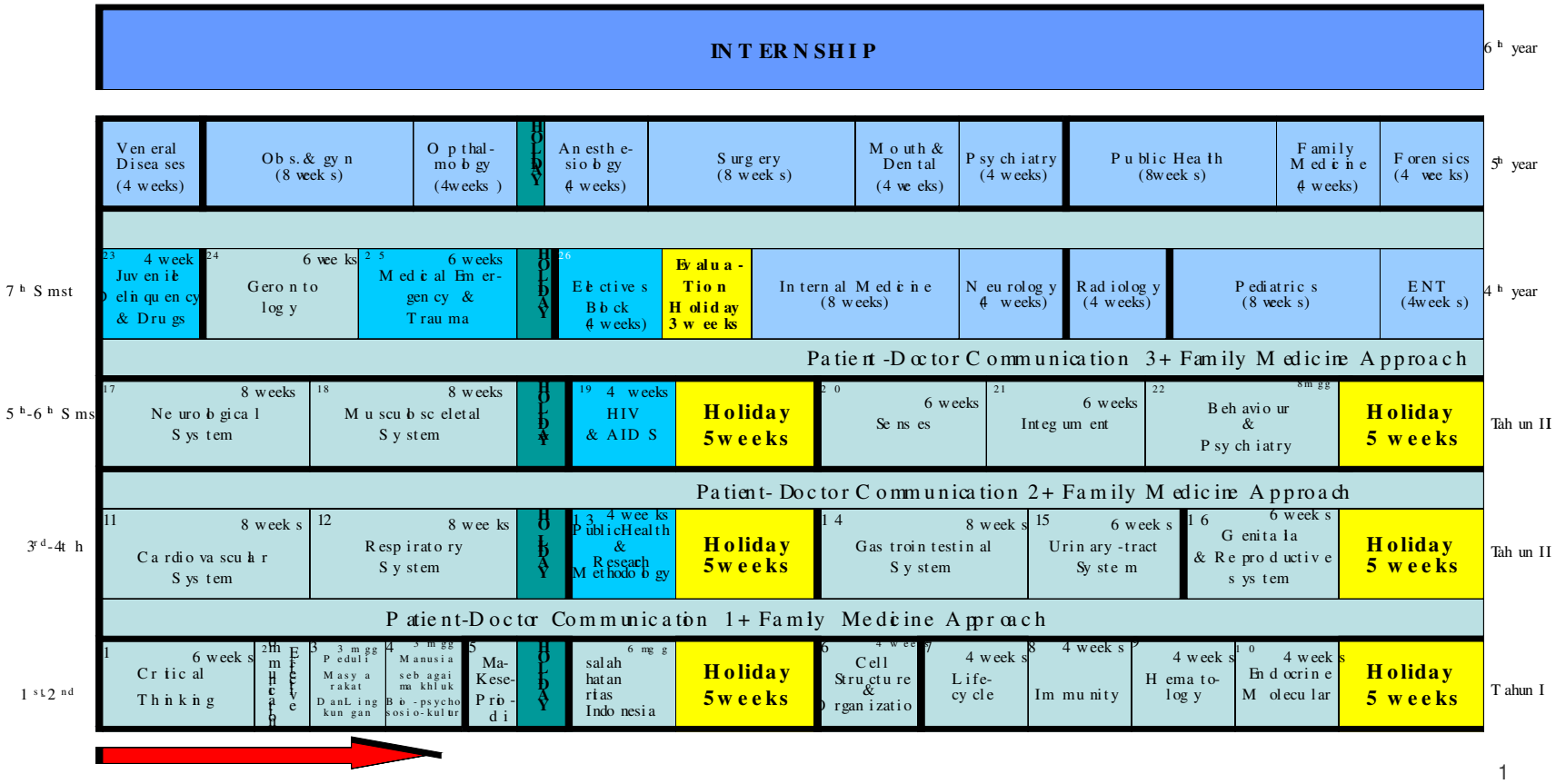

Figure 3. The map of competence-based curriculum developed in 2005 and firstly implemented in 2006

The Herbal Medicine Block

Block 26 comprises of sub-blocks: Herbal Medicine, Entrepreneurship and Disaster Management. Students are free to choices which sub-block he or she wants to apply. The main objective Herbal Medicine Block is to introduce students on the use and further development of herbal medicine. The duration of the block is 4 weeks and during the study students will learn 3 scenarios:

1. Marketing of Phytomedicine. The learning objective of the scenario is to introduce the phytomedicine and its usage in the market.
2. Diarrhea (hygienic aspects of herbal medicine). In this scenario, the students learn some important aspects of preparation, producing and using of herbal medicines, especially simplicias.
3. Failure in Newborn-babies (toxicological aspects of herbal medicine). This scenario gives a broad picture of the toxicological aspect of herbal medicine to be considered by the students.

Students discuss and learn by themselves the topics of the scenarios. In the field practice, students are showed the nursery and herbal plantation, and preparation of herbal medicine. At the end of the block's session, students take multiple choice examinations.

Some students developed a media communication using Facebook (see Fig. 4). They use the social-networking as a tool for exchanging ideas, information on herbal medicine. And they asked the lecturer as the moderator. We also used the media to inform them on certain issues, also it was used for a survey on the perception of the students about the Block.

Box.1. Perception and Suggestions from students about the Block of Herbal Medicine

One students say: "I like this block, I learn that herbs have been used quite sometimes by our ancestors and yet now we are using them, but it needs more scientific evidences through research."

Other student says: "I learn new things on herbal medicines, and herbal medicine can be a great help to modern medicine. And I think (medical) students should make small-scale research on herbal medicine that maybe can improve the quality of health service in Indonesia."

"Too much lecturing, and lack of practical examples are things should be considered for the improvement of the block," other student comments.

Future Plans

As it is planned, the Block 26 will be given prior to clinical practice, therefore the students are prepared to carry out what they have in the pre-clinical years. The three subjects offered in this Block (Herbal medicine, Disaster Management and Entrepreneurship) are indeed as important as other subjects such internal medicine, pediatrics, ENT, surgery, neurology, etc. However, there is no department in the teaching hospital ready yet for practicing the herbal medicine. Therefore, it is important to have department of herbal medicine in the teaching hospital which will give service for patients who seek complementary and alternatives medicine to relieve their health problems. This department should also do research and clinical trials for fulfilling the requirement for registration of the herbal medicine to the legal authority. With regards to education we will revise the scenarios and focusing the theme of the scenarios on What, when and how to use phytopharmaca.

Students setted-up their networking through Facebook as it is shown in Figure 4.

Figure 4. Networking of the students who took the Herbal Medicine Block

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ANTI-HYPERCHOLESTEROLEMIC ACTIVITIES OF *ARTOCARPUS ALTILIS* LEAVES INFUSION

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Abstract: This research was carried out to evaluate the effect of breadfruit (*Artocarpus altilis)* leaves infusion on the cholesterol and triglyceride level in hypercholesterolemic rats. The *Sprague Dawley* strains of white rats were induced hypercholesterolemically using a combination of high-fat diet and 0.01% Propylthiouracyl (PTU). Samples were administered orally with 3 different dosages (1, 2 and 3 ml/200 g body weight), once daily for 28 days and compared with Simvastatin (0.86mg/200g body weight) as a positive control. The results showed that the sample of *A. altilis* leaves infusion reduced triglycerides level (17-30mg/dl) equivalent to the positive control (22.2mg/dl). Although the *A. altilis* leaves infusion did not reduce total cholesterol, it decreased the LDL cholesterol level (38-40 mg/dl) and increased HDL cholesterol level (81-89.9 mg / dl) in all of the groups treated respect to LDL cholesterol (43.53mg/dl) and HDL cholesterol level (70.7mg/dl) of the positive control. These findings indicated that the sample of *A. altilis* leaves infusion may be useful for the treatment of hypercholesterolemia.

Keywords: Antihypercholesterolemic, *Artocarpus altilis* leaves, infusion.

INTRODUCTION

High serum lipid levels, including total cholesterol, low density lipoprotein (LDL) cholesterol and triglycerides, are a major cause of coronary atherosclerosis. Any LDL cholesterol concentration above 100 mg/dL appears to be atherogenic and the higher the level, the greater the risk. Although elevated LDL cholesterol plays a role in the development of the coronary plaque instability, lowering LDL cholesterol stabilizes plaques and reduces the likelihood of acute coronary syndromes. Lowering serum cholesterol reduces the risk of coronary heart disease (Talati *et al.*, 2009).

Breadfruit has long been an important staple crop and a primary component of traditional agro forestry systems in Oceania including Indonesia, where numerous varieties are grown. The trees begin bearing in 3–5 years and are productive for many decades. They are easy to propagate, require little attention and input of labor or materials, and can be grown under a wide range of ecological conditions. In addition to producing abundant, nutritious, tasty fruits, this multipurpose tree provides construction materials, animal feed and medicine [Ragone, 2006]. Diethyl eter extract of breadfruit woods showed anticancer properties in human breast cancer (T-47D) cells. The extract exhibits decreasing cell viability in a concentration-dependent manner. Altered cell morphology after treatment with the extract demonstrated that cells experienced apoptosis. Cell cycle analysis indicated that the number of cells in sub-G1 phase rose with increasing concentrations of the extract (Arung *et al*., 2009). Then, isoprenoid substituted flavonoids and stilbenes isolated from *A. altilis* fruit have shown inhibitory effects on melanin biosynthesis, inhibit 5ß-reductase activity, anti-inflammatory and anti-platelet activity (Amarasinghe and Jayasinghe, 2007). The research was undertaken to evaluate the effect of breadfruit (*Artocarpus altilis*) leaves infusion on the cholesterol and triglycerides' level in serum of hypercholesterolemic rats.

MATERIALS AND METHODS

Drugs and chemicals

Propylthiouracyl was purchased from Indofarma. All of the biochemical kits used in this experiment were obtained from DiaSys diagnostic, Germany, and all of the other chemicals used were of analytical grade.

Experimental animals

Male Sprague Dawley (SD) rats weighing 200–250 g (bred in the Animal House, BPOM-DEPKES) were used in the present experiment. The animals were housed in a temperature controlled room at 22-25^{0}C, with lighting 12 hours, from 6 AM to 6 PM, and the humidity was maintained at 40-70%.

Plant material. The fresh leaves *A. altilis* were collected from Puspiptek Garden Serpong. The 100 gr of dried leaves was boiled within 1L of water for 10 minutes, and then the infusion was filtered and allows it to cool further.

Hypercholesterolemic induction

Hypercholesterolemic induction were performed by combination of exogen and endogen induction. Exogen induction using high cholesterol diet which include 1 kg normal diet, enrichment with 0.25 L of fat, 2.5 g cholesterol, 125 g wheat flour and 150 g tapioca flour, while endogen induction using 0.01% Prophyl-thiouracyl (PTU) which prepared with diluted of 100 mg of PTU within 1L of water. Hypercholesterolemic induction was doing once daily for 28 days of trial.

Administration of sample

The sample was administered orally using a specialized feeding needle. All doses were administered orally once daily for a period of 28 days together with hypercholesterolemic induction.

Experimental design

A total number of 30 rats were used in the experimental, and they were divided randomized into 6 groups of 5 rats each.
Group I : non-hypercholesterolemic rats
Group II : hypercholesterolemic rats treated with 1ml/200g bw of sample
Group III : hypercholesterolemic rats treated with 2ml/200g bw of sample
Group IV : hypercholesterolemic rats treated with 3ml/200g bw of sample
Group V : hypercholesterolemic rats treated with 0.18mg/200g bw of simvastatin
Group VI : hypercholesterolemic rats with received no treatment

Collection of blood samples

Blood samples were collected at the end of 28 days of the trial, from the orbital sinus using capillary tubes after partly anaesthetizing the rats with 0.04ml/200g bw of Deazepan and 0.0.2ml/200g bw of Ketamin. Blood samples were collected into 2 ml tubes with EDTA as an anticoagulant. The plasma samples were separated by centrifugation at 2.000 rpm for 10 minutes. The HDL and LDL cholesterol concentrations in the plasma and plasma fractions were measured spectrophometrically using kits and calibrators (DiaSys Diagnostic Systems, Germany) on a Shimadzu 1200 spectrophotometer.

Biochemical and pathological estimation.

Total cholesterol was determined by CHOD-PAP method and triglycerides concentration was determined by GPO-PAP method. At the end of 28 days of the trial, the organ liver was examined visually for any abnormalities.

Statistical analysis

The mean and standard error for all the groups was calculated. The mean values were compared with using students' t-test at 5 % level of significance.

RESULTS AND DISCUSSION

Total cholesterol analysis on the initial trial before received any treatment showed that the level of total cholesterol on the six groups of test animals varied between 52-70 mg / dl (Figure 1). Statistical analysis indicated that the total cholesterol level of six groups of test animals distribute with normal curve and also distribute homogenously ($\alpha > 0.05$). Thus, all experimental animals in the six groups have total cholesterol level with statistically uniform. The biochemical findings correlated well with that of observe on liver organ visually which revealed no fat stack in the liver, and the liver has red colour and found no fat nodule (Table 1).

While total cholesterol analysis at the end of the trial after 28 days treatment showed a significantly increase of the level of total cholesterol in all groups , even in Group I where the total cholesterol level were about 103-175 mg/dl. Total cholesterol level resulted from the Group V which received simvastatin treatment statistically equivalent to the total cholesterol level on the Group I as the normal group. Group which received the sample of *A. altilis* leaves infusion in all doses did not appear to reduce the level of total cholesterol (Figure 2).

Even thought the addition of sample together with cholesterol enriched diet resulted in the increasing the HDL cholesterol level in comparison with Group I as a normal control. Group III which received sample at doses of 2ml/200g bw significantly elevating the HDL cholesterol (89.94 mg/dl) higher than Group V (70.67 mg/dl) which treated with simvastatin drugs (Figure 3).

Observation of the liver organ visually indicated that hypercholesterolemic induction affected the abnormalities of liver such as changing from the red colour to the brown, induced the fat nodule and stacking fat in the liver (Table 1). Sample *A. alitis* leaves infusion could inhibit fat nodule and also decreasing the stacking fat in the liver.

Sample of *A.altilis* leaves infusion also affected the level of Low density Lipoprotein (LDL) cholesterol as presented in Figure 4. LDL cholesterol decrease significantly in all of the groups treated with sample (Group II, III, and IV) compared to the Group VI which received no sample. The level of LDL cholesterol on Group II was 38.39 mg/dl, opposite to Group VI with the level of LDL cholesterol was up to 53 mg/dl.

The beneficially of sample on declining the LDL cholesterol statistically equivalent to Group V which received simvastatin with the level of LDL cholesterol was up to 43 mg/dl. Besides declining the LDL cholesterol the sample also exhibit decreasing of triglycerides' level respected to the normal control (Group I) which received no sample (Figure 5). Group II and IV which administered with 1ml/200g bw and 3ml/200g bw of sample significantly decreasing the triglycerides' level in the range of 17mg/dl, in contrary the triglycerides' level of Group I was 94.68 mg/dl.

Although sample of *A. altilis* leaves infusion did not affected the total cholesterol level, it exhibit decreasing the LDL cholesterol level and increasing the HDL cholesterol level, and also decreasing the triglyseride level. According to Movahedian et al. (2007) that hyperlipidemia, particularly elevated serum cholesterol and LDL cholesterol level, is a risk factor in the development of atherosclerosis heart disease, therefore the sample of *A. altilis* leaves infusion have beneficially to reduces the risk factor of atherosclerosis.

The active compound involved in the sample *A. altilis* leaves infusion still unknown, Syah *et al*. (2006) isolated two geranylated flavonoid derivatives, namely 2-geranyl-2',4',3,4-tetrahydroxydihydrochalcone (1) and 8-geranyl-4',5,7-trihydroxy-flavanone (2), from methanol extract of the *A. altilis* leaves. Five geranyl dihydrochalcones and four known geranyl flavonoids were isolated from the leaves of

Artocarpus altilis, and three of the nine compounds exhibited moderate cytotoxicity against SPC-A-1, SW-480, and SMMC-7721 human cancer cells (Wu *et al.*, 2007).

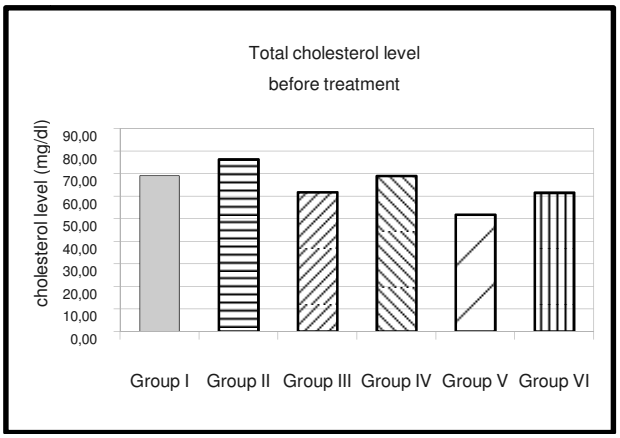

Figure 1. Diagram of the total cholesterol level of six experimental animal groups before hypercholesterolemic induction and sample treatment.

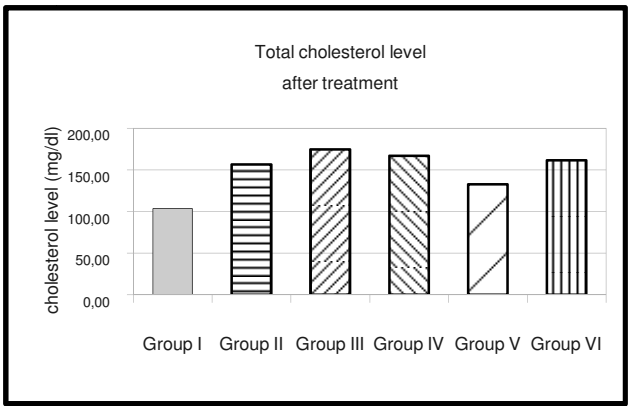

Figure 2. Diagram of the total cholesterol level of six experimental animal groups at 28 days after hypercholesterolemic induction and sample treatment.

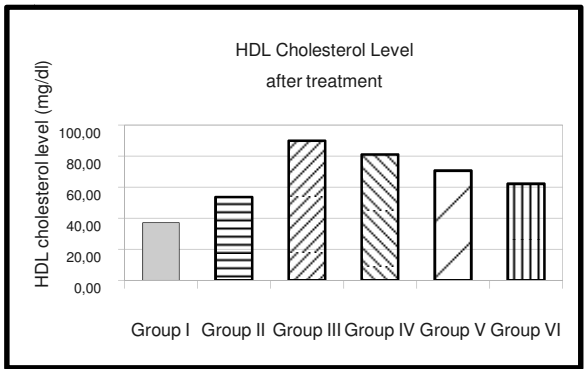

Figure 3. Diagram of the HDL cholesterol level of six experimental animal groups at 28 days after hypercholesterolemic induction and sample treatment.

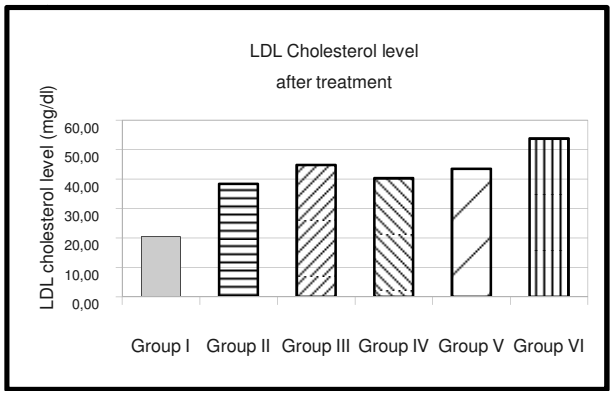

Figure 4. Diagram of the LDL cholesterol level of six experimental animal groups at 26 days after hypercholesterolemic induction and sample treatment.

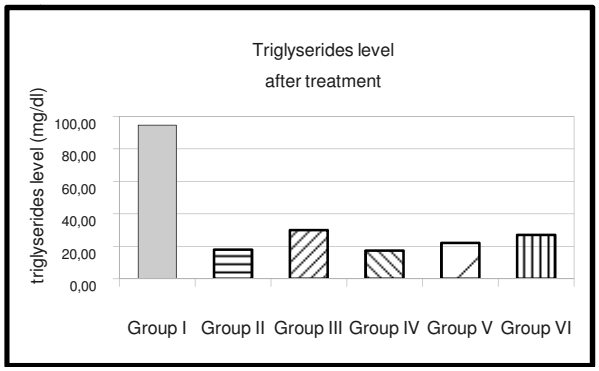

Figure 5. Diagram of the triglyserides level of six experimental animal groups at 28 days after hypercholesterolemic induction and sample treatment.

Tabel 1. Observation of the liver organ visually of six experimental animal groups at 28 days after hypercholesterolemic induction and sample treatment.

Treatment	fat in the liver	liver colour	white fat nodul
Group I	-	red	-
Group II	+++	brown	-
Group III	++	brown	-
Group IV	++	brown	-
Group V	+	brown	-
Group VI	+++	brown	+

Note : - = no fat or nodul, + = few fat or nodul ++ = much fat or nodul, +++ = very much fat, found in the liver.

Mechanism of action of the *A. altilis* leaves infusion to reduce cholesterol still not yet clear. Wu *et al*. (1998) reported that a traditional Chinese medicine (Kampo medicine) Ogi-Keishi-Gomotsu-To-Ka-Kojin (OKGK) suppressed cholesterol absorption through the intestine and stimulated excretion of cholesterol into feces as bile acid. Tea-leaves' saponin exhibited anti hypercholesterolemic activity by inhibiting cholesterol absorption in the intestines (Matsui *et al.*, 2009).

CONCLUSION

The present study revealed that the *A. altilis* leaves infusion possess significant antihypercholesterolemic effects in diabetic rats. However, the exact mechanism and the active compounds involved remains to be elucidated. In addition, the study also confirmed the non-toxic effects of *A. altilis* leaves infusion in both hypercholesterolemic as well as non-hypercholesterolemic rats when administered 1-3 ml/200g body weight for 28 days.

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CALLUS INDUCTION OF *SONCHUS ARVENSIS* L. AND ITS *IN-VITRO* ANTIPLASMODIAL ACTIVITY

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Abstract: The aims of this study was to establish an effective protocol for callus induction from leaf explant of *Sonchus arvensis* L. and to investigated its *in-vitro* antiplasmodial activity. The effect of growth regulator were investigated. Growth regulator effect was studied by using 1 mg/L of Indolyl-3-acetic acid (IAA), Indolyl-3-butiric acid (IBA), Naphthalene acetic acid (NAA), and 2,4-dicchlorophenoxy-acetic acid (2,4-D) alone or in combination with 0.5mg/L 6-benzyladenin (BA). Combination of 1mg/L 2,4-D and 0.5 mg/L BAP produced the highest callusing, and had the best quality of callus. The times required for callusing is shortest. Callus of 4 weeks old was extracted by methanol. *In-vitro* antiplasmodial activity of callus extract was investigated with IC_{50}=0.343 µg/ml.

Keywords : *Sonchus arvensis* L. antiplasmodial, *Plasmodium falciparum*, callus, growth regulator, metanaol extract.

INTRODUCTION

Malaria is caused by a single celled protozoan parasites called plasmodium and transmitted to man thought the *Anopheles mosquito*. It is one of the major fatal diseases in the word, especially in the tropics and is endemic in some 109 countries. Reported at 2006, there are 247 million case of malaria from 3,3 billion world population at risk and caused 1 million death (WHO, 2008). In Indonesia the death by malaria diseases are reported increase year by year. Indonesia at the 26th rank among malaria endemic countries with prevalence value 919,8/ 100.000 peoples (WHO, 2007).

Some program had been done to solve the malaria problem, such as Rool Back Malaria (RBM) which leading by United Nation. This program is in half in progress but WHO data show that the mortality are caused by malaria diseases are increase (Attaran, 2004).

Malaria disease is still complex problem because malaria problem depend on: 1) parasite aspect that cause malaria diseases, Plasmodium, 2) vector aspect, Anopheles, 3) environment aspect, 4) population behavior (Depkes RI, 2009), and climate change that introduce malaria line resistant toward ant malarial drug and resistant Anopheles line toward insecticide (Fahmi & Khairunnisa, 2009; Martins *et al*., 2009 dalam Ekasari, 2010).

Resistantcy problem toward first line drugs has been cause many problem to solve malaria diseases. To find new drug is needed to decrease the malaria mortality value. This situation push researcher to find new drug from synthetic or natural product.

Eighty percent of the world population still use natural product for medicine and 75% human with malaria choose traditional medicine to curate the malaria diseases (Wright, 2004 dalam Ekasari, 2010). The old malaria medicine is gotten from *Chincona succirubra* L. plant. The new malaria drug generation, artemisinin is gooten from *Artemisia annua* L plant too (Wright, 2005).

Tempuyung (*Sonchus arvensis* L.) is Indonesia traditional medicine plant has active compound, such as flavonoid, saponin, and polifenol that is used as antioksidan, hepatoprotektor, diuretic drug, and anti-malarial material potential (Yunita & Lestari, 2008).

Using plant material from the nature can cause genetic resources lost. By tissue culture, we can produce secondary metabolite that identically from nature. For scale commercial, we can produce secondary metabolite by bioreactor system (Radji, 2005).

The objective of this research is to establish an effective protocol for callus induction from leaf explant of *Sonchus arvensis* L. and to investigated its *in-vitro* antiplasmodial activity.

MATERIAL AND METHODS

Plant Material

Fresh leaves obtained from *Sonchus arvensis* L. is located at the Park of Sains and Technology Faculty, Airlangga University. The 2nd and 3rd leaf of plant are used as explant.

Surface Sterilization of Explants

Fresh leaves were brought to laboratory then washed and cleaned carefully with continuous running tap water for 5 minutes. Next the leaves were disinfected by fungiside (500mg fungiside/500ml distilled water) and shaked continuously for 10 minutes, and then the leaves were rinsing by distilled water twice. Finally the leaf explants were soaked in 10% clorox for 10 minutes by three times rinsing with sterile distilled water.

Media Preparation

The medium used for callus culture was MS medium (Murashige & Skoog, 1962). Supplemented with 30g/L sucrose and 8 g agar. All media used in this experiment were adjusted to pH 5,8, and then distributed in 50 culture bottles covered with alminium foil before autoclaving at 121°C for 15 minutes under pressure of 1 atm. The media were left to the media room until used.

Inoculation of Explants Material

Under Laminar Air Flow Cabined, sterile leaves were cut into many pieces. The measurement of one pieces of explants was 1cm^2. Next the explants were placed abaksial side down into bottle culture containing 20 ml of MS media. Then the bottle culture were placed at incubator room without lamp (dark incubation). The callus would be harvested at 4 weeks old.

Effect of Growth Regulator On Callus Growth

The experiment was designated with 1 mg/g of Naphthalene Acetic Acid (NAA), Indolyl-3-Acetic Acid (IAA), Indolyl-3-Butiric Acid (IBA), and 2,4-dichlorophenoxy-Acetic Acid (2,4-D), combination with or without 0,5 mg/L of 6-Benzyl Adenine BA. MS medium without growth regulator as control.

MS medium supplemented with 8 g/L agar was used throughout the experiment. All the experiment treatments were replicated at least 3 times. Data for the callusing degree, time of callusing and percentage of callusing were recorded every week until 4th week of culture.

Extractions and In-Vitro Antiplasmodial Assays

The best callus were extracted whit methanol at room temperature. The methanol extract was evaporated, and then 100, 10, 1, 0.1, 0.01 μg/L methanol extract were used in the in-vitro antiplasmodial assays. *Plasmodium falciparum* 3D7 was used in the in-vitro antiplasmodial assays.

RESULT AND DISCUSSION

Callus Induction

Growth regulator of auxsin (2.4 D, IAA, IBA, NAA) and cytokinin (BAP) are used to callus induction. The effect of several combination of growth regulator to Sonchus arvensis L. callus induction is showed in table 1.

Table 1 Time of callus induction, morphology of callus, percentage of callusing, and degree of callusing at 4th week of culture compact callus

Growth regulator hormon treatment	Time of callusing (week)		Precentage of callusing (%)	Degree of callusing	Morphology of callus
	Dark incubation	Light incubation			
MS_0	0	0	0	-	No callus
MS_1	2	3	100	++	Compact callus, green, the end of the week callus grow to plantlet
MS_2	2	2	100	++	Compact callus, green
MS_3	2	3	100	++	Compact callus, green, at the end of the week callus grow to plantlet
MS_4	2	3	100	++	Compact callus, green, at the end of the week callus grow to plantlet
MS_5	2	2	100	++	Compact callus, green, at the end of the week callus grow to plantlet
MS_6	2	3	100	+	Compact callus, green
MS_7	2	3	100	++	Compact callus, at the end of the week callus grow to plantlet
MS_8	2	3	100	+	Compact callus, green
MS_9	2	3	100	++++	Friabel callus, yellow

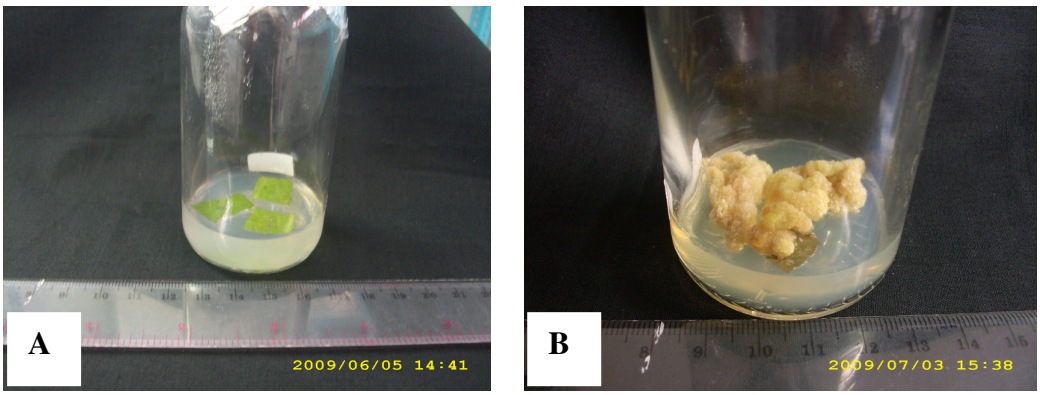

Figure 1. Growth of *Sonchus arvensis* L. callus on MS medium with 2,4D 1 mg/L + BAP 0,5 mg/L treatment.(A) the first week of culture; (B) the 4th week of culture.

MS_1= BAP 0,5 mg/L; MS_2 = IAA 1 mg/L; MS_3=IAA 1mg/L +BAP 0,5 mg/L; MS_4 = IBA 1 mg/L; MS_5 = IBA 1 mg/L + BAP 0,5 mg/L; MS_6 = NAA 1 mg/L; MS_7 =NAA 1 mg/L + BAP 0,5 mg/L; MS_8 = 2,4D 1 mg/L; MS_9 = 2,4D 1 mg/L + BAP 0,5 mg/L; MS_0 = wthout growth regulator hormon

Table 2. Growth percentage and inhibition percentage of methanol extract callus toward to *Plasmodium falciparum* 3D7.

Concentration (µg/mL)	R	% Parasetemia		Growth percentage	Inhibition percentage	Average inhibition percentage
		0 hour	48 hours			
Controle (-)	1	1.22	9,08	7,86	-	-
	2	1.22	9,19	7,97	-	
100	1	1.22	1,45	0,23	97,47	96,59
	2	1.22	1,56	0,34	95,71	
10	1	1.22	2,34	1,12	85,86	83,33
	2	1.22	2,74	1,52	80,81	
1	1	1.22	3,99	2,77	65,03	66,48
	2	1.22	3,76	2,54	67,93	
0.1	1	1.22	6,50	5,28	33,33	33,90
	2	1.22	6,41	5,19	34,47	
0,01	1	1.22	8,12	6,90	12,88	13,13
	2	1.22	8,08	6.86	13,38	

Combination 2,4 D 1mg/L and BAP 0.5 mg/L is the best combination to *Sonchus arvensis* L. callus induction, The time of callusing is 2 weeks in dark incubation. At the first the growth, the color of callus is yellow and friable callus but at the end of growth the color of callus is going brown (figure 1).

Growth regulator hormone 2,4 D is the best choice for callus induction (Indrianto, 2003), like for callus induction of sugar cane (Yamani, 2009) and callus induction of tea plant (Sutini, 2008), although the respond of plant depend on genotype of each plant (George and Sherington, 1992). Saptowo et al., (2004) more higher concentration of 2,4-D, more easier to callus induction, especially combination with BA.

Data of in vitro antiplasmodial assays is analyzed with Probit Analyzed to get the IC_{50} value. From the Probit Analyzed, IC_{50} value of methanol extract callus of *Sonchus arvensis* L. is 0.343 µg/ml. Weenen (1990) says that extract which have IC_{50} value 1-10 (µg/ml) is material has good antiplasmodial activity. In China, extract plant has IC_{50} value 0.008-15,38 (µg/ml) is used as antiplasmodial material (Tan et al., 1998 in Aryanti et al., 2006).

IC_{50} value of methanol extract callus of *Sonchus arvensis* L. is lower than IC_{50} value leaf extract *Cassia siamea* Lamk. (Ekasari, 2001), IC_{50} value leaf extract *Erytrina variegata* L.(Herlina, *et al*.,2010), IC_{50} value leaf extract *Arthocarpus champeden* Spreng (Widyawaruyanti *et al*., 2010), and IC_{50} value of 5 fraction of *Artemisia annua* (Aryanti et al., 2006). According the data, important to explore the antiplasmodial activity *Sonchus arvensi* L.

CONCLUSION

From the data and discussion could be concluded that combination of 1mg/L 2,4-D and 0.5 mg/L BAP produced the highest callusing, and had the best quality of callus. The times required for callusing is shortest. *In-vitro* antiplasmodial activity of methanol

extract of *Sonchus arvensis* L. is potential as antiplasmodial material with IC_{50}=0.343 µg/ml.

ACKNOWLEDGMENTS

This work was supported by Hibah Strategis Nasional, Directorat Jenderal of High Education, Education Departemen of Indonesia.

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DETECTION OF TOXIC SUBTANCES OF *SWIETENIA MAHAGONI* JACG. STEM BARK BY A*RTEMIA SALINA* BIOASSAY

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Abstract : Detection of toxic substances of *Swietenia. mahagoni* Jacg stem bark by Artemia salina bioassay has been conducted. This research aims to increase the value of *S. mahagoni* stem bark, to be used as anticancer. The research was carried out in three part : chloroform and methanol extraction from dried stem bark of *S. mahagoni*, determination of compounds content with GCMS methode and cytotoxic experiment against *Artemia Salina* Leach with BST method. The toxic activities was determined with LC_{50} (*Lethal Concentration*). The result showed that chloroform extract has no toxic activity with LC_{50} 1034,25 ppm and methanol extract has good toxic activity with LC_{50} 609,91 ppm. The results of GCMS analysis from chloroform extract was asam palmitat, asam linoleat, n-heneikosana, 1-oktadekanol, tetrakontana, (3α, 5α)-3-tiosianat koleston, stigmasterol, F-sitosterol, sitostenon and methanol extract was o-metoksi fenol, 1,2-benzenadiol, 4-metil-1,2-benzenadiol, 2,6-dimetoksi fenol, 1,2,3-benzenatriol, 1,3,5 benzenatriol, 4-propil-1,3-benzenadiol, 1.3,4,5-tetrahidroksi sikloheksana karboksilat, 2-hidroksi-4-metil benzaldehid. The methanol extract of *S.. mahagoni* stem bark has toxic activity against *Artemia Salina* Leach and the expected potential as anticancer.

Keywords : *Swietenia mahahoni* Jacq stem bark, *Artemia Salina* Leach, GCMS.

INTRODUCTION

Some studies mention that the seeds of mahagoni (*Swietenia mahagoni* Jacg) contain bioactive compounds such as flavonoids, alkaloids, saponin and phenols. (Anonym, 2000). According to Sugiyanto (2003), group these bioactive compounds associated with antioxidant and anticancer activity. It shows that mahagoni seeds contain potential compounds as anticancer.

The other parts of the mahagoni that has not been exploited is the bark. A same species of plant usually have the same chemical compounds with different quantities, so that the bark of mahagoni which is estimated to contain bioactive compounds similar to the seed. Content of bioactive compounds showed mahagoni bark is potential for anticancer drugs. So far have not done the research potential of stem bark of mahagoni as a source of ingredients and sources of useful chemicals.

Based on this, detection of toxic substances of *Swietenia mahagoni* Jacg stem bark by *Artemia salina* bioassay have been done.

EXPERIMENTAL

Material

Mahagoni bark, larval shrimp, distilled water, seawater, SiO_2GF-254, n-hexane, ethyl acetate, ammonia, chloroform and methanol, butanol, as glacial acetic, Dragendorf reagents, $KMnO_4$., Mayer reagent, Sitoborat reagent.

Tools

Glass ware, microscopes, evaporator, object glass, tweezers, flakon, water bath, a set of soxhlet, tustel, paper filter, vacuum pump, funnel burner, oven, and deksikator, TLC plate.

Plant material and extraction

The plant material used in this study were held from Purwokerto. A dried mahagoni stem bark powder were extracted with solvents chloroform and methanol using soxhlet method. until the content of compounds in the extract are interested in them in a solvent. Extracts obtained evaporated until dry and weighed to obtain the extract in a certain amount.

Assay of Toxic activity

Brine shrimp letality test by Artemia salina bioassay was used. Stock solution of chloroform and methanol extract were prepared. Serial dilution of each extract were prepared in test tube and given in a number group :

Group 1: extract with 1000 ppm concentration
Group 2: extract with 500 ppm concentration
Group 3: extract with 100 ppm concentration
Group 4: extract with 10 ppm concentration
Control: 0.5 ml of solvent

Eggs hatched *Artemia salina* Leach sectional box (dark and exposed to light) which contains sea water that has been filtered for 48 hours. Sea water containing 10 larvae were put into 4 ml of each test tube. The volume of sea water in a test tube until 5 ml into each tube inserted a drop of yeast solution (3 mg / ml sea water) as nutrients. Test tubes were incubated in an open state gets sahaya for 24 hours. The number of surviving larvae were counted and determined the percentage of mortality with MLD (Median Lethal Dose) analisys.

Gas chromatography / Mass spectrometry (GCMS)

Chloroform and methanol extract of mahagoni stem bark was dissolved in appropriate solvent and then analyzed using GCMS. Parameters measured were the percentage of mortality of shrimp larvae Artemia salina Leach.

Methods of Analysis (Mursyidi, 1985)

Data analysis was planned using the toxicity test for MLD (median dose lethall). Percentage mortality of larvae shrimp *Artemia salina* Leach noted, and then searched using the table probitnya price. Probit value (y axis) dilotkan versus log dose (x axis) is used. Drawn following the best line of bullets through the existing points (line A). from the y-axis at the probit value of 5 horizontal line is drawn cut line A, then a vertical line drawn from the intersection so that the obtained value of x (log dose), if this value is in antilogkan, dosage will be obtained stating the value of 50% mortality of shrimp larvae Artemia salina Leach .

RESULTS AND DISCUSSION

The plant of mahagoni stem bark (*S. mahagoni* Jacq.) were taken and collected from the area Cilongok Purwokerto. Cytotoxicity tests conducted to ensure the toxic properties of chloroform extract and methanol extract of stem bark of mahagoni with the BST method to determine LC50 values. The test results obtained from the chloroform extract BST are shown in Table 1.

Table 1. Mortality records of *Artemia salina* exposed to various concentration of chloroform extract of *Swietenia mahagoni*

No.	Concentration (ppm)	Mortality (%)	Log	Probit
1.	1000	53.4	3	5.09
2.	500	40.0	2.6989	4.74
3.	250	30.0	2.3979	4.48
4.	125	26.7	2.0969	4.37
5.	62.5	23.4	1.795	4.27

Chloroform extract of mahagoni stem bark with various concentrations tested its toxicity against larvae Arthemia salina Leach. BST test result obtained relationship between log concentration and probit values by the equation 0.6633 x + 3.0004 with r = 0.963 graph of the equation, obtained LC_{50} = 1034.246 ppm.

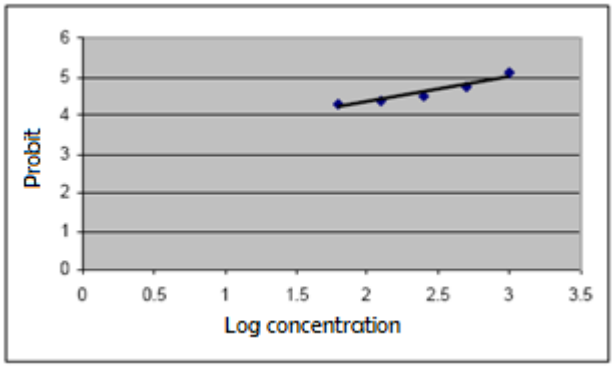

Figure 1. LC_{50} of chloroform extract of *Swietenia mahagoni*

The test results obtained from the methanol extract BST are shown in Table 2.

Table 2. Mortality records of *Artemia salina* exposed to various concentration of methanol extract of *Swietenia mahagoni*

No.	Concentration (ppm)	Mortality (%)	Log	Probit
1.	1000	56.7	3	5.17
2.	500	46.7	2.6989	4.91
3.	250	36.7	2.3979	4.65
4.	125	26.7	2.0969	4.37
5.	62.5	13.3	1.795	3.89

Methanol extract of stem bark of mahagoni with various concentrations tested its toxicity against larvae *Artemia salina* Leach. BST test result obtained relationship between log concentration and probit values by the equation 1.0333 x + 2.122 with r = 0.989. graph of the equation, obtained LC50 = 609.910 ppm.

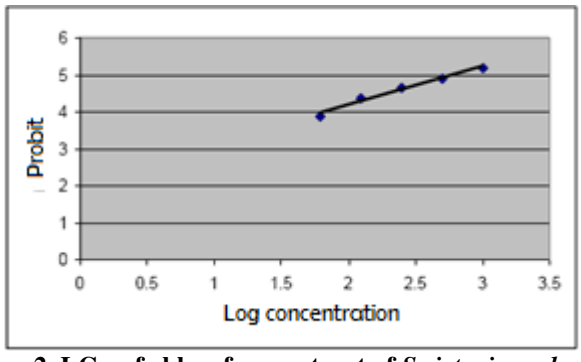

Figure 2. LC_{50} of chloroform extract of *Swietenia mahagoni*

Based on data obtained showed that the percentage mortality of larvae of *A. salina* Leach., decreases with increasingly small concentration of methanol extract of stem bark of *S. mahagoni* Jacq. given. This shows that the potential of ethanol extract of stem bark ketoksikan S. mahagoni Jacq. is dose dependent (depending on dose). According to Mc. Laughlin et al. (1998), if the LC50 <1000 tg / ml of the extract is said that the acute toxic to larvae of A. salina Leach. Thus, the methanol extract of stem bark of S. mahagoni Jacg contain bioactive compounds that can kill (acute toxicity) of shrimp

larvae *A. salina* Leach. Meanwhile, the fraction of chloroform due to its LC50 values> 1000 ppm, the content of existing compounds, have no cytotoxic activity.

The main content of mahagoni bark extract is determined by the number of the highest peaks are formed in the chromatogram. If a component is in a high percentage in the mixture being analyzed, the peaks that appear on kromatogramjuga has a large area. The type and number of fragments of molecules that are formed from a chemical compound of each peak in the chromatogram is determined based on spectrum-mass spectrum. Mass spectrum of chemical components of the extract obtained from the analysis were identified by way of comparison with the mass spectrum contained in a data bank (Libraries in the GCMS instrument).

Results of analysis by gas chromatography chromatogram showed many peaks, with nine main peaks. The main peak was analyzed peaks of relative abundance of more than 1.20%. GCMS chromatogram of giving information concerning the molecular weight and fragmentasinya. The result of fragmentation of the molecular weight of the main components of the chloroform extract of stem bark of mahagoni are shown in Table 3.

Table 3. Mass spektrum of principal components of chloroform extract of *Swietenia mahagoni*

No.	Peak number	M	m/z (intensities relative to base peak in parentheses)	Identity of compound
1.	15	256	41(77), 43(100), 60(90), 73(98), 85(22), 98(15), 115(11), 129(33), 143(2), 157(10), 171(9), 185(8), 213(16), 227(2), 256(49)	Palmitic acid
2.	22	280	41(82), 55(80), 67(100), 81(75), 95(51), 100(19), 123(8), 136(4), 150(2), 164(1), 182(1), 207(0.5), 227(1), 264(1), 280(3)	Linoleat acid
3.	48	296	41(26), 43(58), 57(100), 71(68), 85(50), 99(15), 113(10), 127(6), 141(4), 155(3), 169(1), 183(1), 197(1), 211(1), 225(0.5), 239(0.5), 296(1)	n-heneicosane
4.	49	270	41(50), 43(79), 55(82), 57(100), 83(90), 97(78), 111(42), 125(20), 139(5), 154(4), 168(3), 224(2), 253(2)	1-octadecanol
5.	59	619	41(13), 43(67), 57(100), 71(70), 85(46), 99(28), 113(12), 127(10), 141(5), 155(4), 169(2), 183(2), 197(2), 211(2), 225(1), 239(1)	Tetratetracontane
6	69	429	41(40), 43(100), 57(48), 81(50), 91(30), 105(42), 121(28), 133(20), 147(56), 165(90), 177(5), 190(2) 205(8), 227(2), 241(1), 255(14), 275(12), 288(12), 302(2), 354(2), 381(12), 396(50), 430(80)	(3α, 5α)-3-thio-cyanate cholestone
7.	73	412	41(42), 55(100), 69(56), 83(72), 97(34), 123(24), 145(24), 159(28), 173(10), 213(18), 255(39), 271(29), 300(20), 351(18), 364(18), 412(32)	Stigmasterol
8.	74	414	41(40), 43(100), 57(52), 81(42), 95(42), 107(44), 119(30), 133(26), 145(34), 161(21), 173(10), 185(6), 199(8), 213(19), 231(16), 241(2), 255(18), 273(15), 283(1), 303(20), 329(30), 341(1), 354(2), 371(1), 381(15), 396(20), 414(38)	Γ-sitosterol
9.	79	412	41(26), 43(56), 57(22), 81(20), 95(25), 107(20), 124(100), 135(18), 147(18), 159(5), 173(3), 187(4), 203(2), 229(28), 271(4), 389(15), 314(1), 327(1), 355(2), 370(6), 397(4), 412(16)	Sitostenon

The result of fragmentation of the molecular weight of the main components of methanol extract of stem bark of mahagoni are shown in table 4.

Table 4. Mass spektrum of principal components of methanol extract of *Swietenia mahagoni*

No.	Peak number	M	m/z (intensities relative to base peak in parentheses)	Identity of compound
1.	3	124	39(10), 53(26), 57(10), 81(74), 109(100), 124(82)	o-methoxyphenol
2.	6	110	39(10), 53(14), 64(36), 81(14), 92(10), 110(100)	1,2-benzenediol
3.	9	124	39(13), 51(20), 65(10), 78(70), 95(10), 106(16), 124(110)	4-methyl-1,2-benzenediol
4.	11	154	39(25), 51(18), 65(30), 97(11), 93(46), 111(34), 139(54), 154(100)	2,4-dimethoxyphenol
5.	13	126	39(12), 52(42), 63(3), 80(40), 97(10), 108(20), 123(30), 126(100)	1,2,3-benzenetriol
6	18	126	41(21), 69(33), 80(19), 85(20), 98(10), 126(100)	1,2,5-benzenetriol
7.	19	152	39(2), 51(20), 67(6), 77(23), 94(2), 105(6), 123(100), 152(22)	4-propyl-1,3-benzenediol
8.	20	192	39(11), 43(90), 60(100), 71(71), 84(20), 100(14), 112(38), 150(2), 156(2)	1,3,4,5-tetrahydroxy-cyclohexane carboxylic acid
9.	24	136	39(20), 51(10), 67(11), 79(10), 89(12), 107(10), 118(1), 136(100)	2-hydroxy-4-methyl benzaldehide

CONCLUSION

1. BST test result against Artemia salina Leach, was obtained:
   a. Chloroform fraction did not contain compounds that are cytotoxic
   b. Methanol fraction cntain of cytotoxic compounds, with LC50 values at 609.910 ppm.
2. GCMS analysis of the results obtained with the active compound
   a. Chloroform extract from the bark of mahagoni is palmitic acid, linoleic acid, n-heneikosana, one-oktadekanol, Tetratetra kontana, (3α, 5α) -3 - koleston thiocyanate, stigmasterol,-sitosterol Γ, sitostenon.
   b. Methanol extracts from stem bark of mahagoni is o-methoxy phenol, 1,2-benzenadiol, 4-methyl-1 ,2-benzenadiol, 2,6-dimethoxy phenol, 1,2,3-benzenatriol, 1,3,5-benzenatriol , 4-propyl-1 ,3-benzenadiol, 1,3,4,5-tetrahidroksi cyclohexene carboxylate, 2-hydroxy-4-methyl benzaldehyde.

Based on scientific information obtained in this study, it is necessary to conduct further research on the components of the methanol extract of bioactive compounds that have mahagoni bark as anticancer activity by analyzing the cytotoxicity against cancer cells.

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PRELIMINARY SCREENING OF MARINE ALGAE FROM SOUTH SULAWESI COAST FOR CYTOTOXIC ACTIVITY USING BRINE SHRIMP *ARTEMIA SALINA* LETHALITY TEST

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Abstract: Fifteen algae extracts, isolated from seven species of marine algae belonging to the classes Phaeophyta (*Padina boergesenii, Sargassum prismaticum, Rosenvingea orientalis Dictyopteris acrostichoides*), Chlorophyta (*Codium dwarkense*), and Rhodophyta (*Sarconema filiforme, Wrangelia tanegana*) were investigated for their cytotoxic activity against brine shrimp *Artemia salina*. The algae extracts were prepared successively first by n-hexan followed by dichloromethane (DCM) and finally with ethyl acetate (EtOAc). Of all the extracts tested, only the dichloromethane (DCM) extract of *Sarconema filiforme* showed no significant activity with LC_{50} value >1000 µg/mL. Other 12 extracts such as *Rosenvingea orientalis* (n-hexan, DCM), *Wrangelia tanegana* (n-hexan, DCM), *Padina boergesenii* (n-hexan, DCM, EtOAc), *Codium dwarkense* (n-hexan), *Sargassum prismaticum* (n-hexan, DCM) and *Dictyopteris acrostichoides* (DCM, EtOAc) showed significant activity with LC_{50} value below 62.5 µg/mL. The n-hexan extract of *Dictyopteris acrostichoides* and the dichloromethane extract of *Codium dwarkense* exhibited intermediary cytotoxicity with LC_{50} value between 62.5 and 250 µg/mL.

***Keywords*:** Marine algae, cytotoxicity, brine shrimp *Artemia salina* lethality test.

INTRODUCTION

Over the past several decades, algae and their extracts have generated an enormous amount of interest in the pharmaceutical industry as a fresh source of bioactive compounds with different bioactivity such as cytotoxic (Tang *et al*., 2002), antibacterial (Vallinayagam *et al*., 2009), antifungal (Aliya and Shamaeel, 1999), antiviral (Serkedjieva, 2004; Garg *et al*., 1992), antitumour (Kaori, 2002), antioxidant (Yuan and Walsh, 2006) and larvasidal (Thangam *et al*., 1993). Until now more than 2400 marine natural products have been isolated from seaweeds of subtropical and tropical populations (Faulkner, 2001; Munro and Blunt, (1999).

In the world, also in Indonesia, diseases crisis caused by non-controlled cell proliferation as tumour- and cancer cells have a significant position. National Cancer Institute USA reported that almost one third of human death caused by cancer disease have correlation with human diet. As reported by Harvard School of Public Health in USA that pre-menopause women in Japan have a chance on severe breast cancer three times lower than that severe by American women. This caused by they always include algae on their food menu. Algae have a big potential as food and medicine since long ago since they rich on vitamin, mineral, crude fibrous, protein and polysaccharide (Ito and Hori, 1989; Lahaye, 1991; Darcy-Vrillon, 1993). Algae have a low fat content with unsaturated form which can protect human from cardiovascular pathogen. Their non-toxic phycocolloid role as low calorie nutrition and stabilization agent in food industry (Van den Hoek *et al*., 1993; Critchley and Ohno, 1998; Lee, 1999). In Japan traditional food, algae were used as sushi wrapper, seasonings, condiments and salad or vegetables which composed 10-25% of food consume from most Japan people (Skibola, 2004; Teas, 1981).

Global economy crisis, high price of medicinal drugs, difficult access of population on medical treatment and pharmacy, and side effect of synthetic chemical drugs, are the reasonable factor to use herbal medicine for disease treatment. Many

experts suggest consuming various foods help to prevent the developing of cancer cells or reduce the tumour cells size. Algae have ingredients like ß-carotene, protein, vit B12, fibrous, chlorophyll, and essential fatty acid which protect women from breast cancer. The omega-3 fatty acids (eicosapentaenoic- and stearidonic acid) and iodine in seaweed is thought to reduce breast cancer risk (Vinayak *et al,*.2010). Diets with three algae, *Porphyra tenera*, *Laminaria religiosa* and *L. japonica* var. *ochotensis*, showed an inhibitory effect on mammary tumorigenesis with tumor incidences of 35%, 35%, and 50%, respectively, while in control only 69% (Yamamoto *et al.,* 1987).

Most anticancer and antitumour drugs for chemotherapy treatment are synthesized from the synthetic chemical compounds and for the long term chemotherapy, many cancer patients could not be stand from suffering the drugs side-effect and can finally die. It is therefore urgent to find a new anticancer drug which safely for the long term therapy. For this, non-synthetic or natural product especially marine natural product could be a promised resource. Several study showed that marine environment which cover almost 70% of earth is a rich sources on bioactive compounds, many of them have unique structure different with those from terrestrial one. Marine secondary metabolites are organic compounds produced by marine organisms like microbes, sponges, and seaweeds. The organism biosynthesizes these compounds to protect themselves and to maintain homeostasis in their environment (Selvin and Lipton, 2004). The problem which always appears in marine drug discovery is raw material supply for industrial scale. Marine algae or seaweeds are the renewable resources that easy to cultivate in Indonesia. With the coast line of ±81.000 km, Indonesia has a big challenge or a high potential on marine algae cultivation. Until now there are 555 species of algae that have been found in Indonesia water (Bengen, 2001) and one of the areas which has a big potential for algae culture is South Sulawesi. The aim of this study was to assess the cytotoxic effects of organic crude extracts of marine algae collected from South Sulawesi coast, towards the nauplii of the brine shrimp *Artemia salina*, as a potential source of marine bioprospecting for anticancer drugs candidate.

Brine shrimp bioassay has been used in this study since it is known as an efficient, safe, fast and reproducible procedure to assess biological and pharmacological potential of new compounds (Meyer *et al*., 1982; Manilal *et al*., 2009a) and requires only a small amount of the assayed substance (Svensson *et al*., 2005). This assay has presented satisfactory correlation with citotoxicity property to some solid human tumours (Badisa *et al*., 2007). Moreover, it is an inexpensive test and without ethical constraints (Aristides *et al*., 2008).

MATERIALS AND METHODS

Algae Collecting

Algae were collected during the low tide along the coasts of Takalar and Pangkep in South Sulawesi, Indonesia. The materials were washed with cleaned sea water and put into plastic bags before kept in an ice box to prevent photolysis and thermal degradation during transportation.

Sample Preparation

In the laboratory of Marine Science and Fishery Faculty of Hasanuddin University, algae species were washed with filtered seawater to remove the epizoones, epiphytes, animal castings, sand, calcareous and other adhering detritus matters. Small samples of the species were separated for identification and the rest were washed with freshwater to remove the salt. After draining off the water, the cleaned plant materials were wiped with a blotting sheet and were sun-dried carefully under shade for 24-48 hour. Dried materials were weighed and cut into small pieces before finely grounded in a mechanical grinder. The powdered algae were kept airtight in plastic bags and put in the room temperature for further experiment.

Extraction of Algae

Extraction of algal materials was conducted as described previously (Zainuddin, 2006). 50 g of finely powdered algal material were extracted with 500 mL n-hexan in a 1-L capacity round bottom flask (1:10, w/v). The extraction was run on a stirrer plate for 24 h under room temperature. The extracts were filtered through a Whatman no. 1 filter paper then evaporated under reduced pressure in a rotary evaporator until 5-10 mL volume. The concentrated extracts were kept on small vials and let dry under room temperature to yield thick oily crude extract and stored airtight at -20ºC for further analysis. The algae residue from n-hexane extraction were dried at room temperature for 24 h and re-extracted successively with higher polarity solvents (dichloromethane and ethyl acetate) using the method as described above.

Brine Shrimp Lethality Test

Brine shrimp *Artemia salina* eggs were hatched in a flask containing filtered seawater (1 g cyst per litre) under continuous illumination at 27–30ºC for 48 h. The air stone was placed in the bottom of the jar to ensure complete hydration of the cysts. After incubation period, newly hatched nauplii were collected for the assay.

Brine shrimp cytotoxicity assay was performed using the freshly hatched free-swimming nauplii of *Artemia salina*. Stock solution was prepared by dissolving ten mg of crude algae extract in 1% methanol in an eppendorf and filled with filtered seawater until 1 mL volume. Test solutions were prepared by diluting the stock solution with filtered seawater to obtain two-fold serial dilution (1000, 500, 250, 125 and 62.5 µg). Ten brine shrimp nauplii were transferred into vials containing 4 mL filtered seawater. One mL of the test solution contained each extract concentration were added into vials to make 5 mL of total solution. Parallel positive (only methanol and seawater) and negative (only seawater) controls were included in experiment set up. The cytotoxicity was determined after 24 h exposure under constant illumination. The number of live brine shrimp nauplii in each vial was determined with a hand lens, and the mortality rate was calculated. The mortality end point of the bioassay was determined if no internal or external movement of larvae was observed during 30 seconds. Each test was run in duplicate and it was repeated until two or three times.

Based on the percent mortality, the results were expressed as LC_{50} value. It was defined as the concentration needed to cause half of the tested brine shrimp died within 24 h. If the LC_{50} value is <1000 µg/mL the assayed compound was regarded as cytotoxic and if the value is >1000 µg/mL, the assayed compound was regarded as non-toxic (Meyer *et al*., 1982; Badisa *et al*., 2007; Parra *et al.,* 2001).

RESULTS

The algae species are collected along the coasts of Takalar and Pangkep, South Sulawesi Province, Indonesia. They are distributed in the intertidal zone of sandy beaches and belonging to classes Phaeophyta, Chlorophyta and Rhodophyta. Five species consist of four Phaeophyta (*Padina boergesenii, Sargassum prismaticum, Rosenvingea orientalis, Dictyopteris acrostichoides*) and one Chlorophyta *(Codium dwarkense)* were collected from Takalar coast, whereas two Rhodophyta (*Sarconema filiforme* and *Wrangelia tanegana*) were collected from Pangkep coast (Table 1).

Percent of dry biomass ranging from 6.57% to 19.62%. The highest percent were shown by brown alga *Rosenvingea orientalis* and red alga *Sarconema filiforme* (both have dry weight of 19.62%) whereas the lowest was shown by green algae *Codium dwarkense* with 6.57% (Table 2).

Table 1. Figure and classification of marine algae collected from Takalar and Pangkep coast of Sulawesi Selatan

No.	Algae Species	Class, Order and Family (Location)	Algae Figure
1.	*Padina boergesenii*	Phaeophyta, Dictyotales, Dictyotaceae (Takalar)	
2.	*Sargassum prismaticum*	Phaeophyta, Fucales, Sargassaceae (Takalar)	
3.	*Rosenvingea orientalis*	Phaeophyta, Scytosiphonales, Chnoosporaceae (Takalar)	
4	*Dictyopteris acrostichoides*	Phaeophyta, Dictyotales, Dictyotaceae (Takalar)	
5	*Codium dwarkense*	Chlorophyta, Bryopsidales, Codiaceae (Takalar)	
6	*Sarconema filiforme*	Rhodophyta, Gigartinales, Solieriaceae (Pangkep)	
7	*Wrangelia tanegana*	Rhodophyta, Ceramiales, Wrangeliaceae (Pangkep)	

Table 2. Wet- and dry-weight of marine algal biomass

No.	Algae Species	Class	Wet Weight (ww) (g)	Dry Weight (dw) (g)	Percent of dw/ww
1	*Padina boergesenii*	Phaeophyta	540	58.22	10.78
2	*Sargassum prisnaticum*	Phaeophyta	597.3	74	12.39
3	*Rosenvingea orientalis*	Phaeophyta	265.04	52	19.62
4	*Dictyopteris acrostichoides*	Phaeophyta	1008	110,9	11
5	*Codium dwarkense*	Chlorophyta	1129	74.14	6.57
6	*Sarconema filiforme*	Rhodophyta	158	31	19.62
7	*Wrangelia tanegana*	Rhodophyta	514	78.67	15.31

A total of 15 organic extracts of seven marine algae species were obtained by extraction with three different polarities of solvent. The dry weight of crude extracts ranging from 110.5 mg to 662 mg (0.22-1.32%). The highest percent of dry weight was shown by dichloromethane extract of brown alga *Dictyopteris acrostichoides* (1.32%), whereas the lowest one was shown by n-hexane extract of red alga *Wrangelia tanegana* (0.22%.) (Table 3).

The fifteen organic extracts were evaluated for their cytotoxicity using brine shrimp *Artemia salina* lethality test. Twelve of 15 organic extracts showed high cytotoxicity with LC_{50} <62.5 µg/mL, whereas two extracts only presented intermediary cytotoxicity with LC_{50} ranging from 62.5 to 250 µg/mL. Of all the organic extracts tested, dichloromethane extract of *Sarconema filiforme* exhibited no cytotoxic activity against *Artemia salina* larvae with LC_{50} >1000 µg/mL (Fig. 1).

Table 3. Percentage of crude extracts obtained from 50 mg of biomass dry weight in 500 mL organic solvent.

No.	Algae species	Class	Extracts	Crude Extracts (mg)	Crude Extracts (%)
1	*Rosenvingea orientalis*	Phaeophyta	Hex	218.1	4.36
2	*Wrangelia tanegana*	Rhodophyta	Hex	110.5	2.21
3	*Padina boergesenii*	Phaeophyta	Hex	226.9	4.54
4	*Codium dwarkense*	Chlorophyta	Hex	147.5	2.95
5	*Sargassum prismaticum*	Phaeophyta	Hex	215.7	4.31
6	*Dictyopteris acrostichoides*	Phaeophyta	Hex	519.6	10.39
7	*Codium dwarkense*	Chlorophyta	DCM	222.4	4.45
8	*Sarconema filiforme*	Rhodophyta	DCM	142.4	2.85
9	*Dictyopteris acrostichoides*	Phaeophyta	DCM	662	13.24
10	*Rosenvingea orientalis*	Phaeophyta	DCM	213	4.26
11	*Padina boergesenii*	Phaeophyta	DCM	144.4	2,89
12	*Wrangelia tanegana*	Rhodophyta	DCM	361.3	7.23
13	*Sargassum prismaticum*	Phaeophyta	DCM	238.6	4.77
14	*Dictyopteris acrostichoides*	Phaeophyta	EtOAc	328.4	6.57
15	*Padina boergesenii*	Phaeophyta	EtOAc	282.6	5.65

Table 4. Cytotoxicity of organic extracts of marine algae collected from South Sulawesi coast against *Artemia salina* nauplii.

No	Algae species	Class	Extracts	LC_{50} value	Cytotoxicity Levels
1	*Rosenvingea orientalis*	Phaeophyta	n-Hexan	<62.5 µg/mL	High Cytotoxicity
2	*Wrangelia tanegana*	Rhodophyta	n-Hexan	<62.5 µg/mL	High Cytotoxicity
3	*Padina boergesenii*	Phaeophyta	n-Hexan	<62.5 µg/mL	High Cytotoxicity
4	*Codium dwarkense*	Chlorophyta,	n-Hexan	<62.5 µg/mL	High Cytotoxicity
5	*Sargassum prismaticum*	Phaeophyta	n-Hexan	<62.5 µg/mL	High Cytotoxicity
6	*Dictyopteris acrostichoides*	Phaeophyta	n-Hexan	125-250 µg/mL	Intermediary Cytotoxicity
7	*Codium dwarkense*	Chlorophyta	DCM	62.5-125 µg/mL	Intermediary Cytotoxicity
8	*Sarconema filiforme*	Rhodophyta	DCM	>1000 µg/mL	No Cytotoxicity
9	*Dictyopteris acrostichoides*	Phaeophyta	DCM	<62.5 µg/mL	High Cytotoxicity
10	*Rosenvingea orientalis*	Phaeophyta	DCM	<62.5 µg/mL	High Cytotoxicity
11	*Padina boergesenii*	Phaeophyta	DCM	<62.5 µg/mL	High Cytotoxicity
12	*Wrangelia tanegana*	Rhodophyta	DCM	<62.5 µg/mL	High Cytotoxicity
13	*Sargassum prismaticum*	Phaeophyta	DCM	<62.5 µg/mL	High Cytotoxicity
14	*Dictyopteris acrostichoides*	Phaeophyta	EtOAc	<62.5 µg/mL	High Cytotoxicity
15	*Padina boergesenii*	Phaeophyta	EtOAc	<62.5 µg/mL	High Cytotoxicity

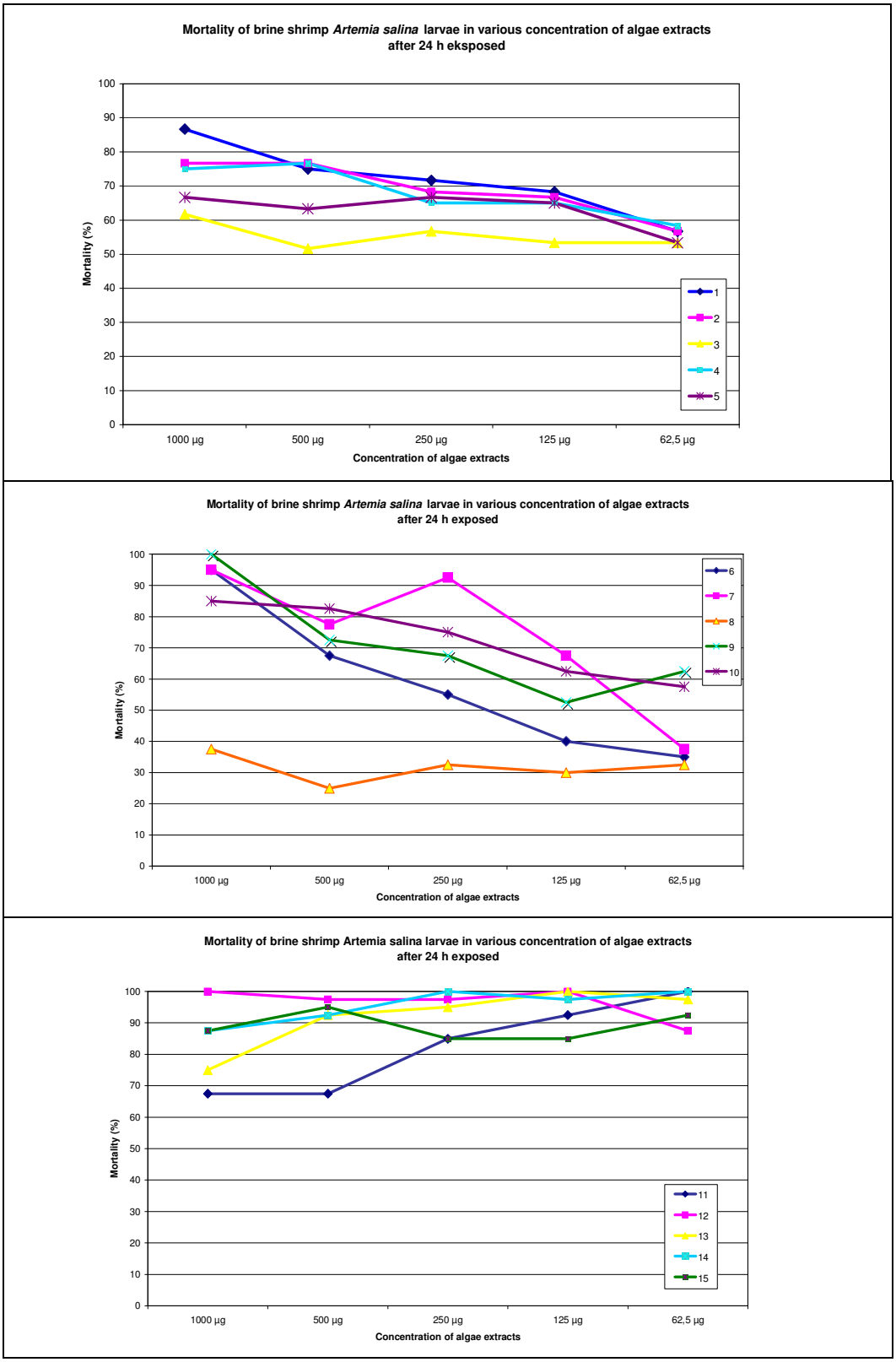

Figure 1. Graphic of percent mortality of *Artemia salina* nauplii after 24 h exposed in various concentrations of algae extracts (number on the graphic related to the number in Table 4).

Of six n-hexan extracts, only n-hexan extract of brown alga *Dictyopteris acrostichoides* had intermediary activity towards the nauplii of the brine shrimp *A. salina*

(LC_{50} value between 125-250 µg/mL), whereas others showed high cytotoxicity with LC_{50} below 62.5 µg/mL. Two dichloromethane extracts obtained from red alga *Sarconema filiforme* and green alga *Codium dwarkense* had no lethal effect (LC_{50} >1000 µg/mL) and intermediary cytotoxicity (LC_{50} occurred between 62.5 and 125 µg/mL) respectively. Ethyl acetate extracts of two brown algae *Dictyopteris acrostichoides* and *Padina boergesenii* showed a potent cytotoxic effect against *Artemia salina* nauplii (LC_{50} value below 62.5 µg/mL) (Table 4).

DISCUSSION

A number of studies have demonstrated that marine algae secondary metabolites from different geographical region had cytotoxic effects against *Artemia salina.* The algae, *Ulva fasciata* and *Hypnea musciformis* collected from India coast showed moderately cytotoxicity against brine shrimp (Selvin and Lipton, 2004). El-Baroty *et al.* (2007) demonstrated that the powdered red alga *Asparagopsis taxiformis* showed cytotoxic activities on *Daphnia magna.* In our study, almost all extracts from seven species of marine algae exhibited high and moderate cytotoxic activites against *Artema salina* nauplii.

In this study, the brown algae were established as a rich source of cytotoxic compounds since 10 of 14 potential extracts were obtained from these species. These results are comparable to the results of other cytotoxic study of algae, which five ethanolic extracts of brown algae species namely *Stoechospermum marginatum, Sargassum swartzii, Sargassum binderi, Spatoglossum asperum, Stokeyia indica* showed significant cytotoxicity on brine shrimp assay (Ara *et al*., 1999). From the other study with brown algae *Sargassum ringgoldianum* and *Porphyra yezoensis*, a potential antitumor activity to ehrlich carcinoma and meth A fibrosarcoma was detected (Noda *et al.,* 1989). Beside brown algae, many of the secondary metabolites produced by the marine red algae are well known for their cytotoxic property against cancer and tumor cells. As noted by Harada and Kamei (1997) that the extract of red alga, *Amphiroa zonata* exhibited strong cytotoxicity to human leukemic cell line.

In this study, from 15 non-polar extracts, almost all were effective against *Artemia salina* nauplii. This may be due to a non-polar structure of cytotoxic compound(s). Of all tested extracts, the hexan extracts performed high and moderate cytotoxic activities against the nauplii of *Artemia salina*. From the study of antitumor activity of 42 japanese seaweeds species against K562 human leukemia cells, significant cytocidal activity was found in hexane-extract from *Protomonostroma undulatum, Enteromorpha linza, Monostroma latissimum*, *Scytosiphon lomentaria, Hizikia fusiformis* and *Sargassum thunbergii*, while cytostatic activity was indicated in hexane-extract from *Colpomenia sinuosa, Ecklonia cava, Undaria pinnatifida* and *Sargassum muticum* and *Chondria crassicaulis*. The hexane-extract of *Ecklonia cava* (Phaeophyceae) had the highest antitumor activity which inhibited cell growth by 45, 92.1 and 96.4% at 10, 20 and 50 MU.g/ml, respectively. Hexane extract of *E. cava* also induced apoptosis of K562 cells (Kaori, 2002). This is also related with our study, where the hexane extracts of brown algae (Phaeophyceae) showed more potential than the other algae. The dichloromethane extracts showed also good potential against *Artemia* nauplii. Five of seven DCM-extracts exhibited high cytotoxic activity against *Artemia salina* nauplii (LC_{50}= <62.5 µg/mL). From the other study, the crude dichloromethane:chloroform extract of *Stypopodium zonale,* a Brazilian coastal seaweeds, showed good cytotoxic activity against the C32 cell line (Rocha *et al.,* 2006). In this study, all ethyl acetate extracts of brown algae showed high cytotoxicity against the nauplii of *Artemia* salina. This was also performed in the other studies, where the ethyl acetate extracts of *Gracilaria salicornia* and *Hypnea flagelliformis*, collected from Persian Gulf showed potent cytotoxic effect against *Artemia salina* nauplii with LC_{50} values of 3 and 4 µg/ml, respectively (Saeidnia *et al.,* 2009). The ethyl acetate extracts of *Sargassum thunbergii*

and *Dictyopteris divaricata* showed excellent cytotoxic activity against the HL-60 cell line. Furthermore, the *Sargassum thunbergii* extract also exhibited good cytotoxic activity against the HT-29 and B16F10 cell lines. These results suggest that *S. thunbergii* and *D. divaricata* have great potential value as food additives, medicinal supplements for patients with chronic diseases and preventive agents against cancer (Kim *et al.,* 2009).

Other than the extracts, many specific compounds also have been found in algae. Fucoidan, laminarin and terpenoids stated to possess anticancer, antitumor and antiproliferative properties (Smith, 2004). These cytotoxic compounds could be further explored as novel leads in cancer chemoprevention and complementary chemotherapy and necessitates further investigation (Vinayak *et al*., 2010). Fucoidan, a sulphated polysaccharides isolated from brown algae *Laminaria cichorioides,* exerts chemopreventive effects. It inhibits neoplastic cell transformation induced by epidermal growth factor or a tumor promoter (12-O-tetradecanoylphorbol-13-acetate) (Anonim, 2009). Beside fucoidan, other highly sulphated polysaccharide isolated from *Gracilaria dominguensis* inhibited the transplantation of Ehrlich ascites carcinoma in mice (Fernandez *et al.,* 1989). The polysaccharide DAEB was isolated and purified from *Enteromorpha* intestinalis and had potent antitumor activity which may be associated with its potent immunostimulating effect (Jiao *et al*., 2009). The *in vitro* and *in vivo* antitumor properties of a sulfated polysaccharide isolated from the alga *C. feldmannii* (Cf-PLS) has some interesting anticancer activity that could be associated with its immunostimulating properties (Lins *et al*., 2008).

Many species from genus *Sargassum,* such as *Sargassum micracanthum (Mori et* al., 2005), *Sargassum caryophyllum* (Tang *et al*., 2002) and *Sargassum tortile* (Numata *et al*., 1991) exhibited cytotoxic activity against cancer cell lines. The sulphated polysaccharides of *Sargassum* act as a potent anticancer agent (Dias *et al*., 2005). In our studies, one of genus *Sargassum* (Sargassaceae), *Sargassum prismaticum* showed high cytotoxic activity on *Artemia* nauplii.

CONCLUSION

High price of the medicine and difficult access to the pharmacy could be the reason for poor people in the coastal community to provide their drug with marine medicinal plant surround their area. As we know, culture location of algae or seaweeds is in the sub-tidal zone with 2 m depth. Like terrestrial plant, marine algae require sun light for their metabolisms and Indonesia is one of the tropical countries which obtain sun light through the year. This advantage makes the algae is easy to be cultivated in Indonesia regions (marine bioprospecting). With ±75% of marine area (5,8 million km2), ±81.000 km coast line and 17.500 big and small island, make the marine and fishery industries have good expectations. Sulawesi Selatan is one of the east Indonesia regions which has a potential area to develop the algae culture. Almost half of total national productions of algae commercial are come from South Sulawesi area. Therefore, the aim of the study is to explore the bioactive compounds of marine algae from South Sulawesi as marine bioprospecting resources. In conclusion, the results of this study showed that marine algae from South Sulawesi can be used as medicinal marine plant for anticancer drugs candidate.

ACKNOWLEDGMENTS

We sincerely thank to Indonesia government for the financial support through competitive grant DIPA DP2M-DIKTI Stranas 2010.

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STUDY OF CELL AND CALLUS CULTURE OF *ERYTHRINA VARIEGATA* L. FOR SECONDARY METABOLITE PRODUCTION AS ANTIMALARIA HERBAL MEDICINE

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Abstract: Malaria desease is one of deseases that to be solved in Indonesia. *Erythrina variegata* known as traditional medicine as anti malaria medicine. Plan regeneration of *E. variegata* is slowly and time consuming. Alternative technology i.e tissue culture technology could open a possibility both plant regeneration and providing herbal medicine material. The objectives of research are (1) to study cell and callus induction from *E. Variegata;* (2) to get sufficien and good quality of callus; (3) to identify active compound based callus. Several Experimental Design were used in this research including sterilisation procedure, basic media, explant factor, growth regulator related to callus growth. Result showed that 2,4-D between 0.5 mg/L until 1,5 mg/L could induce callus growth from two type of *E. Variegata* leaf explant. The best concentration of 2,4-D was 1,5 mg/L for obtaining optimum callus size and biomass. Secondary Metabolite content form Callus has been analyzed. Result showed that callus derived leaf dan callus derived leaf stem indicated antimalarial activity to *P. falciparum* IC_{50} 40 and 62 □g/mL, respectively.

Keywords : *Erythrina variegata*, tissue culture, secondary metabolite, antimalaria, phytochemistry

INTRODUCTION

Malaria is a major public health problem in Indonesia with 6 million clinical cases and 700 deaths each year (Laihad, 2000). In 1998, it was reported that 46.2% of the total Indonesian population of 210.6 million lived in malaria endemic areas (Ministry of Health and Social Welfare, 2001). The appearance of drug-resistance *Plasmodium falciparum* since 1960 has made the treatment of malaria increasingly problematic, and apparently the battle has not been successful (Najila *et al.,* 2002) . This could be attributed in part to the widespread problem of parasite drug-resistance (Kalauni *et al*, 2006) . In recent years, attention was focused on traditional system of medicine that has become a topic of global importance plants to provide new and novel antimalarial agents. Although modern medicine may be available in developed countries, herbal medicines (phytopharmaceuticals) have often maintained popularity for tradition and cultural reasons.

Progress in biotechnology particularly methods for culturing plant cell and organ, should provide new means for the commercial processing of even rare plants and the chemicals they provide. These new technologies will extend and enhance the usefulness of plants as renewable resources of valuable chemicals. There has been considerable interest in plant cell cultures as a potential alternative to traditional agriculture for the industrial production of secondary metabolites (Dicosmo and Misawa, 1995). Plant cell culture technologies were introduced at the end of 1960s as a possible tool for both studying and producing plant secondary metabolites. Different strategies using cell cultures systems have been extensively studied with the objective of improving the production of bioactive secondary metabolites. Cell culture systems could be used for the large scale culturing of plant cells from which secondary metabolites can be extracted.

The Dadap Ayam (Heyne, 1987) (*Erythrina variegata* L.) is known under the common name Dadap blendung (Sundanese), Dadap ayam, Dadap laut (Java); Theutheuk

(Madura); Galala kokotu (Ternate); Lola kohori (Tidore). Part of *E. variegata* such as stem, leaf, root, and seed has been applied as traditional medicine (Chawla, *et al.*, 1988). Tanaka, *et al.*, 2000 and Sato, *et al.*, 2000 has been reported that part of *E. variegate* has alkaloid, flavonoid and isoflavonoid.

In the course of our continuing search aiming for novel bioactive compound from Indonesian plants, the methanol or extract of the seed and leave of *Erythrina variegata* (Leguminosae) showed significant paralytic and antimalaria activity.

The objectives of research are (1) to study sel and callus induction from *E. Variegata;* (2) to get sufficien and good quality callus; (3) to identify active compound based on callus.

MATERIAL ADN METHODS

Explant and Plant material

All plant growth regulators, standard media and agar used for cell and tissue culture were obtained from several supplier. Two types of plant *E.variegata* was used as sources of explants. Stem section of leaf of approx. 0.5 cm long and leaf section of approx. 1,0 cm^2 taken from plants grown in the field in Bandung and around campus Universitas Padjadjaran Jatinangor, Sumedang. The explants were cultured in bottle (75 mm high dan 50 mm diameter) containing 10.0 ml Murashige and Skoog (1962)-liquid nutrient medium (MS) with 2,5% sucrose, wirh several concentration of (2,4-D). All explants were maintained in a light regime of 16 h under daylight fluorescent lamps (Philips TLT 40W/ 54 R. S.), at average temperature of 24^oC.

Parasite strain

In this study two strains of *P. falciparum* were used, culture of 3D7 (chloroquine sensitive and K1 (chloroquine resistant).

General Experimental Procedure

Melting points (mp) were uncorrected. The IR spectra were recorded with a Perkin-Elmer 1760 X FT-IR spectrophotometer, and the UV spectra were recorded with a Hitachi model U-3210. Mass spectra were recorded with JEOL JMS-DX300 instrument. The ^1H- and ^{13}C-NMR spectra were obtained with JEOL JNM GX 270 and JNM A-500 spectrometer. Chemical shifts are given on a δ (ppm) scale with TMS as an internal standard. Column chromatography was carried out using Merck Kieselgel 60 (70-200 mesh), and thin layer chromatography (TLC) analysis was performed on precoated Si Gel plates (Merck Kieselgel GF_{254}, 0.25 mm 20 x 20 cm).

Drug dilution

The lactate dehydrogenase (LDH) method was performed using 96-well microtitreplates (flat bottom). The initial concentration of the extract, chloroquine (Sigma Chemical, USA) and artemisinin (Sigma Chemicals, USA) were 1000 µg/ml and 1 µg/ml, respectively. The samples were then serially diluted in culture medium supplemented with 10% human serum 19 times.

Extraction and isolation

The dried callus derived leaves and stem of leaves of *E. variegata* were soaked in methanol. The crude methanolic extract was then assayed for anti-malarial activity via the LDH method. Evaporation of the methanol extract gave concentrated aqueous extract, which was extracted with dichloromethane. The resulting dichloromethane extract was partitioned between *n*-hexane and methanol containing 10% of water, and then lower layer was concentrated and extracted with ethyl acetate. The ethyl acetate layer was subsequently dried over anhydrous sodium sulfate, filtered, evaporated to dryness, and assayed for anti-malarial activity

Antimalarial activity

In vitro testing of the anti-malarial activity was carried by measuring the LDH activity of the parasite. Briefly, continuous culture of the 3D7 sensitive chloroquine and K1 resistant chloroquine, were maintained in a suspension consisting of RPMI 1640 culture medium supplemented with HEPES, *N*-2-hydroxyethylpiperazine-*N*'-2-ethane-sulfonic acid (25 mM), sodium bicarbonate (0.2%) and gentamycin (40 µg/ml) at pH 7.4, and O type red blood cell. For each LDH test, a blood suspension of 1% parasitemia and 2% haematocrit were prepared. Control reading of parasitised red blood cells devoid of plant extracts or drugs and non-parasitised red blood cells were done simultaneously. After the plate has been prepared, it was placed in a candle jar and incubated for 48 h at 37^{o}C. After 48 h, 100 µl of Malstat (Flow Inc., Portland, OR), was dispensed into a new microtitreplate. To it was added 25 µl of NBT-PES (Sigma Chemicals, USA) mixture. Twenty microliters of blood suspension was transferred into the plate containing the Malstat and NBT-PES. Any air bubbles were eliminated as it could interfere with the absorbance reading. Absorbance was read at 630 nm using an ELISA plate reader (MRX Microplate Reader, Dynex Technologies, USA). Chloroquine and artemisinine functioned well as positive controls [2].

Analysis of results

Percentage inhibition of parasite viability was determined and the mean of least IC_{50} values was calculated using the curve fitting analysis in Grafit (Grafit v.4.09, Erithacus Sofware Limited).

RESULT AND DISCUSSION

Callus Growth Initiation

Murashige and Skoog (MS) media without 2,4-D could not initiate callus growth, but MS media containing 2,4-D 0,5 mg/L (d_2), 1,0 mg/L (d_3), 1,5 mg/L (d_4), dan 2,0 mg/L could stimulate callus growth (Tabel 1). There was no significant result among 2,4-D concentration 0.5 mg/L (d_2), 1,0 mg/L (d_3), 1,5 mg/L (d_4), and 2,0 mg/L (d_4) to initiate callus growth, 11,67; 12,17; 12,00; 12,50 days after culture respectively. Jacobsen (1990) reported that callus initiation effected by endogenous hromon as well as exogenous hormone, and translocation and it metabolism. Caboche *et al.* (1984) has also demonstrated that 2,4-D absorbed by mesophyl tissue of tobacco slowly.

Tabel 1. Effect of 2,4-D on two types of explants sources to Callus Initiation

Treatment Genotype	Average Callus Initiation (days after culture)
a_1	3.44 a
a_2	3.47 a
2,4-D	
d_1	0.00 a
d_2	11.67 b
d_3	12.17 b
d_4	12.00 b
d_5	12.50 b

Note: Value capital font on the same row and small font on the same coloum : no significant based on Duncan 5% test.
a_1 = leaves type A; a_2 = Leaves type B; d_1 = MS + 2,4-D 0 mg/L; d_2 = MS + 2,4-D 0.5 mg/L; d_3 = MS + 2,4-D 1.0 mg/L; d_4 =MS + 2,4-D 1.5 mg/L; d_5 =MS + 2,4-D 2.0 mg/L

Friable callus structure was obtained from two types of each leaves explants in after three wekks of culture (Figure 1.)

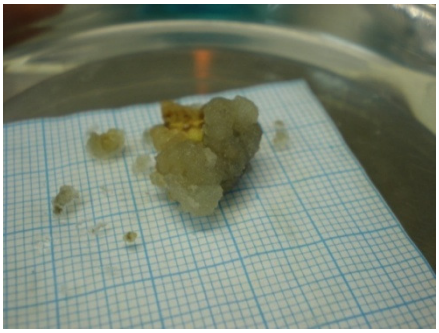

Figure 1. Friable callus structure from leaf explant of *E.variegata*

Callus Size

Callus size can be used as indicator for cell proliferation. Callus size related to activity of dividing cells, response of the cells on media containing, nutrient, growth regulator and physical factors. Callus size normally cam be measured by clay model.

There was interaction between types of leaf explant and 2,4-D concentration (Tabel 2). Based on Duncan test 5% showed that 0,5 mg/L (d_2) 2,4-D was the best concentration for callus size induction, 2,33 clay and 11,50 clay, respectively.

Table 2. The Effect of 2,4-D on *E. variegata* leaf to Callus Size 70 DAC

Concentration 2,4-D	Types of leaf explant	
(D)	A1	A2
MS + 0 mg/L 2,4-D	0.00 a	0.00 a
(d_1)	A	A
MS + 0.5 mg/L 2,4-D	12.33 d	11.50 d
(d_2)	B	B
MS + 1.0 mg/L 2,4-D	10.00 c	8.33 b
(d_3)	B	A
MS + 1.5 mg/L 2,4-D	9.00 b	8.33 b
(d_4)	B	A

Note: Value capital font on the same row and small font on the same coloum : no significant based on Duncan 5% test.
a_1 = leaves type A; a_2 = Leaves type B; d_1 = MS + 2,4-D 0 mg/L; d_2 = MS + 2,4-D 0,5 mg/L; d_3 = MS + 2,4-D 1.0 mg/L; d_4 =MS + 2,4-D 1.5 mg/L; d_5 =MS + 2,4-D 2.0 mg/L

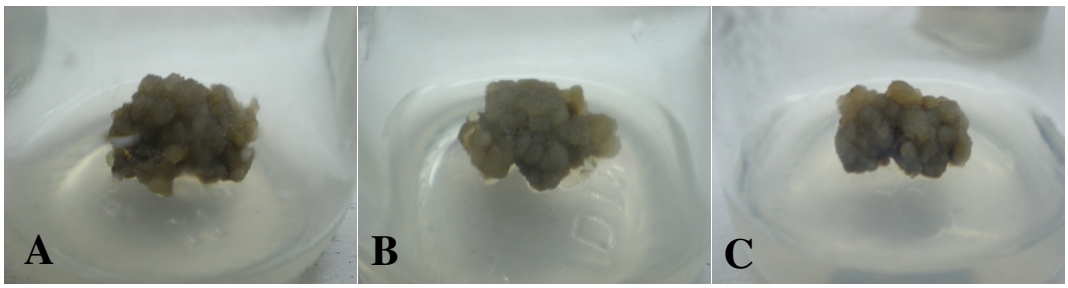

Figure 2. Calllus performance derived different leaf type and 2,4-D concentration in MS medium. Leaf A1 (MS + 0.5 mg/L 2,4-D) (A), Leaf A2 (MS + 0.5 mg/L 2,4-D) (B), and Leaf A2 on MS media containing 1.5 mg/L 2,4-D (C).

Callus size from leaf type A_1 better then callus derived leaf type B_2 on MS medium containing 1,5 mg/L 2,4-D. Figure 2. shown calllus size from different types leaf and 2,4-D concentartion on medium.

Secondary Metabolite

Callus production from leaf have to be optimized. Low production of callus is main problem. Different growth regulator and physical factor has to be improved. In contrast with this achievement showed that callus derived leaf dan callus derived leaf stem indicated antimalarial activity to *P. falciparum* IC_{50} 40 and 62 μg/mL, respectively.

CONCLUSION

The best concentration of 2,4-D was 1.5 mg/L for obtaining optimum callus size. Callus derived leaf dan callus derived leaf stem indicated antimalarial activity to *P. falciparum* IC_{50} 40 and 62 μg/mL, respectively.

ACKNOWLEDGEMENTS

We are grateful to DGHE for funding this research via Riset Unggulan Strategsi Nasional 2009.

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ACUTE TOXICITY STUDY OF THE METHANOL EXTRACT OF *RHODOMYRTUS TOMENTOSA* LEAVES IN MICE

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Abstract : *Rhodomyrtus tomentosa* (local name: Karamunting) is a medicinal plant, which is traditionally used as antidiabetic agent in South Kalimantan, Indonesia. The objective of the study was to evaluate the acute toxicity of the methanol extract of the leaves of *R. tomentosa*, in Balb/c mice. In this study, fifty male and female Balb/C mice were randomly distributed into one control group and four treated groups. The methanol extract of *R. tomentosa* leaves were administered orally to treated groups of mice with dosages of 5000, 7500, 10000, and 15000 mg/kg body weight to determine the acute toxic effects and the median lethal dose (LD_{50}). The evaluation of the toxic symptoms and mortality was done for 14 days. The LD_{50} of the methanol extract of *R. tomentosa* leaves was found to be 12450 mg/Kg of body weight. It was categorized as moderately toxic. Histopathological examination of control group after administration of 0.5 % CMC Na showed no lesions on all tissues examined. However, marked changes were observed in the liver and kidney in form of centrolobular necrosis, tubular degeneration, inflammation in blood circulation area, congestion, and swollen after given to all dosage levels.

Keywords: methanol extract, *Rhodomyrtus tomentosa*, LD_{50}, histopathology.

INTRODUCTIONS

Rhodomyrtus tomentosa is evergreen shrub of Southeast Asian origin which usually grows to 2 m tall, but occasionally to 4 m tall. Locally, the plant is known as "Karamunting". Its leaves are opposite, simple, entire, elliptic-oval, to 3 in. long. The leaves are glossy green above and densely soft-hairy below, with 3 main veins from the leaf base (Figure 1). In South Kalimantan, the leaves are traditionally used as an antidiabetic agent and for the treatment of diarrhea and burn wound. Phytochemicals reported in the plant are the alkaloids, flavonoid, steroid, triterpenoid, tannin, and quinon. There is a lack information about the toxicological potentials of the extracts, and the possible hepato/nephrotoxicity of the methanol extract of the leaves has not been reported. Therefore, the present study is aimed at investigating the toxicity of the methanol extract of the leaves of *R. tomentosa* on some indices of liver and kidney function in Balb/c mice.

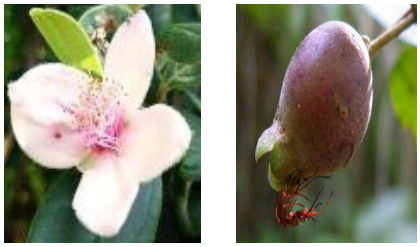

Figure 1. ***Rhodomyrtus tomentosa***

MATERIALS AND METHODS

Plant material

Part of the plant used for the study was the leaves. They were collected between the months of April and June in Banjarmasin, South Kalimantan, Indonesia and identified by a botanist in the Department of Biological Sciences, University of Lambung Mangkurat, Banjarmasin, Indonesia.

Animals

Healthy Balb/c mice of both sexes, weighing 25-35 g, obtained from the University of Gadjah Mada. A total of 50 mice were used, divided into one control group and four treated groups.

METHODOLOGY

Extract preparation

The fresh leaves of the plant collected were sun dried and processed into fine powder by many rounds of grinding and sieving. 400 g of the fine powder was extracted with 96% methanol using a soxhlet extractor (50^{o}C).

Acute toxicity study in mice and histopathology

The methanol extract of *R. tomentosa* leaves were administered orally to treated groups of mice with dosages of 5000, 7500, 10000, and 15000 mg/Kg body weight to determine the acute toxic effects and the median lethal dose (LD_{50}). The evaluation of the toxic symptoms and mortality was done for 14 days.

Small pieces of liver and kidney, fixed in buffered formalin were processed for embedding in 4% paraffin. Section were cut and stained with hematoxylin and eosin and examined for histopathological changes under the microscope. The images were taken at original magnification of 100-400x.

RESULTS AND DISCUSSION

Observation of clinical symptoms by such as motoric, stretch, silence tendency edge cage, tremors and liveliness to the stimuli during the 14 days. Treatment groups that obtained the highest score is the percentage of treatment group doses of 15,000 mg/KgBW.

Group I (negative control/NaCMC 0.5%) weight changes which tend to be less stable. Group II (doses 5000 mg/KgBW) showed that body weight tended to be less stable, this is indicated by body weight decreased from day 1 to day 7. The range of greatest weight increase occurred on day 9 until day 10 that reached 1.34 g. Group III (doses 7500 mg / KgBW) showed weight loss tends to be less stable. The range of greatest weight loss occurred from day 5 to 6 reached 1.62 g. Group IV (doses 10 000 mg/KgBW) showed weight loss tends to be less stable. The range of greatest weight increase occurred on day 7 to 8 that reached 0.66 g. Group V (doses 15 000 mg/KgBW) showed weight loss tends to be less stable. The range of greatest weight loss occurred from day 12 to 13 reached 2.46 g (the effect presented in Table 1). The effect of administration of methanol extract of the leaves of *R. tomentosa* on mortality of the mice is presented in Table 2. The LD_{50} of the methanol extract of R. tomentosa leaves was found to be 12.450 mg/Kg of body weight.

Histopathological examination of control group after administration of 0.5 % CMC Na showed no lesions on all tissues examined. However, marked changes were observed in the liver and kidney in form of centrolobular necrosis, tubular degeneration, inflammation in blood circulation area, congestion, and swollen after given to all dosage levels (Table 3 and Figure 2).

Table 1. Body weigh mice after use methanol extract karamunting (*R. tomentosa*) leaves long times of 14 days.

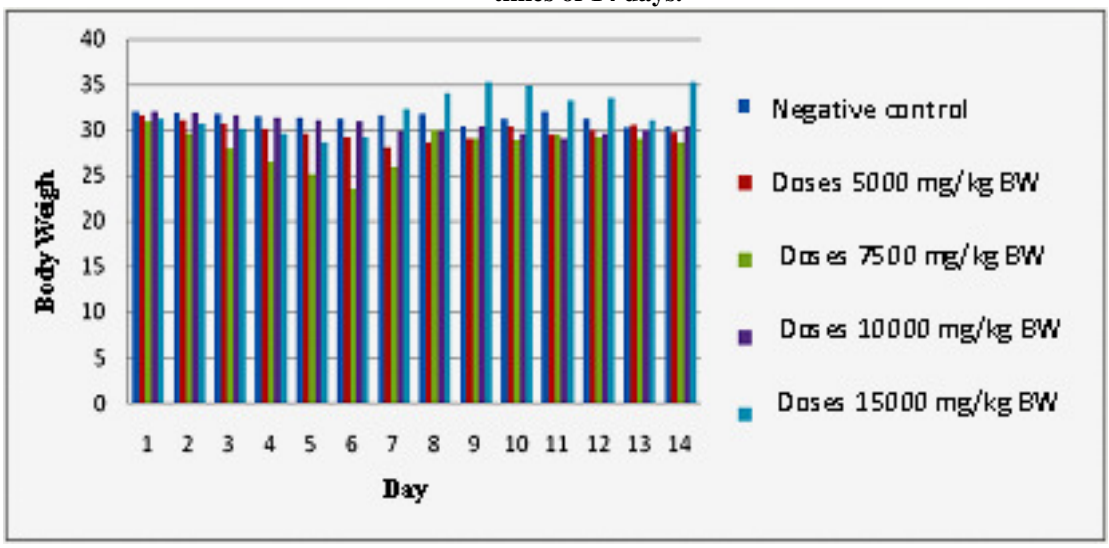

Table 2. The effect of administration of methanol extract of the leaves of *R. tomentosa* on mortality of the mice

Treatmens (doses)	N	Dead mice	Respond (%)	LD_{50}
CMC-Na 0.5%	10	-	-	12.450 mg/kgBW
5.000 mg/kgBW	10	1	10	
7.500 mg/kgBW	10	3	30	
10.000 mg/kgBW	10	2	20	
15.000 mg/kgBW	10	7	70	

Table 3. Histopatological changes occurred in the treatment groups by treatment with *R. tomentosa* methanol extract

Treatment	Liver				Kidney			
	Vacuolar Degenera-tion	Necrosis	Conges-tion	Inflam-mation	Tubulus degenara-tion	Swoller	Conges-tion	Inflam-mation
I	-	-	-	-	-	-	-	-
II	+	+	+	_	++	-	-	-
III	++	++	++	+	+	+	+	-+
IV	++	++	++	+	+++	+-	+	-+
V	+++	+++	++	-	++	+	-	+

Note
- : Normal
+ : Decrease of 25%
++ : Decrease of 50%
+++ : Decrease of 75%

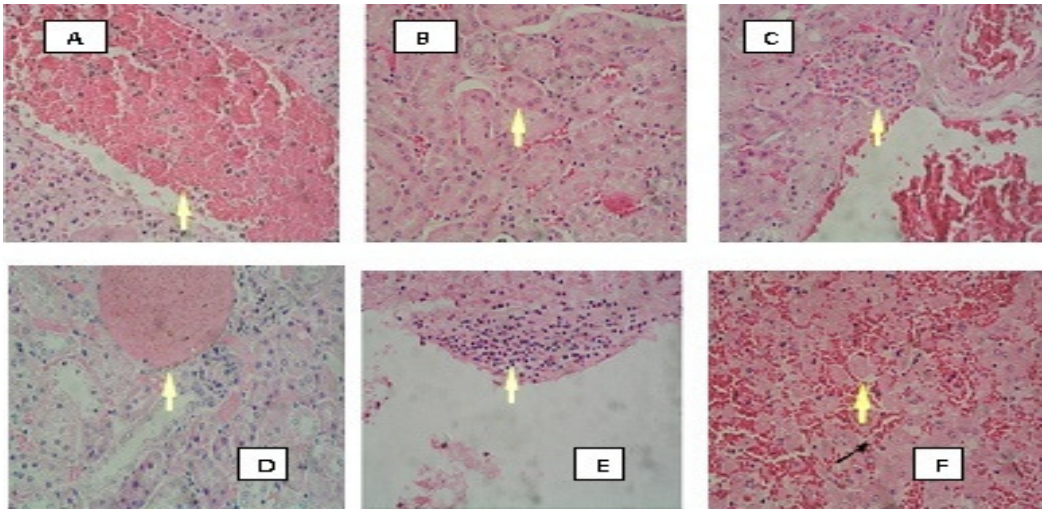

Figure 2 : Histopatological changes occurred in the treatment groups by treatment with R. tomentosa methanol extract . (A : congesti in liver cell, B : Tubullus degeneration in kidney cell, C : glumerullus swollen in kidney cell, D : Congesty in kidney cell, E : inflamation in liver cell, and F : Degeneration vacuolar and necrosis in liver cell)

CONCLUSION

1. The LD_{50} of the methanol extract of *R. tomentosa* leaves was found to be 12450 mg/Kg of body weight. It was categorized as moderately toxic.
2. Histopathological examination of control group after administration of 0.5 % CMC Na showed no lesions on all tissues examined. However, marked changes were observed in the liver and kidney in form of centrolobular necrosis, tubular degeneration, inflammation in blood circulation area, congestion, and swollen after given to all dosage levels.

ACKNOWLEDGEMENTS

Thanks To DIKTI who have helped in funding the research through grants budget character in 2009, making it one of a series of research activities can be performed well.

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CYTOTOXICITY EVALUATION OF MEDICINAL PLANT *ANDROGRAPHIS PANICULATA* IN BREAST CANCER CELL LINES

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Abstract: Andrographis paniculata is widely used as a medicine to treat many different diseases in Indonesia and other Asian countries. In this present study, methanolic and ethanolic extracts of *Andrographis paniculata* (known as **Sambiloto**) collected from Medicinal Plant Research Centre (B2PTO, Balai Besar Penelitian Tanaman Obat), Tawangmangu, Central Java have been tested againts breast cancer cell lines of T47D and MCF-7 and normal fibroblast cell line of HFL-1 using enzymatic reaction of 3-(4,5-dimethylthiazoyl-2-yl) 2,5 diphenyltetrazolium bromide (MTT). *In vitro* assay performed on normal fibroblast of HFL-1 cell line showed that 50 ppm of methanolic extract of *Andrographis paniculata* did not inhibit cell growth. However, methanolic and ethanolic extracts of *Andrographis paniculata* gave relatively low of IC-50 on breast cancer cell lines which were 111 ppm and 122 ppm on the MCF-7 cell lines and 70 ppm and 197 ppm on the T47D cell lines. In addition, the mixture of *Andrographis paniculata* extract containing 25% of *Thyponium divaricatum* (known as **Keladi tikus**) and *Anredera cordifolia* (known as **Binahong**) extracts confered greater growth inhibition on breast cancer cell line of MCF-7, where IC-50 values were 68 ppm and 34 ppm, respectively. To sum up, the total methanolic or ethanolic extract of medicinal plant *Andrographis paniculata* collected from Tawangmangu, Central Java have potency as a source of anti cancer compounds and need to be further studied.

Keywords: *Andrographis paniculata* extract, MTT, normal cell line, cancer cell lines and anti cancer.

INTRODUCTION

The genus Andrographis is a widely distributed in South Asia and medicinally important member of Acanthaceae, consisting of approximately 40 species. *Andrographis paniculata* Nees (known as "**Sambiloto"** in Indonesia) is an herb well known in the South East Asian as traditional medicine and its beneficial actions are many and varied. Over the century, mostly the leaves and the roots have been used traditionally not only in Asia but also in Europe as folklore remedy for wide spectrum of ailments or supplements for health support as listed in Table 1 (Jarukamjorn and Nemoto, 2008).

Phytochemical studies resulted in isolation of flavonoid and andrographolides from *A. paniculata* (Rao *et al* 2004). Furthermore, three new compounds of *ent*-labdane diterpenoids, namely 19-norandrographolides A–C (compounds 1–3), were also isolated from the ethanolic extract of *A. paniculata*. (Zhang et al. 2006). Recent studies demonstrated that of *A. paniculata* have a potency as an antimicrobial activity (Sule *et al.* 2010). Previous research also reported that *A. paniculata* has anticancer and immunostimulatory effect (Kumar *et al.* 2004), anti-hyperglycemic and renal protective activities (Rao, 2006). *Andrographis paniculata* are the most common traditional herbal that is used to achieve lower blood glucose in diabetic pasients by exhibits insulin-releasing actions (Wibudi *et al*, 2008). Hydroalcoholic extract of *A. paniculata* was reported as antioxidant, antilipid peroxidative and antiischemic activity and was used in ischemic heart diseases (Ojha, 2009). In addition, leaf extract of *A. paniculata* has ability

to suppress arsenic -provoked toxicity in human peripheral lymphocyte culture (Ghopalkrisnan & Rao, 2008). In this present study, we evaluated the methanolic and ethanolic extracts of *Andrographis paniculata,* collected from Medicinal Plant Research Centre, Tawangmangu, Central Java, againts breast cancer cell lines of T47D and MCF-7 and normal fibroblast cell line of HFL-1 using enzymatic reaction of 3-(4,5-dimethylthiazoyl-2-yl) 2,5 diphenyltetrazolium bromide (MTT). In addition, we also tested the mixture extract of *A. paniculata* Ness with *Thyponium divaricatum* (known as Keladi tikus) as well as with *Anredera cordifolia* (known as Binahong).

Table 1. Traditional Uses of *Andrographis paniculata*

Country	Native Names	Traditional Uses
Traditional Chinese Medicine (TCM)	Chuan-Xin-Lian Chunlianqialio Yiqianxi Si-Fang-Lian Zhanshejian	Fever, Common cold Laryngitis, Pharyngitis, Tonsilitis Pneumonia Respiratory infections Hepatitis
Traditional Indian Medicine	Kalmegh Kiryato Maha-tikta Bhunimba	Diabetes Dysentrery, Enteritis Helminth infection Herpes, peptic ulcer, skin infection (topical use), snake- bites (topical uses)
Traditional Thai Medicine	Fah Thai Lai Nam Rai Pangpond	Fever, Commond cold Non-infectious diarrhea
Malaysia	Hempedubumi Sambiloto	Diabetes Hypertension
Japan	Senshinren	Fever, Common cold
Scandinavian	Green Chiretta	Fever, Common cold

MATERIAL AND METHODS

Reagents

Unless stated otherwise, all reagents used in this experiment was purchased from Sigma Aldrich (USA). RPMI-1640 medium, heat-inactivated fetal bofine serum (FBS), EDTA-trypsin was purchased from Gibco BRL (NY, USA). MTT powder (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) was purchased from Promega (USA).

Extract Preparation

The aerial parts (stems and leaves) of *Andrographis paniculata Nees*, *Thyponium divaricatum* and leaves of *Anredera cordifolia* used in this study were collected from Medicinal Plant Research Centre, Tawangmangu, Central Java – Indonesia. The plant samples were dried at room temperature and was finely powdered. Suitable amounts of the powdered materials were soaked in 95% ethanol or methanol (1 L per 100 g), and the solvent was evaporated at 40 °C to dry up under reduced pressure using a rotary evaporator to produce crude extracts. The extract was then collected and stored at -20 °C for further testing. The High Performance Liquid Chromatography (HPLC) analysis HPLC profile of *Andrographis paniculata Ness* was carried out to detect the presence of active compound of Andrographolide (C18 volumn,□□ = 223 nm, flowrate = 0.5-1 ml/minute, 20 minutes).

Cell culture

The human breast adenocarcinoma cells (MCF-7 and T-47D) and the human normal lung fibroblast cell lines (HFL-1) were used to determine the cytotoxicity of the extract. Cells were cultured in T-flask containing RPMI 1640 medium supplemented with

10% heat inactivated foetal bovine serum (FBS), 50 IU/ml penicillin and 50 µg/ml streptomycin. The cells were maintained in the CO_2 incubator at 37ºC in a 5% CO_2 with 95% humidity. After reaching the confluency of approximately 70-80%, exponentially growing of the cells were washed twice with magnesium and calcium free phosphate buffer saline (PBS). The buffer solution was decanted and cells were detached with 0.025% trypsin-EDTA solution by incubating the cells at 37ºC in a 5% CO_2 with 95% humidity for couple of minutes, then cell culture growth medium were added to a volume of 5 ml. The cell suspension was then transferred to the falcon tube and the cell pellet was obtained by centrifugation (1000g , 5 min). The cells were then resuspend in 10 ml of medium to make single cell suspension. The viable cells were counted by trypan blue exclusion assay in haemocytometer.

Cytotoxicity Assay

Cells were seeded in 96-well plate in 100 □l of cell culture growth medium to a final concentration of $5x10^3$ cells/well and incubated to allow for cell attachment. After 24 h incubation, a partial monolayer was formed (confluency of 70-80%) then 100 µL of the medium containing the plant extract (initially dissolved in DMSO, those were: 250, 100, 50, 20 and 10 µg/mL) were added to the cells (normal and cancerous cell lines) and re-incubated for the next 24 h. After incubation with the plant extracts for overnight (24 hours), 100 □l of the media was aspirated and the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) (Promega, USA) solution (5 □g) in culture media of 100 □l was added. The incubation time for cells-MTT solution was 4 hours in which blue crystal were formed, then 100 µL of the stop solution (SDS 10%) were added and cells were then incubated further for the next 24 h. Reduced MTT was assayed at 550 nm using a microplate reader. Culture medium containing 0.1% DMSO was used as a solvent control and untreated cells were used as a negative control

RESULT AND DISCUSSION

High Performance Liquid Chromatography (HPLC) analysis of the ethanolic extract of *A. paniculata* Ness shows that the extracts containing the active compound of Andrographolide. The andrographolide concentration was various depend on the solvent composition. Apparently, the retention time (RT) was 2.422 minutes and the maximum concentration of andrographolide was achieved when sample was extracted using ethanol 70 % (Figure 1). The andrographolide content was calculated based on the amount of extract being injected (1000 ppm) and the values are listed in Table 2.

Table 2. Andrographolide content of samples extracted using various solvent composition

Solvent Composition	Area under curve	Andrographolide (ppm)	% Andrographolide
96% ethanol	490398	15.97	1.6
Ethanol : H_2O = 70 : 30	3851355	96.25	9.6
Ethanol : H_2O = 50 : 50	1469925	69.85	7
H_2O (100 %)	2032409	6.75	0.7

Note: Sample extract injected: 1000 ppm at λ= 223 nm

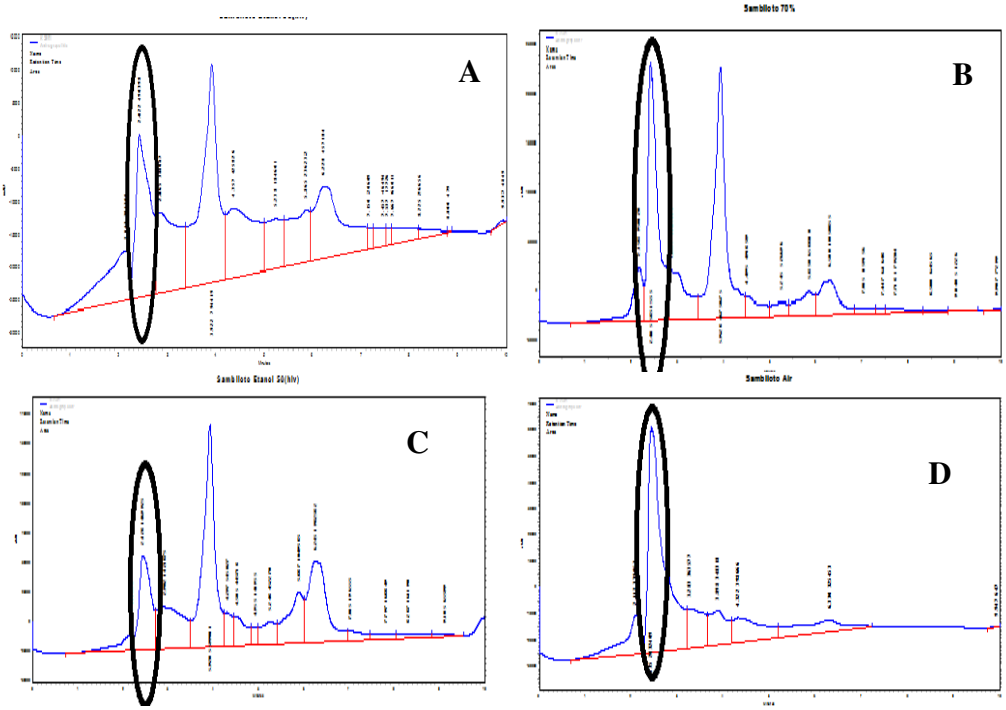

Figure 1. HPLC profile of *Andrographis paniculata* Ness extracts containing andrographolide; A. ethanolic extract of 96 %, B. etahnolic extract of 70%, C. ethanolic extract of 50 % and D. Decoction (H_2O extract).

In vitro cytotoxicity assay performed on normal fibroblast of HFL-1 cell line showed that 50 ppm of methanolic and ethanolic extract of *A. paniculata* did not inhibit cell growth, where the cell viability was about 101 % on the methanolic extract and 94 % on the methanolic extract. In this regards, we could simply assume that the extract has no cytotoxic effect on normal cell line of HFL-1. However, methanolic and ethanolic extracts of the sample gave IC-50 relatively low on breast cancer cell lines of MCF7 which were 122 ppm and 111 ppm, respectively. The growth inhibition of the extract in MCF7 was dose dependent manner. As shown in Figure 2, the proliferative inhibition activity indicated that increasing extract concentration resulted in an increasing the percent proliferative inhibition. Furthermore, cytotoxicity of the methanolic and ethanolic extracts on other breast cancer cell line of T-47D showed an IC-50 value of and 70 ppm and 197 ppm respectively. The proliferative inhibition activity pattern of the extracts on T-47D cell line similar with the proliverative inhibition activity on MCF-7 cell line, done dependent manner, where increasing of the extract concentration treated followed with an increased the proliferative inhibition activity (Figure 3).

Our present study on *A paniculata Ness* extract was in agrement with the results from previous researcher using another cell line, which reported that 10 ug/ml methanolic extract of *A. paniculata* inhibited proliferation of HT-29 (colon cancer cell) by 50%. In addition, the petroleum ether and dichlormethan extracts inhibited the proliferation of HT-29 cell with IC-50 value of 46 ug/ml and 10ug/ml, respectively (Kumar *et al*. 2004). Furthemore, from the ethanolic extract of the aerial parts of *A. paniculata* resulted in the isolation of 14 compounds including flavonoids and labdane diterpenoids, where the compound 6 *was* rich source for the active compound of andrographolide. The bioactivity assays showed that metabolites 1-4 and 6-8 exhibited moderate cytotoxic activity against Jurkat, PC-3 and Colon 205 cell lines, where compound 6 had IC_{50} values of 0.05, 0.07 and 0.05 mm, respectively. Further, among these effective compounds, 3 and 6

selectively blocked the cell cycle progression at G0/G1, while 1, 2, 4, 7 and 8 metabolites blocked the same at G2/M phase of the Jurkat cell line (Geethangili *et al.* 2008),

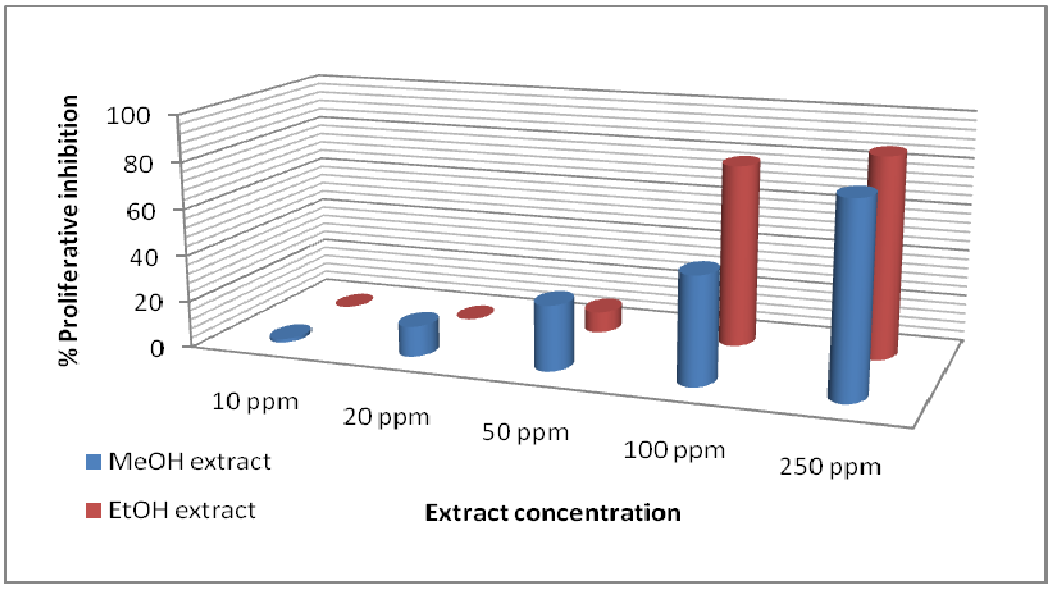

Figure 2. Effect of *A. paniculata* methanolic and ethanolic extract on proliferation of MCF-7 cancer cell line.

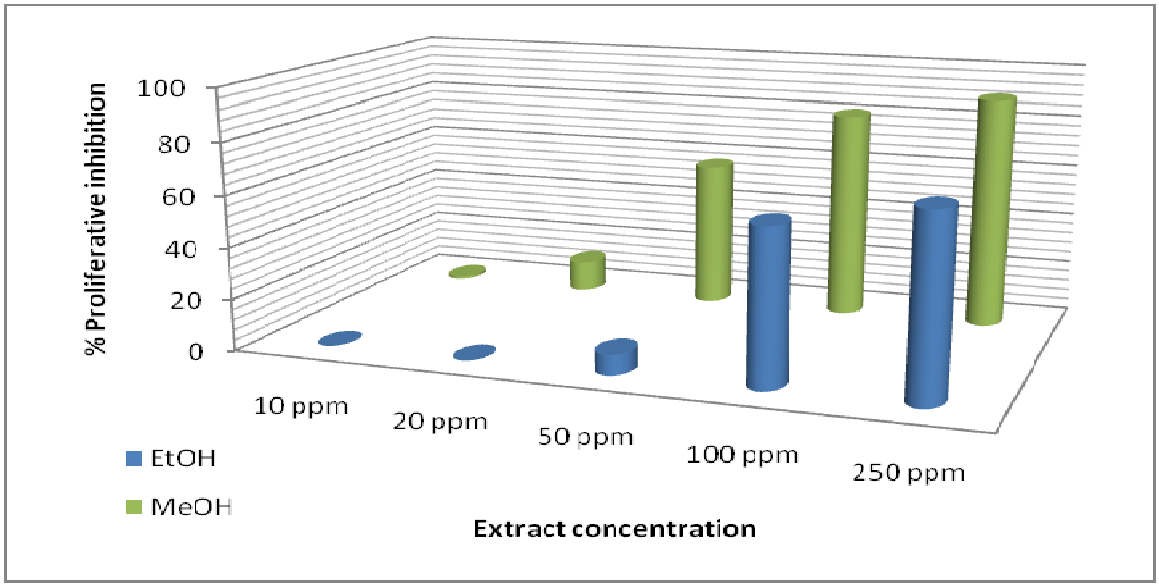

Figure 3. Effect of *A. paniculata* methanolic and ethanolic extract on proliferation of T-47D cancer cell line.

Subsequently, the mixture of 75% *A. paniculata* extract with 25% of *Typhonium divaricatum* confered greater proliferative inhibition on MCF-7 cell with gave IC-50 values of 68 ppm. Moreover, the combination of 75% *A. paniculata* extract with 25% *Anredera cordifolia* extracts resulted in lowest IC-50 value of 34 ppm. The pattern of proliverative inhibition of the mixture of A. paniculata with *Typhonium divaricatum* and *Anredera cordifolia* was shown on Figure 4. This result revealed possibility any synergistic action between the constituents resulted from the *A. paniculata* and from the *T. divaricatum* or the *A. cordifolia.* This result was also supported with previous study which reported that several fractions of the hexane and dichloromethane extracts of

Typhonium divaricatum were found to inhibit the growth of NCI-H23 non-small cell lung carcinoma cell line significantly, with IC_{50}<15µg/ml, and several fractions from this extract were also found to inhibit the growth of non-tumorigenic BALB/c 3T3 mouse fibroblast cell line. This particular fraction was not only less cytotoxic to the non-tumorigenic cells, where the IC_{50} was 48.6 µg/ml compared to IC_{50} 7.5 µg/ml for NCI-H23, but it was also found to induce apoptosis in the cancer cell line. GC–MS analysis revealed that D/F21 contains hexadecanoic acid, 1-hexadecene, phytol and a derivative of phytol (Lai *et al.* 2008). Subsequent studies reported that purification of the chemical constituents was guided by the antiproliferative activity using MTT reagent on NCI-H23 (lung cancer) and HS578T (breast cancer) cell lines. Four pheophorbide related compounds, namely pheophorbide-a, pheophorbide-a′, pyropheophorbide-a and methyl pyropheophorbide-a were identified in the most active fraction, D/F19. These constituents exhibited antiproliferative activity against cancer cells and the activity increased following photoactivation. The inhibitory effect of the fractions was apoptotic in the absence of light. Other chemical constituents that have been identified in this study include hexadecanoic acid, oleic acid, linoleic acid, linolenic acid, campesterol, stigmasterol and β-sitosterol (Lai *et al.,* 2010).

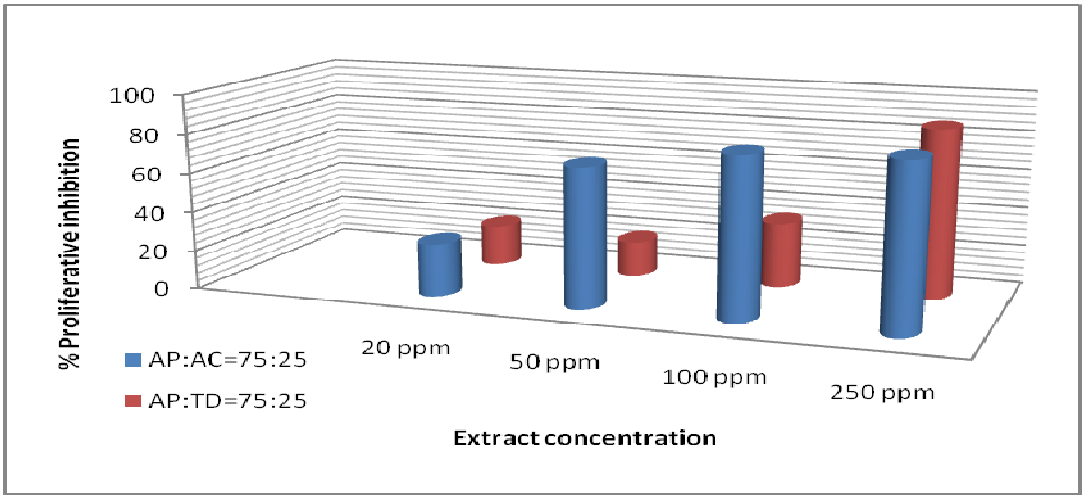

Figure 4. Effect of the mixture of *A. paniculata* with *T. divaricatum* and *A. cordifolia* ethanolic extract on proliferation of MCF-7 cancer cell line. AP : *Andrographis paniculata*, AC: *Anredera cordifolia*, TD: *Thyponium divaricatum*.

CONCLUSION AND FUTURE WORKS

The total methanolic or ethanolic extract of medicinal plant *Andrographis paniculata* collected from Tawang-mangu, Central Java have potency as a source of anti cancer compounds and need to be further studied to understand the mechanism of action of the extracts against cancer cell.

ACKNOWLEDGEMENT

The authors would like to kindly acknowledgement to the Centre for Pharmaceutical and Medical Technology- Deputy for Agro-industry and Biotechnology-BPPT for the funding of this research.

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SEPARATION OF CHLOROFORM FRACTION OF STEM BARK OF *BRUGRUIERA GYMNORHIZA* USING BIOASSAY GUIDED FRACTIONATION AND CYTOTOXIC EFFECTS ON CANCER CELL LINES

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Abstract : Utilization of mangrove plants as ingredients of traditional medicine has long been used by people in some therapies such as anti-cancer diseases. *Bruguiera gymnorhiza* is one of the mangrove plants that have not been much studied as an anti-cancer potential. The purpose of this study was fractionate stem bark of *Brugruiera gymnorhiza* using bioassay guided fractionation and to examine its cytotoxic effects on Raji cells. Chloroform extract was fractionated using column chromatography method on the basis of the polarity of the solvent mixture. Each fraction had preparative thin layer chromatography. The same fractions were collected, and the cytotoxic test was then performed on Raji cells. The kinetics of toxic fraction was then determined using MTT method. The results of fractionation with chloroform solvent showed that methanol extract was cytotoxic with IC50 value of 14.184 mcg / ml, and inhibited cell division process in the 24-hour incubation period and caused cell death marked by shrinkage, chromatin condensation and fragmentation of DNA.

Keywords**:** *B. gymnorhiza, Bioassay Guided Fraktination*, cytotoxic

INTRODUCTION

Bruguiera gymnorhiza is a family rhizoporaceae which a mangrove plant. Local peaple use the leave and steam bark of plant as anti microba, antifungal, antivirus, anticancer, insecticide and antileuchemia (Soetarno, 2000). The research on the effect of extract ethanol 70% of *B. gymnorhiza* was cytotoxic effect of Myelona and raji cells line with IC_{50} 301.78 µg/ml and 582 µg/ml (Warsinah,2005). Steam bark of *B. gymnorhiza* was activity of antibacteria *Aeromonas sobria, Pseudomonas stutzeri* and *Vibrio parahaemolyticus* of *udang windu*. Result of the research of diklorometana extract was hight activity of n- hexan extract and metanol extract, diklorometana extract was LC50 96 jam was 0.00414% (b/v) (Susanti, 2004). Chen and coworkers (2007) have isolated chemistry compound fermentation of root *gymnorrhiza* with *peniccilium thomi*, there was twelf compound is 4',5- dihydroxy -2-3- dimethoxy -4-(hydroxypropyl)-biphenyl(1) and 11 compounds is used cytotoxic effect to 3 cancer cell (A549, HepG2 dan HT29). Homhual *et al* (1996), isolated was sulfur of *B. gymnorrhiza* of flowers, the result of the research were tree compounds, it is cyclic 4- hydroxy-dithiosulfonate (bruguisulfurol), hidroksidithiolane 1-oxida (bruguierol) end isobruguierol. Trees compound was activity antiocsidan elemen (ARE) with luciferae and value nilai EC50 56.7, 3.7 and 1.8 mikroM. Two compounds of bruguierol and isobruguierol was inhibitor of phorbolester-induksi NF-kappaB (nuclear factor –kappaB) luciferase denganwith value IC_{50} 85 and 14.5 µM and bruguierol was inhibitor of enzim COX-2 with value IC_{50} 6.1 µM.

MATERIAL AND METHODS

Chemicals ----- medium RPMI (RPMI 1640 (Sigma), Natrium Bikarbonat (Sigma) dan Hepes (Sigma)), *fetal bovin serum* (FBS) (Gibco) 10% (v/v), penisilin-streptomisin (Gibco) 1% (v/v), fungison (Gibco) 0.5% (v/v), aquades steril, etanol 96% v/v, DMSO,

Acridine Oranye, Etidium Bromida, Etanol 70% (E Merck, Sodium Dodesil Sulfat (SDS) 10% dalam HCl), pelat Silika gel GF 254, MTT (3-(4,5- dimethyltiazol)2,5-diphenyltetrazolium bromide) 5 mg/mL in FBS, glacial acetic acid (E Merck), ammonia (E Merck), methanol, chloroform, n-hexane, ethyl acetate

Cell line and culture condition ----- the cell lines used in this study, Hela cell were provided by parasitologi laboratories of faculty medicine Gadjah Mada University. Hela cell was culture d in RPMI 1640 medium suplemented with 10% fetal bovin serum at 37^{o}C in 5% CO_2.

Extraction of steam bark ------- Five hundred gram steam bark pelvis in maceration with methanol was minced 3 x 24 hr. The aqueous solution was evaporation with evaporator, residue of the methanol layer obtained was 53.6 gram dry weight. The active VLC were separated with preparative colom chromatography. The colon was subsequently eluted with 4:6 (v/v) chloroform/ etilacetat. The fractions that showed similar on TLC were combined and evaporated under reduced pressure.

Catatonic effect and antiproliferative effect with MTT methods---- Heal cell line was used catatonic and antiproliferative assay**.** Near**-**confluent cell was collected and diluted to a concentration 2 x 10^4/100 cells / ml. one hundred up aliquot of cell suspension were dispensed into each well of 96- *well plate*. The residue from extract steam bark *B. gymnorhiza* was redisolved in DMSO1.25%**,** the concentration extract were 1000 µg/ml, 500 µg/ml, 250 µg/ml, 125 µg/ml and 62.5 µg/ml. was added to each cell suspension well to give each test concentration. After 24 hr of incubation at 37^{o}C in 5% CO_2. The controlled by 100 µl HeLa cell. In each of doxorubicin with concentration 0,2 mg/ml, 0.1 mg/ml, 0.05 mg/ml, 0.025 mg/ml and 0.0125 mg/ml. and control of 100 µl suspension cell in medium. was incubated at 37^{o}C of 24 hr. In each were added to 10 µl MTT 5mg/ml in RPMI, alter incubation in 4 hr at 7^{o}C. reaction to stop with SDS and solution incubated for 24 jam. The absorbance of each well was measured 595 nm using *Ellisa reader.*

Detection of apoptosis Induction ------ Apoptosis in cells were detected by ethidium bromide- acridine orange. Near**-**confluent cell was collected and diluted to a concentration 3 x 10^4/100 cells / ml. one hundred micro liter aliquots of cell suspension were dispensed into each well on cover slip of 24 –well plate. The extract residue in DMSO and was diluted between 30, 15 and 7.5 microliter were then incubated 24 hr the DNA was analysis by microscop flourescense, the cover slips was added with ethidium bromide – acridine orange.

RESULTS

At level of 136.5 µg/ml the crude extract chloroform released the cytotoxic effect . However, they had different levels cytotoxic. The fraction 9 (F9) caused was higher inhibition of the concentration ,but the F9 was partitioned with F1-F8 to separate the non polar compound in chloroform fraction. The same compound also separated from chloroform fraction. All of chloroform fraction were collected and used for fractionation using TLC. Five fraction had cytotoxic effect and the other fraction not active. The fraction of chloroform fraction with gradient polarity aliquot produced ten fractions, the fraction showed figture 1.

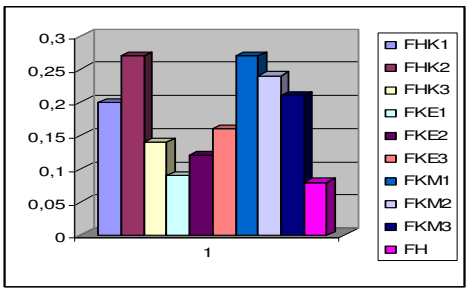


Figure 1. Weight fraction of Bioassay Guided Fractionation

Initially, were examined the cytotoxic effect of the fraction obtained from steam bark of *B. gymnorrhiza* in human cell lines, the fraction showed cytotoxic effect towards HeLa cell lines used study, with un IC_{50} value of 223, 299019, 84, 5334, 1218981, 56, 161, 82, 14 and 29 µg/ml. Respectively, the fraction showed cytotoxic effect F9 higher effec of value 14 µg/ml.

To investigated its proliferative activity futher, the fraction from chloroform fraction steam bark *B. gymnorhiza* was subyected to cell proliferation assay using HeLa cells. HeLa cells was treated with the clorofrom fraction (14 µg/ml), and cell proliferation was examined at interval by the MTT assay. As shown in figure 2, the antiproliferation of the cells in creased in a time manner, similar ti antiproliferative of cells treated with doxorubicin was used positif control because it is known to induce apoptosis in various cell lines.

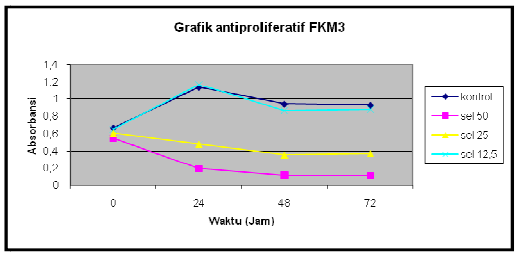


Figure 2. Cell proliferation from F9 HeLa cells were incubated at 24 hr on 37°C.

Morphological changes in the cells caused by the chloroform fraction were observed by microscopy, as shown in figure 3, after a 1 hr incubation with the F9, the HeLa cells had swelled in comparison to control cells. After 24 hr , the cells that had died owing to the F9 were not fragmented, and they maintained a sircular form. In contrast, there were no morphological changes in the cells incubated with doxorubicin for 1 hr.

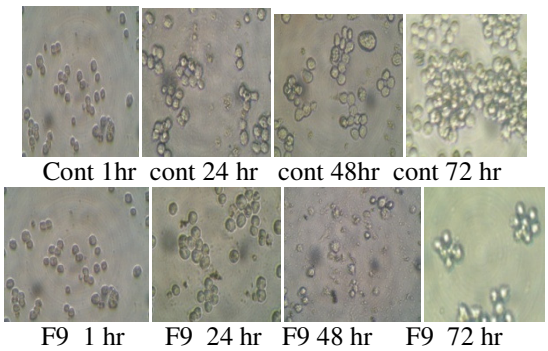
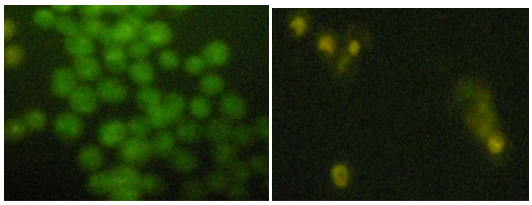


Figure 3. Morfological change in HeLa cells incubated with F9

Next, the Hela cells were exposed to 14,321 µg/ml f9 fraction for steam bark *B. gymnorhiza* for 24 hr and the DNA was ectracted, DNA framented showed by ethidium

bromide - acridin orange was performen ed and typical DNA ladder pattern of apoptosis was onserved. This result comfirm that F9 fraction steam bark *B. gymnorhiza* can induce apoptosis of HeLa cells (figure 4).



Control apoptosis induction

Figure 4. The result of apoptosis induction.

DISCUSSION

The research on the effect of extract ethanol 70% of *B. gymnorhiza* was cytotoxic effect of Myelona and raji cells line with IC_{50} 301.78 µg/ml and 582 µg/ml (Warsinah, 2005). Steam bark of *B. gymnorhiza* was aktifity of anti bakteri *Aeromonas sobria, Pseudomonas stutzeri* dan *Vibrio parahaemolyticus* of udang windu. Result of the research of diklorometana extract was hight activity of n- hexan extract and metanol extract, diklorometana extract was LC50 96 jam was 0.00414% (b/v) (Susanti, 2004). Chen, *et al*., 2007, have isolated chemistry compound fermentation of root *B. gymnorhiza* with *peniccilium thomi*, there was twelf compound is 4',5- dihydroxy -2-3- dimethoxy-4-(hydroxyprophyl)-biphenyl(1) and 11 compounds is used cytotoxic effect to 3 cancer cells (A549,HepG2 dan HT29). Homhual and coworker (1996), isolated was sulfur of *B. gymnorhiza* of flowers, the result of the research were tree compounds, it is cyclic 4-hidroksi-dithiosulfonat(bruguisulfurol), hidroksidithiolane 1-oxida (bruguierol) end isobruguierol. Trees compound was activity antioxidant elemen (ARE) with luciferae and value nilai EC50 56.7, 3.7 and 1.8 µM. Two compounds of bruguierol and isobruguierol was inhibitor of phorbolester-induksi NF-kappaB (nuclear factor –kappaB) luciferase denganwith value IC50 85 and 14.5 µM and bruguierol was inhibitor of enzim COX-2 with value IC50 6.1 µM.

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OPTIMIZATION OF EXTRACTION SOLVENT USING SIMPLEX CENTROID WITH AXIAL DESIGN TO OBTAIN *PHYLLANTHUS NIRURI* HPLC PROFILE

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Abstract: Optimization of extraction solvent combination was conducted to obtained informative profile of chemical constituent from *Phyllanthus niruri*. Simplex centroid with axial design applied for extraction solvent combination contained varying proportion of Methanol, ethyl acetate, and dichloromethane. The chemical constituent separated on High Performance Liquid Chromatography with photodiode array detector using C_{18} column (5 µm, 250 mm x 4 mm) and isocratic mobile phase acetonitrile:water (55:45). Number of peak observed at 225 nm evaluated to determined the best solvent combination. The most numbers of peak ware 12 and given by Ethyl acetate and methanol:ethyl acetate:dichloromethane (1/6:2/3:1/6) extract. While evaluation of the whole result using MINITAB software concluded that the optimum condition gave by combination of methanol:ethyl acetate (1,86:98,14).

Keywords: Optimization, extraction solvent, simplex centroid, HPLC profile.

INTRODUCTION

Phyllanthus niruri is a medicinal plant widespread in tropical country, including Indonesia. This plant is used as hepatoprotector (Harish and Shipanindappa, 2006; Sabir and Rocho, 2008), antihepatitis (Shin *et al.*, 2005) and antioxidant (Harish and Shipanindappa, 2006; Sabir and Rocho, 2008). Quality and efficacy of *P. niruri* is varied according to varieties of plants and growing conditions.

Quality of this plant as row material for some traditional medicines monitored by analysis of marker compound such as lignan (Murugaiyah and Chan, 2007) and tanin (Colombo *et al.*, 2009). In other hand, a marker compound does not give a complete picture of a herbal product. Multiple constituents are usually responsible for its therapeutic effects (Liang *et al.*, 2004; Xie and Leung, 2009). Quality control based on High Performance Liquid Chromatography (HPLC) propile is an alternative for monitoring the quality of *P.niruri*, HPLC profile is able to represent complete picture of active compounds contained in *P.niruri*.

Informative HPLC profile is need for *P.niruri* quality control model. Since the HPLC profile depend on extraction process and separation system, therefore we need suitable extraction solvent that able to give good HPLC profile. Good experimental design is important to support this work. In this preliminary investigation, combination of extraction solvent was optimized with mixture design, simplex centroid with axial design. HPLC profile then evaluated to determine the optimum extraction solvent.

MATERIAL AND METHOD

Material

P.niruri collected from Tawang Mangu, Central Java Indonesia, methanol, ethyl acetate and dichloromethane for extraction solvent, C_{18} LiChospher column (5 µm, 250 mm x 4 mm) from Merck, acetonitrile and H_2O from Merck as HPLC mobile phase, membrane

filter (Millipore®), HPLC Shimadzu LC-20 AD with diode array detector used as instrument.

METHOD

Extraction was conducted with maceration method using 10 combination of extraction solvents based on simplex centroid with axial design (Figure 1). 25 gram of *P.niruri* extracted with 125 mL extraction solvents. Each extraction conducted 24 hours and repeated three times. The extracts were concentrated and dried using vacuum rotary evaporator at temperature 60 ˚C or less. Dried extracts weighed gravimetrically to determine the yield.

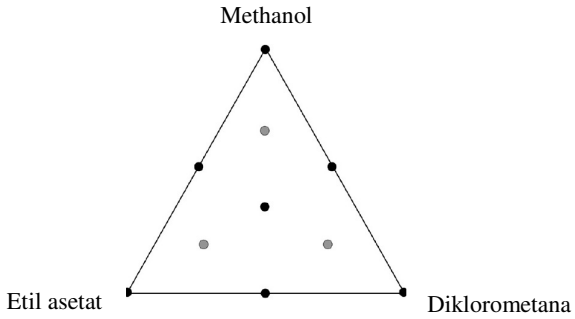

Figure 1 Extraction solvent combinations based on simplex centroid with axial design.

The mobile phase, acetonitrile: H_2O (55:45), was filtered through a 0.45 μm membrane filter (Millipore®) and sonicated before use. Each dried extracts diluted in HPLC mobile phase. After filtered through 0.45 μm membrane filter (Millipore®), 20 μL extracts injected to HPLC shimadzu LC-20 AD and separated with 1 mL/minute flow rate. HPLC profile obtained from each separation then evaluated and the number of peaks was calculated.

RESULT AND DISCUSSION

Ten combination of extraction solvents based on simplex centroid with axial design gave different extraction yield. Each extraction solvent combinations had different solvent strength, selectivity and polarity, therefore the type of extracted compounds will be varied. Methanol is protogenic solvent that able to form hydrogen bond, ethyl acetate was dipolar aprotic solvent, while dichloromethane is a nonpolar solvent (Marcus, 2004). The highest yield was obtained when methanol used as extraction solvent. It could happen since most compounds of *P.niruri* were soluble in polar solvent. According to Calixto (1998) the major compounds in *P.niruri* were lignans, tannins, polyphenol and flavonoid (Calixto, 1998). Figure 2 shown the extraction yield of each extraction solvents used in this work.

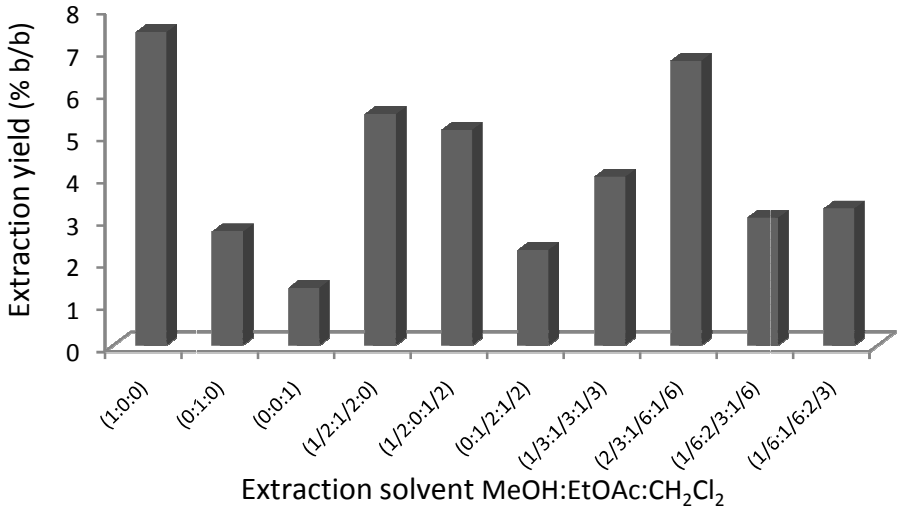

Figure 2 Extraction yield of *P.niruri* using different extraction solvents.

The HPLC profile and numbers of peak for each *P. niruri* extracts separated on reversed phase HPLC and monitored at 225 nm showed in Figure 3 and Table 1. The numbers of peak was calculated based on category signal to noise ratio and resolution.

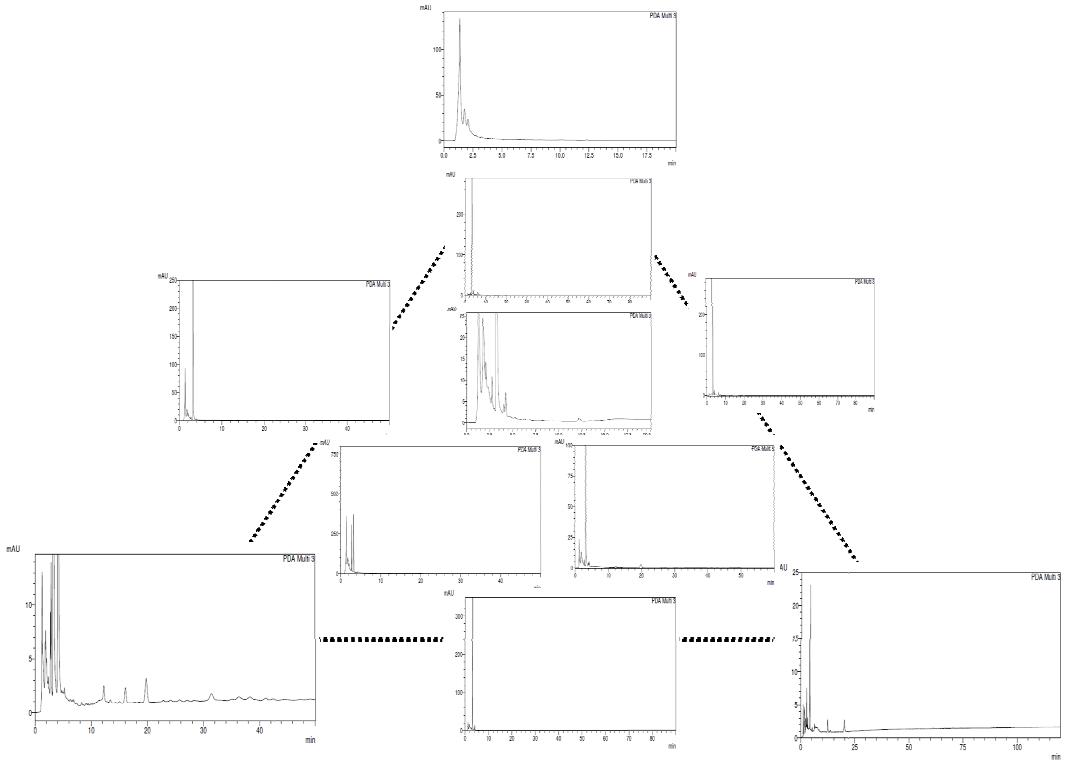

Figure 3. HPLC profiles of 10 *P. niruri* extracts monitored at 225 nm.

From Table 1 we can showed that the most numbers of peak obtained from ethyl acetate extract and methanol:ethyl acetate:dichloromethane (1/6:2/3:1/6) extract. The most numbers of peaks did not obtain from the highest yield extraction solvent, it happened since the amount or yield of extract not always correlated to the kind of compounds type.

Table 1 Numbers of peak from HPLC separation process of *P. niruri* extracts

Methanol	Ethyl acetate	Dichloromethane	Number of peak at 225 nm
1	0	0	3
2/3	1/6	1/6	10
1/6	1/6	2/3	7
1/2	0	1/2	3
0	1	0	12
1/2	1/2	0	5
1/3	1/3	1/3	6
0	1/2	1/2	9
1/6	2/3	1/6	12
0	0	1	10

Statistical evaluation using MINITAB software conducted based on the data from Table 1. The optimum extraction solvent was combination of methanol:ethyl acetate (1,86:98,14) and the surface plot of peak numbers showed in Figure 4.

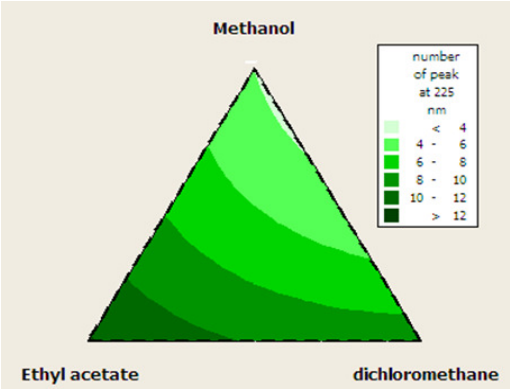

Figure 4 Surface plot profile of *P. niruri* extracts monitored at 225 nm.

CONCLUSION

The most numbers of HPLC peak monitored at 225 nm was 12. This peaks was obtained when Ethyl acetate extract and methanol:ethyl acetate:dichloromethane (1/6:2/3:1/6) extract separated in reversed phase HPLC system and acetonitrile: H_2O (55:45 v/v) as mobile phase. Evaluation of the result using MINITAB software concluded that the optimum condition gave by combination of methanol:ethyl acetate (1,86:98,14) as extraction solvent.

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FORMULATION OF HERB PILLS OF *STENOCHLAENA PALUSTRIS:* AN OVERVIEW OF FINENESS VARIATIONS OF POWDERS, CONCENTRATION OF DISSINTEGRANT, AND DRYING TIME

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Abstract : This study aims to determine the influence of fine degrees of variation powders, the concentration of dissinegrant and drying time of the test uniformity of weight and disintegration time in order to produce a good supply of pills. Herb powder formulation pills kelakai using variation of the degree of fine powder with the sieve numbers 60, 80 and 100, starch concentration Manihot 3%, 4%, 5% as a dissintegrant, Mucilago amili with 10% concentration as a binder, 5% distilled water and glycerin as a wetting materials, as well as lactose as filler. Dried at a temperature of 45^{o}C with time drying four, five and six hours. The test results were analyzed statistically with kolmogorov-smirnov to know the data distribution (P <0.05) means that no normal and normality (P> 0.05) means homogeneous, followed by non-analyzing using parametric Kruskal Wallis test and Mann whitney. Formula. The best is the formula A1 with a type of fine powder of 60 degrees, Manihot amylum with 3% concentration with time as dissintegrant 4 hours of drying, where the uniformity of the test results showed an average weight there are no two pills that deviate 10% from the average weight and none of the pills deviates 20% from the average weight. The average disintegration time of drying hours to 4, 5, and to six or less more than 60 minutes.

***Keywords** : formulation pills, kelakai (Stenochlaena palustris), disintegration time.*

INTRODUCTION

Herb kelakai (*Stenochlaena palustris*) are the types of plants-ferns spread nails used as medicine by the people of Indonesia. Efficacy of this plant is treating diarrhea in a dose of 500 mg/kg (Sutomo et al, 2008). In addition, kelakai can also treat anemia, fever reducer, ageless (antioxidants), and for the periods become irregular [Boon, 1999; Krisdianto, 2007). Kelakai dosage forms that circulate in today's society is still shaped traditional preparations such as vegetables and steeping bulbs. To increase the practicality of its use is necessary to develop other dosage forms such better pill form.

Pill is one of the solid dosage forms of traditional medicine and a small circular containing ingredients and are used for consumption by mouth (orally). Preparation and use of the pill no less than other finished drug dosage in terms of quality and efficacy and the price is relatively cheaper (Ansel, 1989). The quality of a pill is determined from some physical parameters such as degree of fine powders, the uniformity of weight and disintegration time pill. The degree of fine powder is one factor in determining the quality of a preparation of a drug because the degree of fine powders will affect drug absorption in the gastrointestinal tract.

This study aims to formulate herb pill Kelakai which focused on the influence of variations in the degree of fine powders, the influence of the concentration of dissintegrant and the effect of drying time on the uniformity of weight and disintegration time of the pill so it can be a good dosage pill.

MATERIAL AND METHODS

Materials

Materials used for the herb kelakai, Amylum Manihot, mucilago amili, lactose, talc, glycerin, distilled water.

Procedure

Picking and collecting herbs kelakai

Picking and collecting herbs kelakai were done in the area Gambut, Banjarbaru, South Kalimantan. The selected herbs are herbs that are still fresh and which are directly quoted, also herbs that are not too young and not too old.

Processing materials

Herbs kelakai processed by washing and then sliced thinly and dried with aerated. Samples are dried and sorted is separated from foreign particles like dirt. Haksel considered dry when crushed be crushed. Haksel made into powder using a blender a blender, then sieved with the numbers 60, 80 and 100.

Formula Pills

Table 1. Formula A Pill Herb Kelakai

FORM A	A1 (Sieve 60)	A2 (Sieve 80)	A3 (Sieve 100)
Kelakai	0.125 g	0.125 g	0.125 g
Amylum manihot	3 %	3 %	3 %
Mucilago amili	10 %	10 %	10 %
Glycerin	5 %	5 %	5 %
Lactose	2.34 g	2.34 g	2.34 g
Aquadest	qs	qs	qs
Talc	qs	qs	qs
Drying time (hour)	4 ǀ 5 ǀ6	4 ǀ 5 ǀ6	4 ǀ 5 ǀ6
Weight of pill	200 mg	200 mg	200 mg
Total pill	180 pills	180 pills	180 pills

Table 2. Formula B Pil Herb Kelakai

FORM A	A1 (Sieve 60)	A2 (Sieve 80)	A3 (Sieve 100)
Kelakai	0.125 g	0.125 g	0.125 g
Amylum manihot	4 %	4 %	4 %
Mucilago amili	10 %	10 %	10 %
Glycerin	5 %	5 %	5 %
Lactose	2.22 g	2.22 g	2.22 g
Aquadest	qs	qs	qs
Talc	qs	qs	qs
Drying time (hour)	4 ǀ 5 ǀ6	4 ǀ 5 ǀ6	4 ǀ 5 ǀ6
Weight of pill	200 mg	200 mg	200 mg
Total pill	180 pills	180 pills	180 pills

Tabel 3. Formula C Pil Herb Kelakai

FORM A	A1 (Sieve 60)	A2 (Sieve 80)	A3 (Sieve 100)
Kelakai	0.125 g	0.125 g	0.125 g
Amylum manihot	5 %	5 %	5 %
Mucilago amili	10 %	10 %	10 %
Glycerin	5 %	5 %	5 %
Lactose	2.10 g	2.10 g	2.10 g
Aquadest	qs	qs	qs
Talc	qs	qs	qs
Drying time (hour)	4 ǀ 5 ǀ6	4 ǀ 5 ǀ6	4 ǀ 5 ǀ6
Weight of pill	200 mg	200 mg	200 mg
Total pill	180 pills	180 pills	180 pills

Procedure of making the pill includes:

1. Created pill mass by mixing powdered herb kelakai, Amylum Manihot, lactose, distilled water and glycerin and mucilago amili, shear zones all ingredients until smooth and form the appropriate pill mass.
2. After the pill mass is formed, the cylindrical shape made by rolled-Roll with a flat wooden board on board tools pill with the number 180 pill, then roll the pill long-stem cut to the same pill.
3. The mass of a cylindrical pill that has been rolled-Roll cut with a cutter knife on the mold tool pills. then rolled on a board so that the masses become pill round board. To prevent the pill mass attached to the appliance, then the board is paved with material that is talkum sower which is a thin coating for pills no spots.
4. Pill that has been rounded then roll on a board with coated talkum round pill.
5. Pill that has been so included in the oven at a temperature of 45^{o}C with different drying times of 4, 5, and 6 hours.

Pill Testing

Weight uniformity

Weighed as much as 20 pills by weighing 20 pills at once and weigh pills one by one. After that calculated weighted average. Results weighing 20 pills compared to the table, which should not be more than 2 pills a weight deviates 10% from the average weight and weight pills that none deviated 20% of average weight.

Disintegration time

Pill is said to meet the test of time were disintegration by SK Menkes number 661/MENKES/SK/VII/1994, for pills that are not covered should be disintegration in no more than 60 minutes (Menkes, 1994). To determine the disintegration time desintegration pill use tool tester. A total of 5 pills included in the tool. Each tube filled with 1 pill. Equipment included a vessel of water temperature of 36oC-38oC, the water level of not less than 15 cm, so the tool can go up as much as 30 times per minute in the medium regularly. At the top notch plate netting right on the water surface and at the lowest position of the mouth of the tube just above the water surface. Pil otherwise destroyed completely when the remaining inventory left on the screen test device is the software that does not have a clear core. Determination carried out 3 times and calculated the average.

RESULTS AND DISCUSSION

Kelakai pill herb formulations made with reasonable consideration of economic value, making it more efficient in use by the public. With consideration of the pill has the advantage that is easy to use. Making additional substances such as pills necessary to enlarge the volume of filler, binder, material spreader, material destruction and the materials and if necessary added wetting coating materials (Anief, 2006). Basically, additional material should not toxic, available in sufficient quantities, the price is quite cheap, do not have the opposite properties (odor), an inert or neutral, physically and chemically stable in combination with other components, free of microbes, does not interfere with color active substance (Syamsuni, 2006). The additional materials used are Amylum Manihot, mucilago amili, talkum, glycerine, lactose and distilled water.

The degree of fine powder is one factor in determining the quality of a drug dosage. Where the degree of fine powder used for the measurement of particle size range which aims to improve drug absorption in the gastrointestinal tract. (Health Department, 1995). The variations in the degree of fine powder used in the powder 60, 80 and 100. Where powder 60 is roughly half of powder, powder 80 is half fine powder and powder 100 is a fine powder.

Amylum Manihot 5-15% is a destructive material added to facilitate rupture or collapse of the pill into small particles so that the surface area in contact with digestive

fluids enlarged and cause more rapid absorption (Kibbe, 2000). Mucilago amili 50-10% is a binder that serves to enlarge the power of cohesion and adhesion power pill for pill masses attached to each other into a compact mass (Anief, 2006). Talkum powder is a material that serves to minimize friction between the molecules are similar or not similar, so the mass of pills to be not stick to each other. Sticky in question is a sticky on the pill maker or sticky between one pill with another pill (Anief, 2006).

Table 4. Weight Uniformity Measurement and Time Crushed Pills

Form	Drying time (hour)	Weight uniformity ± SD (g)	Disintegration time (s)
A1	4	0.2066 ± 0.00319	25.04
	5	0.2073 ± 0.00308	27.35
	6	0.2047 ± 0.00245	29.74
A2	4	0.2080 ± 0.00196	28.37
	5	0.2083 ± 0.00195	31.15
	6	0.2049 ± 0.00205	36.17
A3	4	0.2101 ± 0.00225	35.07
	5	0.2079 ± 0.00192	32.87
	6	0.2020 ± 0.00307	35.37
B1	4	0.2082 ± 0.00193	29.48
	5	0.2078 ± 0.00158	32.93
	6	0.2026 ± 0.00185	36.03
B2	4	0.2086 ± 0.00188	37.76
	5	0.2070 ± 0.00136	36.16
	6	0.2046 ± 0.00150	44.30
B3	4	0.2091 ± 0.00186	45.26
	5	0.2079 ± 0.00132	47.87
	6	0.2014 ± 0.00333	48.33
C1	4	0.2091 ± 0.00165	52.97
	5	0.2071 ± 0.00118	51.06
	6	0.2024 ± 0.00280	64.10
C2	4	0.2100 ± 0.00170	59.27
	5	0.2079 ± 0.00118	59.32
	6	0.2034 ± 0.00244	64.83
C3	4	0.2102 ± 0.00185	54.99
	5	0.2077 ± 0.00118	58.00
	6	0.2017 ± 0.00385	65.24

Glycerine and distilled water is the wetting material that serves to minimize the contact angle (<90 °) between molecules so that the masses become wet and soft and easy to set up [Depkes RI, 1995]. Lactose is a filler that is added to enlarge the volume of the pill mass to be easily made. The charger is needed if the drug dose is not sufficient to achieve the ideal weight (Anief, 2006).

Weight uniformity

Based on the weight of an average table weight uniformity by varying degrees of fine powder and drying on the formula, there is more weight reduction increased with drying time. The average weight uniformity in the formula A3, B1, B2, B3, C1, C2 and C3 indicate the longer the drying time is less and less weight. This is due to the homogeneity at the time of making the pill and the decrease of water content along with the length of drying time. While the average weight uniformity in the formula A1 and A2 with drying time 4, 5 and 6 hours showed an average weight uniformity formula A1 with a drying time 5 hours greater weight than the drying time of 4 hours and the formula A2 with drying time 5 hours is also greater weight than the drying time of 4 hours. This may be due to different pills that are used at the time of testing uniformity of weight and the pill is not homogeneous when making a pill because of the limitations pill maker can not

make a lot of pills to the scale. How to overcome these problems either before the pill should be made at the time of the mass distribution of the pill were measured for equal distribution to the pill so that the resulting weight is also equally.

Pill formulation was varied, this is due to the influence of variations in the degree of fine powders and length of drying time. Judging from the average weight uniformity of weight, weight pills tend to decrease with the use of subtle variations in the degree of powder and the addition of drying time, the biggest drop occurred on the formula C3 to 6 hours drying time. While the largest average decline occurred in all the formulas that the type of powder 100 (formula A3, B3 and C3), this is because the powder used is too subtle and thus affects the weight reduction pills after drying.

In the weight uniformity test, if the formula pills are made with a weighting of 100 mg to 250 mg 2 pills should not be there that deviate 10% from the weighted average and not a single pill that deviate 20% from the average weight (Depkes RI, 1995). The results show that pill weight uniformity requirements that have been determined.

Disintegration time.

The test results were destroyed when the pill can be seen in Figure 1. Disintegration time is closely related to the ability to crushed pills in the body after consumption of the patient. Under the optimum formula is attempted still meet the requirements of the disintegration time is less than 60 minutes, although there are some pills that do not meet the requirements.

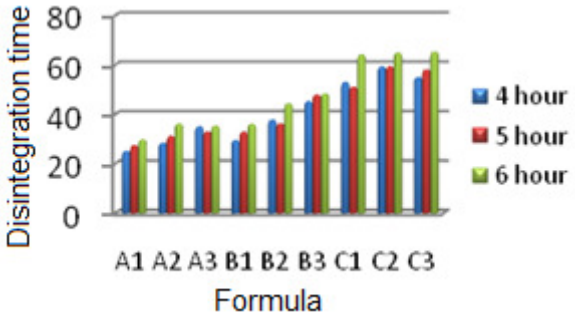

Figure 1. Bar chart average disintegration time with the variation of drying

This test is intended to find out how long does it take pills to destroyed after exposure to water or gastric fluid. Test time was damaged beyond stating that its active ingredient preparations or dissolved completely. Preparations otherwise destroyed completely when the remaining inventory left on the screen test device is the software that does not have a clear core, but part of the coating or capsule shell that is insoluble (Ansel, 1989).

When viewed from the variations in the degree of fine powder is used, then the degree of fine powder type 100 (formula A3, B3 and C3) in all formulas have the power tie stronger and harder to destroy than to the type of degree of fine powder 60 (formulas A1, B1 and C1) and 80 (formula A2, B2, C2). This is due to the fine powder used to make pills that formed the more solid and stronger binding power, causing the destruction of growing old.

Addition Amylum Manihot with concentration of 3%, 4% and 5% can not be functioning optimally as a destroyer. This may be due to the concentration of material destruction on the formula C1, C2 and C3 are used is still not big enough to offset a very fine powder and binder used. But the formula A1, A2, A3, B1, B2 and B3, the binder concentration variation and destruction can still be tolerated by the disintegration time of not more than 60 minutes. The results obtained in testing the disintegration time showed that the disintegration time of the pill still meet the established requirements.

Data Analysis

Disintegration time formula analyzed using non parametric Kruskal Wallis test because it does not meet the requirements for parametric analysis. This is due to data not normally distributed in all formulas (P <0.05), but can be homogeneous in all formulas (P> 0.05). While non-parametric analysis results between formulations using Kruskal Wallis test on each formulation is obtained that all formulas disintegration time was significantly different (P<0.05). Mann Whitney test results for the formula A1 at 4.5 and 6 hours of drying can be seen in Table 3. As for the other formulas have the same results.

Table 3. Mann Whitney test results for the time crushed pill formula A1 with drying time 4, 5 and 6 hours

Drying time (hour)	4	5	6
4	-	B	B
5	B	-	B
6	B	B	-

Based on the results of Mann Whitney test for disintegration time in the various formulas are derived any significant differences between the disintegration time formula with drying time of 4 and 5 hours, where in all formulas showed significant differences (P <0.05). At the time destroyed the formula with drying time 4 and 6 hours and with a drying time of 5 and 6 hours showed significant differences in all formulas (P <0.05). This is due to the longer drying time is used the greater the power of binding and the reduced weight and water content so that when crushed between the formula with drying time of 4 and 6 hours with 5 and 6 h show all formulas significantly different.

While based on the results of the analysis Mann Whitney test on drying time 4, 5 and 6 hours to various formulas show formula A1 to A2, the formula B1 to B2, C1 with C2 formula was not significantly different, this is because the formula A1, B1 and C1 using powder 60 and the formula A2, B2 and C2 using the powder 80, in which the two powders is still a rough and half coarse powder. In the formula A1 to A3, B1 to B3 and the formula for formula C1 with C3 showed significant differences, this is because the formula A1, B1 and C1 using a powder 60 that is a coarse powder and the formula A3, B3 and C3 using the powder 100 is an fine powder. This suggests differences in the degree of fine powder will cause the difference in disintegration time of the pill.

Table 4. Results of Mann Whitney tests were destroyed in the drying time of 4 hours

Form	A1	A2	A3	B1	B2	B3	C1	C2	C3
A1	-	TB	B	B	B	B	B	B	B
A2	-	-	B	B	B	B	B	B	B
A3	-	-	-	B	B	B	TB	B	B
B1	-	-	-	-	TB	B	B	B	B
B2	-	-	-	-	-	TB	TB	TB	B
B3	-	-	-	-	-	-	TB	TB	TB
C1	-	-	-	-	-	-	-	TB	B
C2	-	-	-	-	-	-	-	-	TB
C3	-	-	-	-	-	-	-	-	-

Table 5. Mann Whitney test results were destroyed in the drying time of 5 hours

Form	A1	A2	A3	B1	B2	B3	C1	C2	C3
A1	-	TB	B	B	B	B	B	B	B
A2	-	-	B	B	B	B	B	B	B
A3	-	-	-	B	B	TB	B	B	TB
B1	-	-	-	-	TB	B	B	B	B
B2	-	-	-	-	-	B	B	TB	B
B3	-	-	-	-	-	-	TB	TB	TB
C1	-	-	-	-	-	-	-	TB	B
C2	-	-	-	-	-	-	-	-	TB
C3	-	-	-	-	-	-	-	-	-

Table 6. Mann Whitney test results were destroyed in the drying time of 6 hours

Form	A1	A2	A3	B1	B2	B3	C1	C2	C3
A1	-	TB	B	B	B	B	B	B	B
A2	-	-	B	B	B	B	B	B	B
A3	-	-	-	TB	B	B	B	B	B
B1	-	-	-	-	TB	B	B	B	B
B2	-	-	-	-	-	B	B	B	B
B3	-	-	-	-	-	-	TB	TB	TB
C1	-	-	-	-	-	-	-	TB	B
C2	-	-	-	-	-	-	-	-	B
C3	-	-	-	-	-	-	-	-	-

Description:
B: Different meaning
TB: Not significantly different

In the formula A1 are significant differences with the formula B1 and C1, this is because the formula A1, B1 and C1 using the same type of powder that is powder 60, but using a concentration of destroyer (Amylum Manihot) different. Where the formula A1 uses material concentration destroyer 3%, using the formula B1 and C1 concentration of 4% using a concentration of 5%. This shows that the difference in the concentration of material destruction caused disintegration time difference resulting from the pill.

Formula pill is best formula A1 with a type of degree that is fine powder 60, Amylum Manihot with a concentration of 3% as a destroyer with a drying time of 4 hours of testing showed an average weight uniformity and the average disintegration time requirements in terms of physical quality with test weight uniformity and disintegration time. Where is the drying time of 4 hours showed the fastest disintegration time so that the pill is expected to provide the desired therapeutic effect.

CONCLUSION

The conclusion that can be gained from this research that the best formula pill is a pill that is herbal formula A1 with a type of degree kelakai fine powder 60, Amylum Manihot 3% as a destroyer with a drying time of 4 hours.

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DETECTION OF CHLORAMPHENICOL RESIDUE IN SHRIMP (*PENAEUS MONODON*) BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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Abstract : In spite of its potential toxicity, chloramphenicol at its therapeutic dose is used for the treatment of various infections in human. Since chloramphenicol is used as a preservative in food producing animals and animal feed products, many countries have made a strict regulation to control the residual content of chloramphenicol in meat, including shrimp meat (*Penaeus monodon*). The aim of this study was to develop a method for the detection of chloramphenicol in shrimp meat at the part per billion (ppb) level. The shrimp meat was homogenized in saline buffer, extracted using chloroform, evaporated and then reconstituted in the mobile phase. The resulting solution was injected onto an HPLC-C_{18} column and detected at 265 nm. A mixture of methanol: water (30:70, v/v) was used as a mobile phase. A linear curve of the standard solution ranging between 0.10 and 1.0 μg/ml was constructed. The overall recovery of analyte was found to be 82.77 (± 3.17%). The limit of quantitation (LOQ) and the limit of detection (LOD) of chloramphenicol were 0.28 ng per g of shrimp (0.28 ppb) and 0.09 ppb, respectively.

Keywords: chloramphenicol, shrimp meat, HPLC

INTRODUCTION

Chloramphenicol is a broad spectrum antibiotic that was first isolated from *Streptomyces venezuelae* and it has very effective antibacterial properties, which interferes with protein synthesis of many gram-negative and gram-positive bacteria. Miscellaneous toxic effects are due to the dichloride carbon alpha to the carbonyl group. This carbon readily undergoes substitution with nucleophiles such as those found on proteins as seen in the Figure 1.

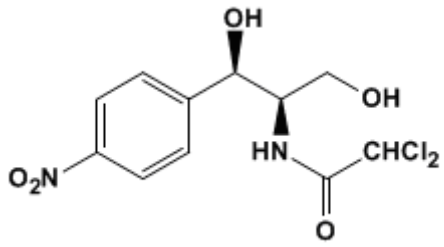

Figure 1. Structure of Chloramphenicol

The main potential human toxicity is depression of red blood cell production in bone marrow leading to aplastic anemia (Neuhaus *et al.*, 2002). Because of the unpredictable effects of dose on different patient populations, many country regulations prohibit its use in food producing animals and animal feed products.

Analytical methods for assaying chloramphenicol in shrimp have been available for a number of years, using many methods. The Gas Chromatography (GC) - Electron Capture Detector (ECD) method has an effective detection limit of 5 parts per billion (ppb) and relies on chromatographic retention time for identification. Liquid Chromatography (LC) using ultra violet (UV) detection was also commonly used which

gave detection and quantitation limits at 5 and 10 ppb respectively in aquaculture tissue. The GC using mass spectrometry (MS) detection was used with detection limits at about 1 ppb. Liquid Chromatography (LC) using MS detection was also commonly used which gave detection and quantitation limits at 0.08 and 0.3 ppb respectively. At least two enzyme linked immunosorbent assay (ELISA) kits have recently been developed which claim to be able to detect chloramphenicol in seafood tissue low ppb region, but the results using these kits have not been published (Stuart, 2002). However modifications and new methods should lower the detection limit to at or below 1 ppb.

A common approach to the analysis of chloramphenicol in seafood tissues was first cleanup utilizing liquid/liquid extraction and solid phase extraction followed by derivatization to form volatile derivatives, and analyzed by GC-ECD. The other method was pulverized with dry ice, extracted with ethyl acetate, evaporated with N2, treated with hexane/aqueous NaCl, extracted back into ethyl acetate, dissolved into methanol-water after evaporation, and injected into an LC/MS. It showed that the preparation sample was difficult and spent a lot of reagent. However, this study was done to develop a simple method for the detection of chloramphenicol in shrimp meat at the part per billion (ppb) level by High Performance Liquid Chromatography using UV detection.

MATERIAL AND METHODS

Reagents

Chloroform (pro analysis), Sodium diphosphat (pro analysis), disodium phosphate (pro analysis), methanol (HPLC Grade), Water (aqua pro injection), and Chloramphenicol (pharmaceutical grade).

Standard Solution

A chloramphenicol standard stock solution of 1,0 mg/mL was prepared by dissolving 100 mg chloramphenicol in 100 mL of methanol. The working solution of 5 µg/mL was made by diluting a stock solution with methanol : water (30 : 70, v/v) as the mobile phase.

HPLC Condition

High Performance Liquid Chromatography analyses were performed on a C18 Licrosorb column (100 x 2 mm i.d.) (E-Merck, USA) using an Hitachi series liquid chromatograph equipped with a isocratic system. Mobile phase was methanol: water (30:70; v/v). The flow rate was set at 1 mL/min and the injection volume was 20 µL.

Sample Preparation

Three hundred grams (300g) of headless, peeled and defrozen shrimp meat was homogenized in saline buffer and extracted using chloroform. The extract dried with waterbath at 45±5°C and diluted into a sufficient volume using mobile phase.The extract was then passed through a 0.2µm membrane filter and ready for analysis.

RESULTS AND DISCUSSION

Sample Preparation:

A major goal for the method development in this study is to avoid using the labor intensive and reagent-consuming procedures as in literatures. In current work, the shrimp meat were destructed with saline buffer, followed by extraction the chloramphenicol with chloroform, which is necessary for chromatographic separation. Thus, the extract was evaporated and then reconstituted in mobile phase, before be taken for HPLC analysis.

Method Performance

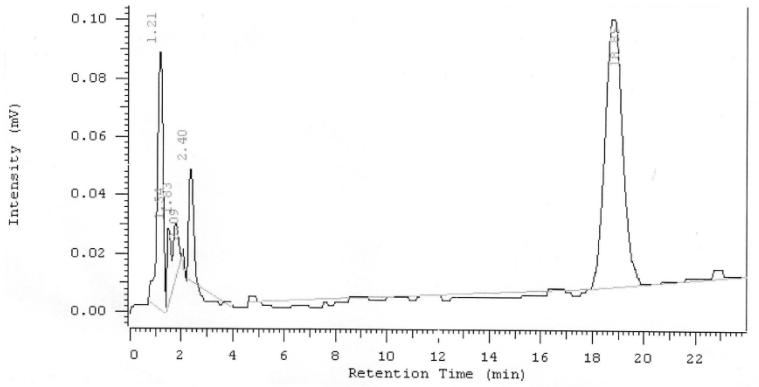

Figure 2. Chromatograms of Chloramphenicol (1 µg/mL) in shrimp meat

Figure 2 shows representative chloramphenicol in shrimp meat chromatograms. It showed that the mobile phase methanol: water (30:70, v/v) was selected to achieve maximum separation and sensitivity. Under these conditions the retention times values of chloramphenicol was 18 minutes and detected at 265 nm (figure 3).

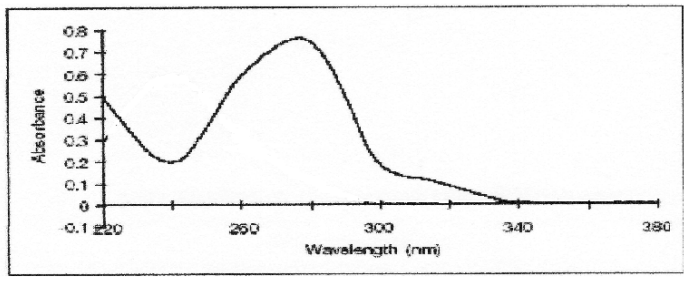

Figure 3. Absorption spectrum of chloramphenicol from 200 – 280 nm

All the traces can be well separated. It should also be noted that the chloramphenicol peak width (at 10% above baseline) is still narrow.

A representative calibration curve from 3 replicate standards interday prepared in mobile phase was shown in Figure 4. Good linearity from 0.1 to 1.0 µg/mL with correlation coefficient of R^2= 0.9969 – 0.9983 was obtained.

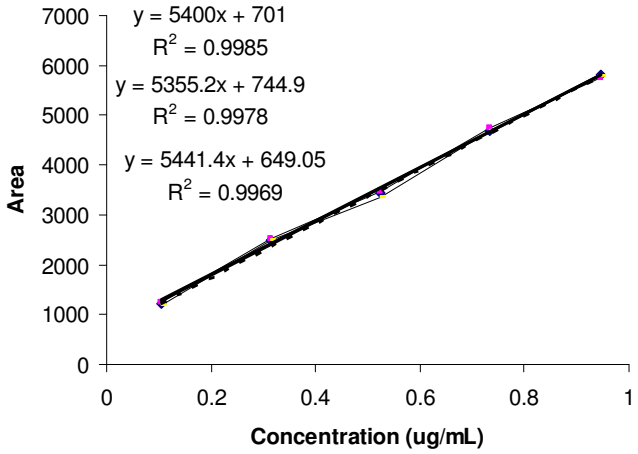

Figure 4. Calibration of three replicate chloramphenicol standard solution interday

The analyte concentration in a typical matrix blank was determined by spiking the chloramphenicol working solution (5µg/mL) into the matrix blank before sample preparation. The determination was reported in Table 1.

Table 1. Accuracy and Precision Data of Chloramphenicol in Shrimp Meat

Replication	Recovery (%)
1	84.4
2	82.8
3	86.7
4	82.6
5	80.9
6	79.2
	82.77 %(±3.17%)

It ranged from 79.2 for the lowest level of recovery to 86.7 for the highest level.

According to the Symposium on Harmonization of Quality Assurance Systems for Analytical Laboratories in Budapest, Hungary, 4–5 November 1999, (Thompson, 2002), the detection limit as estimated in method development may not be identical in concept or numerical value to one used to characterize a complete analytical method. It is accordingly recommended that for method validation, the precision estimate used (S_0) should be based on at least 6 independent complete determinations of analyte concentration in a typical matrix blank or low-level material, with no sensoring of zero or negative results, and the approximate detection limit calculated as $3S_0$. The limit of quantitation (LOQ) and the Limit of Detection (LOD) of chloramphenicol were 0.28 ng per g of shrimp (0.28 ppb) and 0.09 ppb, respectively.

Confirmation

Chloramphenicol is identified by chromatographic retention time over the course of the analysis sets in as many weeks, the retention times were predictable and consistent. For all analyses, the retention time ranged from a high of 18.90 minute to a low of 18.35 minute.

CONCLUSIONS

The method presented here is economical of both time and material. It simultaneously provides reliable determination and confirmation of chloramphenicol in shrimp meat which is useful in a regulatory situation.

ACKNOWLEDGEMENTS

We would like to thanks to Faculty of Pharmacy, Widya Mandala Catholic University for supporting the research facilities and financing.

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TECHNIQUE FOR PURIFICATION OF POLYCHLORINATED TERPHENYL IN RAW PRODUCT OF SYNTHESIS

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Abstract : The lack of polychlorinated terphenyls standards is an obstacle for developing analysis method using GC-MS. Synthesis of standards is only way to solve the problem. The Suzuki-Coupling reaction offers possibility of bonding carbon-carbon within three aromatic rings to form terphenyl backbone. For the purpose, phosphine-based catalyst of $Pd(PPh_3)_4$ in the base condition and in toluene medium was deployed. Up to the formation reaction of six-grade chlorinated terphenyls, the composition of the raw products indicated that some unexpected side-product substances appeared in raw products. The most annoying substance in the purification step to be removed was lower or same chlorinated grade terphenyl of targeted hexachlorinated terphenyls. A good strategy of product purification was indeed needed to obtain more than 90% purity of terphenyls for the purpose of spectroscopic identification. The synthesis of hexachlorinated terphenyls of 2',3,3",5,5',5"-p-PCT, 2,2",3,3",5,5"-m-PCT and 2',3,3",4,4",5'-p-PCT have been done to provide some ortho chlorinated standards. The raw products contain commonly the rest of catalyst, biphenyls, palladium oxide, phosphines, lower grade chlorinated terphenyls and other isomers of hexachlorinated terphenyls. A specific treatment of purification was done by washing, crystallization, some coloumn chromatography treatments. The series of purification step depends on the targeted product. The purification technique for 2,2",3,3",5,5"-m-PCT in the raw products was different with purification for 2',3,3",5,5',5"-p-PCT or 2',3,3",4,4",5'-p-PCT. After purification treatment series with washing, coloumn chromatography and crystallization, the first mentioned congener standard was 92% purity based on GC-MS measurement and the two latest was 98%. All standards were than able to be identification with ^{13}C and ^{1}H NMR and also GC-IR due to sufficient purity for the purposes.

***Keywords*:** Suzuki-Coupling, purification, synthesis, $Pd(PPh_3)_4$, ortho-terphenyl

INTRODUCTION

Polychlorinated terphenyls (PCTs) have three aromatic rings with general chemical formulation $C_{18}H_{14-n}Cl_n$. Their aromatic rings are able to bind each other through C-C bond in which three forms of ortho-, meta- und para terphenyls are exist (Figure 1).

Due to their different backbone and chlorinated structure, the summ of terphenyls are many so that until 8557 congeners are theoretically possible (Remberg *et al.*, 1998). The chlorination of their aromatic rings influences the rotation freedom of the congener backbone through their C-C bond. Non ortho-chlorinated terphenyls (p- and m-terphenyls) are considered as congeners having co-planare structure. It has about 138 congeners with no ortho-position through chlorination.

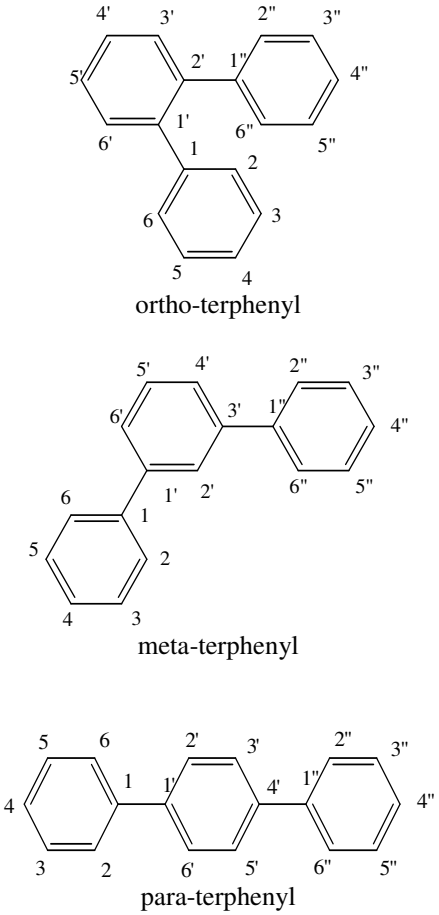


Figure 1. Terphenyl backbone and chlorination number

PCTs are considered yellowish solid and having low conductivity, high resistance of thermal breaks, not water-soluble, soluble in many organic solvents and oils, having corrosive resistance towards chemicals (base, strong acid), thermal stable, and not flammable (Filyk, 2002). Due to their properties, chlorinated terphenyls were applied mostly for industrial use. Basically, PCTs that are chlorinated substances have structure, properties, and similiarity usage like polychlorinated biphenyls (PCBs). PCTs were applied in industry as hydraulic fluids, electricity equipments for example transformators, sealants, lacks, adhesives due to their electrical properties and flame rasistance(Galceran *et al.,*1993). Furthermore, PCTs were used as plasticizer, paper coating, colour printing, vapour reducer for insecticide, as waterproof in cable coating and casting in the fake teeth, clothes jewelries and plane equipments as well (Filyk, 2002).

PCTs have been found in many compartment areas as pollutant, because PCTs have been deployed since many years like PCBs. Therefore, the release of PCT as potential environmental problem in compartment should be indeed considered. Different with PCBs, PCTs have been used mostly in the open system like lubricants and cooler. This organic pollutant were detected in almost all environmental compartments like water, sediments, soils, oil waste and also animals like birds and aquatic organisms. Contamination of PCTs in the food packing was even detected, so that direct contact to human being is also possible (Remberg *et al.*, 1998). Compared to PCBs, information of composition and distribution of PCTs in the environmental is not much reported (Santos, 1996).

Synthesis of PCTs is basically similiar to the biaryl formation through coupling reactions. The principle is connection of two or more aromatics by C-C bond for

biphenyls and three aromatic for terphenyls. With this method, biphenyl and its metabolite for instance hydroxybiaryls since many years have been synthesised with reaction route of Ullman and Cadogan. This principle is condensation reaction of aryl halogen with the present of copper and also removal of copper halogen (Fanta, 1974). The other method to connect two aromatics is by aryl radical like primary amine in the form of azo and diazo derivate or carboxylate acid (Cadogan, 1962). Tetrachlorinated congener 3,3',4,4'-Tetrachlorobiphenyl was synthesised through the method by Nakatsu and coworkers (1982). The synthesis was through diassoziation of 3,3'-Dichlorbenzidine followed by chlorination with copper chloride and salt acid. In the beginning of the year 1980, the method of Suzuki-coupling that are to be used to synthesis biaryl was done with reagent of organometal and aryl halide (Miyaura *et al.*, 1981). The synthesis is able to be built with some reagents of organometal for cross coupling reactions. Organoboronic acid is often used because it has thermally stable, inert towards water and acid and without pre-treatment (Miyaura and Suzuki, 1995). Palladium-(0)-catalyst are commonly used for this cross coupling reaction. For instance, $Pd(PPh_3)_4$ is commonly used compared to other catalysts lke $PdCl_2(PPh_3)_2$, $Pd(OAc)_3$, and PPh_3. Attempt to synthesise chlorinated biphenyls and their metabolite through Suzuki-Coupling have been reported Lehmler und Robertson (Lehmler and Robertson, 2001). The principle is connecting reaction from C-C bonds, in which Palladium-catalyst was used for coupling reaction from organoboronic acid and halogenated aromatics. In this reaction, boronic acid firstly should be activated with base.

The first attempt to synthesise polychlorinated terphenyls through Suzuki-coupling was performed by Bahadir and coworkers (2003). The synthesised congeners are terphenyls with low grade chlorinated (max five chlorinated) like *p*-PCT (3,3",5,5"-tetrachlor-, 2,2",4,4"-tetrachlor-, 2',3,3",5,5"-pentachlor-), *m*-PCT (3,3",5,5"-tetrachlor-) und *o*-PCT (3,3",5,5"-tetrachlor-)

This study are intended to synthesis ortho terphenyls with higher grade of chlorination through Suzuki-coupling using two kinds of catalysts like $Pd(PPh_3)_4$ and $Pd(OAc)_2$. Until recent time, the availability of congeners with higher chlorination grade is still low, due to lack of appropriate method to be deployed for standards synthesise.

EXPERIMENTAL SECTION

Materials

For synthesis of tetra- and hexachlorinated terphenyls, some reagents were used, tetrakistriphenylphosphine-palladium(0) (Chempur, Feinchemikalien und Forschungsbedarf GmbH, Karlsruhe, Germany), palladium acetate (Lancaster Synthesis, England), 1,3-dibrombenzene (Acros Organics, USA), toluene p.a. (SupraSolv®, Germany), 2,3,5-trichlorophenylboronic acid (Lancaster Synthesis, England), ethanol (Merck AG, Darmstadt, Germany), hydrogen peroxide (Merck AG, Darmstadt, Germany), sodium hidroxy (Merck AG, Darmstadt, Germany), dichlormethane (p.a. SupraSolv®, Germany), sodium sulphate (Carl Roth GmbH, Karlsruhe, Germany), sodium carbonate (Merck AG, Darmstadt, Germany), 2,5-dichloro-4-phenyldiamine (Acros Organics, USA), sulfuric acid (Fluka GmbH, Steinheim, Germany), sodium nitrite

(Fluka GmbH, Steinheim, Germany), copper (I) bromide 98% (Acros Organics, USA), Hydrogen bromide acid 47% p.a. (Merck AG, Germany), diethylether p.a. (SupraSolv®, Germany), 3,5-dichlorphenylboronic acid (Lancaster Synthesis, England), n-hexane (SupraSolv®, Germany), n-Heptane (SupraSolv®, Germany). NMP (Acros Organics, USA).

Instrumentation

All synthesised PCTs were characterized by FT-IR, Hewlett-Packard GC 6890/FT-IR 5965 A, equipped HP-5 coloumn with dimension 30 m x 0,32 mm x 0,25 µm, ^{1}H-NMR (300 and 400 MHz) and ^{13}C-NMR (100 MHz) from Bruker AM 400, GC-

MS Shimadzu GC 17A/MS QP 5050, equipped DB 5 MS coloumn with dimension 28 m x 0,32 mm x 0,25 μm.

Synthesis Procedure

Here, synthesis of 2',3,3",5,5',5"-hexachlor-p-terphenyl using $Pd(PPh_3)_4$ catalyst is described. Other terphenyl synthesis is almost similiar. But, purifications of the terphenyl products is not simillar. 2,5-Dichloro-1,4-dibromobenzene as the middle former of the chlorinated aromatic ring of 2',3,3",5,5',5"-hexachloro-p-terphenyl was synthesised from 2,5-dichloro-p-diaminbenzene with Sandmeyer reaction to 2,5-dichloro-p-dibromobenzene.

1,4-dibromo-2,5-dichlorobenzene (mixture with content 69%) und 145.34 mg tetrakistriphenylphosphine-palladium (0) (3 mol%) were mixed in nitrogen condition in 100 mL three-necks flask with 25 mL toluene and stirred. 8 mL solution of sodium carbonate (2 M) was than added. Solution of 0.800 g (4.19 mmol) 3,5-dichlorphenylboronic acid in 20 mL ethanol was than droped. The reaction was held for 21 h at 75-80 °C . After cooling to room temperature, the mixture was added with 1 mL 30% hydrogen peroxide and 2 h stirred. Addition of 10 mL sodium hydroxide (2 M) was done before extraction with five times of 50 mL dichlormethane. Organic phase was than washed with water untill pH-neutral and dried with sodium sulphate. Solvent removal was done with evaporator. Further purifications were done to obtain high purify of terphenyl standards. Isolated products were identified with ^{1}H-NMR and ^{13}C-NMR to ensure the position of chloro binding on each phenyl rings of polychlorinated terphenyl.

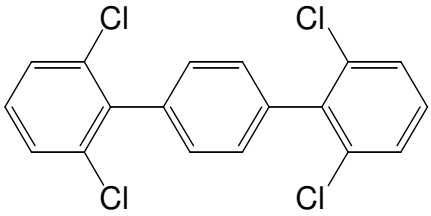
Synthesis using $Pd(OAc)_2$ catalyst was done like the following procedure. 0,350 g (1.15 mmol) of 2,6-dichlor-p-dibrombenzene, 0.480 g (2.52 mmol) 3,5-dichlorboronic acid and 0.552 gr (4 mmol) K_2CO_3 and finally 20 ml NMP/H_2O 19:1 were mixed in 100 ml three-neck flask in nitrogen condition. For reaction, synthese equipment was setted with water cooler. The mixture was heated at 90^{o}C. Pd(II)acetate, that was already in NMP was about 0.5-1.0 mL in tropfted. After cooling in room termperature , water and DCM were added. Extraxtion was than to the mixture done. The organic phase was washed than with water until pH-neutral and than with Natriumsulfat dried und finally filtration was done. The solvent was removed with evaporator.

RESULTS AND DISCUSSION

In this study, the most important we concern is how to obtain polychlorinated terphenyls with high purity to deployed as standards for developing analytical methods using GC-MS in the SIM mode. In our research group, we have synthesised some low-grade-chlorinated and coplanar terphenyls. Due to lack of congeners having non planar structure for separation with coplanar standards using chromatography coloumn, we focused since than synthesis of congeners with non-planar structure. Success of synthesis with higher chlorination in the ortho position of ring aromatics would be considered as good achievement in this study.

Synthesis using $Pd(PPh_3)_4$

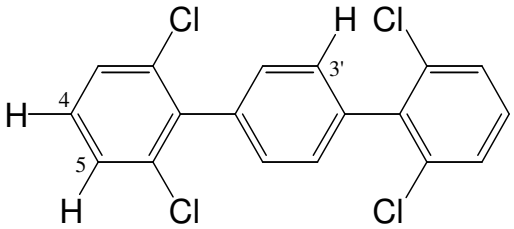
Synthesis of congener 1 through this coupling was aimed to have terphenyls with four chlorination, in which these chlor atoms were held on four ortho positions of side aromatic rings Synthesis of 2,2“,6,6”-tetrachlorinated-p-terphenyl was done by reaction of 2,6-dichlorobrombenzene and 1,4-benzyldiboronic acid. Synthesis of tetrachlorinated-terphenyl through this reaction formed some side products. These side products that are detected in raw products by GC-MS were generally fewer than synthesis of



Congener 1

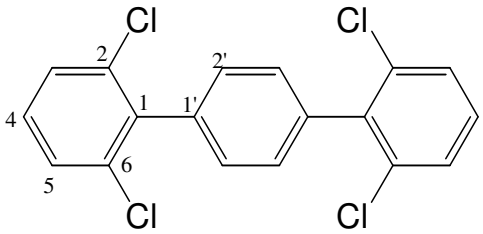
hexachlorinated terphenyls. The side products formed were tetrachlorinated isomer and fewer chlorinated congeners. In this reaction, coupling of three aromatics was fairly easy. The double ortho chlorination of bromobenzene, which are firstly predicted as steric agent was just slightly significant interference. To get fair purify of congeners, coloumn chromatography with siliga gel was enough, but two times colomn is preffered. The isolated product after obtained was with 98% purify by GC/MS.

^{1}H-NMR (400 MHz, in $CDCl_3$):



H-5 : δ = 7.43 (d, 4H);
3*J* (H,H)=7.95 Hz
H-3': δ = 7.35 (s, 4H);
H-4 : δ = 7.24 (t, 2H);
3*J* (H,H)=7.95 Hz

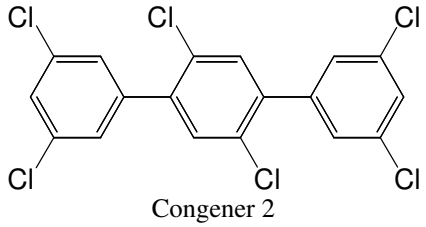
^{13}C-NMR (100 MHz, in $CDCl_3$):



C-1: δ = 139.30; C-1': δ = 136.55;
C-6: δ = 135.00; C-2': δ = 129.42;
C-4: δ = 129.07; C-5 : δ = 128.10

Figure 2. Spectra of 2,2",6,6"-tetrachlorinated-p-terphenyl (^{1}H-NMR und ^{13}C-NMR)

2,2",6,6"-Tetrachlorinated-p-terphenyl as standard for analytical study is classified as non-planar congener (Figure 2). Together with 3,3",5,5"-Tetrachlorinated-p-terphenyl (coplanar congener) and 2,3,5,6-Tetrachlorinated-p-terphenyl (half congener), were used in the preliminary study for separation of coplanar and non-planar terphenyls. In the part of analytical study, we found an appropriate parameter of coloumn chromatography to separate between coplanar and non-planar terphenyls (not reported here)

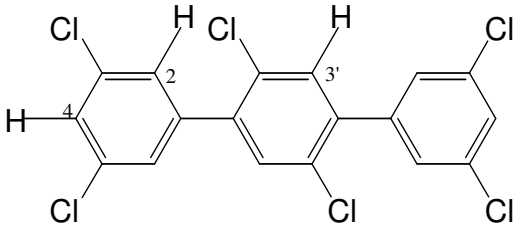


Congener 2

Synthesis of 2',3,3",5,5',5"-Hexachlorinated-p-terphenyl was success with a reaction of 2,5-dichlorinated-p-dibromobenzene und 3,5-dichlorinated-p-benzyllboronic acid. The reagent of 2,5-dichlorinated-p-dibrombenzene that was used in this reaction was firstly obtained from a synthesis with Sandmeyer-Reaction. By this coupling reaction, mixture of its raw products was also yielded. The raw product identified by GC-MS was 2',3,3",5,5',5" -hexachlorinated-p-terphenyl (main product), dichlorinated biphenyls (m/z 222), tetrachlorinated biphenyls (m/z 292), triphenylphosphines (m/z 277),

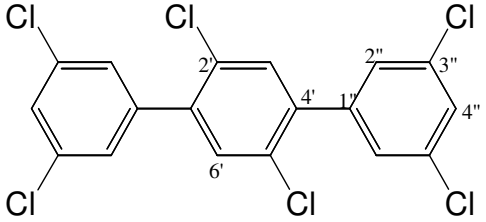
pentachlorinated terphenyls (m/z 402), and hexachlorinated terphenyls (m/z 436). The main product of 2',3,3",5,5',5"-hexachlorinated-p-terphenyl was identified about 38% of the GC-MS peaks (Figure 3). This means that to synthesise hexachlorinated congeners is more difficult than tetrachlorinated ones in term of obtaining congener with high purify for standards. As consequence, to purify hexachlorinated terphenyls are indeed more difficult too than tetrachlorinated terphenyls.

^{1}H-NMR (400 MHz, in $CDCl_3$):



H-3': δ = 7.48 (s, 2H);
H-4 : δ = 7.43 (d, 2H);
4*J* (H,H)=1.90 Hz
H-2 : δ = 7.35 (d, 4H);
4*J* (H,H)=1.90 Hz

^{13}C-NMR (100 MHz, in $CDCl_3$):

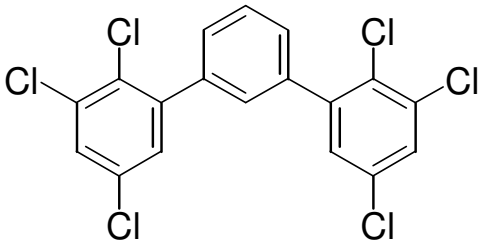


C-1" : δ = 140.15; C-4' : δ = 138.96;
C-3" : δ = 134.98; C-6' : δ = 132.19;
C-2' : δ = 131.02; C-4" : δ = 128.50;
C-2" : δ = 127.80

Figure 3. Spectra of 3,3",5,5',5"-hexachlorinated-p-terphenyl (^{1}H-NMR und ^{13}C-NMR)

Strategy for obtaining main products in this case was by some steps of purifications. Firstly, two times washing with diethylether was done. This steps was to remove side products of biphenyls and triphenylphosphine. Next step is criystalization with DCM. Side products of pentachlorinated terphenyls and hexachlorinated terphenyls was expected to be removed in this steps. Finally, purification with coloumn chromatography using silica gel was performed. Solvent used was firstly diethylether than followed by elution with DCM. The purify of 2',3,3",5,5',5"-Hexachlorinated-p-terphenyl obtained is about 98% by GC-MS.

Compared with the previous synthesis of two congeners, to obtain this standards with high purify was more difficult. The reason is that 2,2'',3,3",5,5"-hexachlorinated-m-terphenyl has meta backbone structure and also coupling with ortho chlorination that affects increasing of side products.

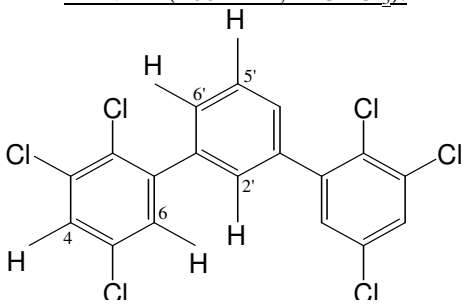


Congener 3

The congener of 2,2'',3,3",5,5"-hexachlorinated-m-terphenyl was synthesised with a reaction of 2,3,5-trichlorobenzylboronic acid and 1,3-dibromobenzene. By this reaction, side products of congeners with various chlorination grade and biphenyls were identified by GC-MS. The content of main congener in raw materials was about 42%. The side products were tetrachlorinated und pentachlorinated terphenyls (both 24%), biphenyls (10%) and hexachlorinated terphenyls, triphenylphosphine and triphenylphospide. The side products of tetrachlorinated, pentachlorinated and hexachlorinated Terphenyls were detected with GC/MS in near retention time that makes obtaining high purify of targeted product difficult. Again, some steps of purifications

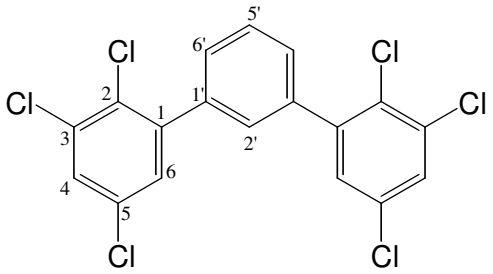
were needed. The first step was two times coloumn chromatography with silica gel. It was expected to remove biphenyls and low-grade chlorinated terphenyls (mono untill trichlorinated terphenyls) in the first coloumn separation, and tetrachlorinated untill hexachlorinated terphenyls in the second coloumn. The last step was crystallization with DCM and washing with hexane. 2,2'',3,3",5,5"-Hexachlorinated-m-terphenyl obtained was with 92% purify by GC-MS

^{1}H-NMR (400 MHz, in $CDCl_3$):



H-5' : δ = 7.53 (td, 1H);
3*J* (H,H)=7.74 Hz; 5*J* (H,H)=0.56 Hz
H-4 : δ = 7.50 (d, 2H);
4*J* (H,H)=2.48 Hz
H-6' : δ = 7.44 (dd, 2H);
3*J* (H,H)=7.74 Hz; 4*J* (H,H)=1.83 Hz,
H-2' : δ = 7.42 (td, 1H);
4*J* (H,H)=1.83 Hz; 5*J* (H,H)=0.56 Hz
H-6 : δ = 7.29 (d, 2H);
4*J* (H,H)=2.48 Hz

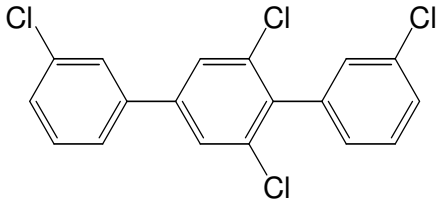
^{13}C-NMR (100 MHz, in $CDCl_3$) :



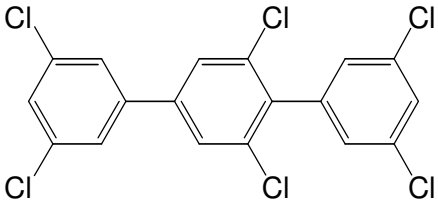
C-1 : δ = 142.96; C-1' : δ = 138,22;
C-3 : δ = 134.48; C-5 : δ = 132,61;
C-2': δ = 129.96; C-2 : δ = 129,85;
C-6 : δ = 129.51; C-4 : δ = 129,37;
C-6': δ = 129.11; C-5' : δ = 128.36

Figure 4. Spectra of 2,2'',3,3",5,5"-hexachlorinated-m-terphenyl (^{1}H-NMR und ^{13}C-NMR)

Synthesis using $Pd(OAc)_2$ catalyst



Congener 4



Congener 5

An alternative synthesis of terphenyls using $Pd(OAc)_2$ catalyst has been performed. The aim is to obtain synthesis method, that offers good recovery of synthesis products. With this method, reaction medium of NMP/Water (19:1) was used. The choice of this method was to consider of usage hidrophilic solvent as medium contact to maximize reaction process. Two congeners having similiar chlorination patterns of tetra- and hexachlorinated terphenyls were separatly synthesised using $Pd(OAc)_2$ catalyst. The result showed that content of both congeners were detected in the raw products just less than 5% (GC-Ms). It means that no use to do isolation of the targeted substances. In this phenomenon, we had no intend to do further study of the causes of this poor reactions using phophine-free catalyst. The conclusion for further synthesis for obtaining some other otho-terphenyls standards is indeed with phosphine catalyst.

CONCLUSION

For further synthesis of ortho-terphenyls, cross-coupling reaction using catalyst

of $Pd(PPh_3)_4$ showed more suitable method. Changing of using $Pd(OAc)_2$ catalyst in the medium of more hydrophilic solvent of NMP/Water (19/1) was no used since no improving reaction contact happened in this ortho-congener synthesis. Appropriate purification should be used in the isolation of targeted terphenyls in the raw products to obtain standards with high purify.

ACKNOWLEDGEMENTS

Specials thanks for Prof. Henning Hopf and Dr. Joerg Gruenenberg (Organic Institute of TU-Braunschweig) for giving good facility and fruitfull helping during the synthesis.

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STUDY OF CLOVE LEAVES VOLATILE OIL AND ITS POTENTIAL AS GROWTH INHIBITION AGAINST *STREPTOCOCCUS MUTANS* AND *STREPTOCOCCUS PYOGENES*

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Abstract :*Streptococcus mutans* and *Streptococcus pyogenes*, two of gram positive bacteria live normally in the mouth and respiratory tract. *Strept. mutans* associated with other bacteria in the mouth can cause dental caries, while *Strept. pyogenes* can cause upper respiratory tract infection. This research aim was to study the clove leaves which yield volatile oil and to evaluate its antibacterial activity against *Strept.mutans* and *Strept. pyogenes*. Microscopically clove leaf consist of epidermis, palisade, lisigen oil cells, and spons tissues, xylem, floem, sclerenchyma tissues, prism and star shaped calcium oxalate crystals and anomocytic stomata. The volatile oil was extracted by Stahl distillation of fresh and dried leaves at a flow rate 2.0-2.5 ml/minute. The percentage of volatile oil obtained from fresh leaves was 1.06% v/w and from dried leaves was 3.85%. The antibacterial activity was measured by the well diffusion method to obtain the zone of growth inhibition. The volatile oil was mixed with DMSO into concentration 10%, 20% and 30% v/v, while ampicillin was used as reference compound and DMSO as a blank control. Clove leaves volatile oil showed zones of growth inhibition against *Strept. mutans* and *Strept. pyogenes*. Compared to ampicillin, the zones of growth inhibition of 10%, 20%, and 30% v/v volatile oil were 50.16%, 54.62%, and 58.71% against *Strept. pyogene* and 45.23%, 48.82% and 53.99% against *Strept. mutans* .The active compounds of clove leaf volatile oil which showed antibacterial activity against *Strept. mutans* and *Strept. pyogenes* were most probably eugenol (Rf 0.56 toluene-ethyl acetate (93-7)) and eugenol acetate. A linear correlation of the concentration test and antibacterial activity for both bacteria was obtained (r_{count} 0.992 and 0.976 > $r_{table\ (0.05)}$ 0.95).

Keywords: *Streptococcus mutans*, *Streptococcus pyogenes*, zone of growth inhibition, well diffusion method

INTRODUCTION

Clove (*Syzygium aromaticum* (L.) Merr et Perry) is widely cultivated in Indonesia, Srilanka, Madagascar, Tanzania, and Brazil (Medline Plus, 2006). It is used in limited amounts in food products as a fragrant, flavoring agent, and antiseptic and traditionally used as cough remedies, reliving toothache, as stimulant, gargle antiseptic, carminativum, local anaesthetic, antispasmodic and also for body warmer (DepKes, 1989). As an antiseptic gargle, the volatile oil is possibly the active constituent. Clove volatile oil was produced from its bud most but from the leaves also had by a smaller degree. The content bud clove volatile oil was approximately 16-20%, while from the leaves was 1.6-4.5% (10 times lower than the bud). Eventhough smaller, the leaves was easier collected than the bud.

At 20% and 30% Clove leaves infuse as a gargle inhibited the growth of *Streptococcus mutans* by solid dilution method (Arifiah, 200), while its bud volatile oil had growth inhibition against *Staphylococcus aureus* and *Escherichia coli* at Rf 0.50 start at 2% for *Staphylococcus aureus* and 3% for *Escherichia coli* by bioautography (toluene-ethyl acetate 93:7 as mobile phase) (Tan, 1999). This research aim was to explore clove leaves as producer of volatile oil and to complete evaluation its bacteriostatic activity

against *Streptococcus mutans* and *Streptococcus pyogenes*. *Streptococcus mutans* caused dental caries and *Streptococcus pyogenes*, microbe caused sore throat.

MATERIALS AND METHOD

The clove leaves was randomly collected from Kebun Raya Purwodadi, Pasuruan, Jawa Timur (± 300 m above sea level and rain rate ± 2271 mm/ year) on December 2003 and determined based of determination by Kebun Raya Purwodadi. The mature leaves (thick, glow & green leaves) was used to produce volatile oil by Stahl distillation (flow rate 2.0-2.5 ml/minutes). The microorganisms (*Streptococcus mutans* & *Streptococcus pyogenes*) were obtained from Faculty of Dental Medicine Airlangga University, Surabaya. *Streptococcus mutans* was cultured at Tryptone Yeast Cystine (TYC), while *Streptococcus pyogenes* was at Blood Agar Base. Tryptone Soya Broth was used as liquid medium for bacterial suspension. All reagents used except cited others were pro analyzed degree.

Bacteriostatic activity was obtained by well diffusion method with ampicillin as standard and DMSO as emulsifying agent and blank control. The oil was made at 3 concentrations 10%, 20% and 30% v/v by DMSO dilution. Ampicillin was used as standard and DMSO as blank. The microbial suspensions were used equally to 1,5 x 10^5 microorganisms/ ml. Incubation All tests were done on 5 replicates.

RESULT AND DISCUSSION

The fresh mature leaves were used in this research since it had more volatile oil than the younger one (Muljana, 1989). From organoleptic, macroscopic and microscopic observation from the fresh leaves used was known that was clove leaves (*Syzygium aromaticum* (L.) Merr et Perry) which characterize as its specific odor, and calcium oxalate crystal at parenchyma tissue. Microscopically clove leaf consist of epidermis, palisade, lisigen oil cells, and spons tissues, xylem, floem, sclerenchyma tissues, prism and star shaped calcium oxalate crystals and anomocytic stomata. The oil percentage from the fresh clove leaves Stahl distillation was known that the leaves produced lower (1.06% v/w) than literally (1.6-4.5%) (DepKes, 1989), while dried leaves was 3.85%. The variation of process and time of harvesting might be the caused of its different. At this research the leaves were collected on December, while rainy season when the water content of the fresh leaves were increase than in dry season.

Table 1. Stahl distillation of clove leaves

Leaves weight (g)	Volume of volatile oil (ml)	Content of volatile oil (% v/w)	Mean±SD
50	0.45	0.9	1.06±0.11
50	0.55	1.1	
50	0.50	1.0	
50	0.60	1.2	
50	0.55	1.1	

Table 2. Measure of growth inhibition diameter (mm) of volatile oil against *Streptococcus mutans* and *Streptococcus pyogenes*

Microorganisms	Mean ± SD of growth inhibition diameter (mm)				
	clove volatile oil concentration			Ampicillin 10 µg/ 20µl	DMSO
	10%	20%	30%		
S. mutans	16.07±0.37	17.34±0.75	19.18±0.76	35.52±1.47	0.000
S. pyogenes	21.56±0.45	23.48±0.73	25.24±0.43	42.98± 3.34	0.000

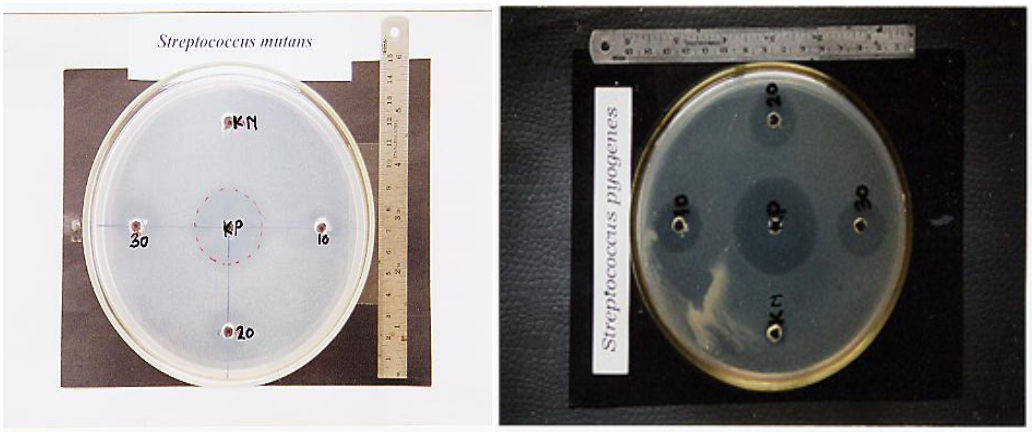

Figure 1. growth inhibition diameter of *Streptococcus mutans* & *Streptococcus pyogenes*

Streptococcus mutans and *Streptococcus pyogenes*, two of Gram positive bacteria were lived normal flora in the mouth and upper respiratory tract in human. Both carbohydrate fermented and produced acid which caused mouth smell. *Streptococcus mutans* stiked to the teeth and produced dextransucrase which changed sucrose from the food to dextran and n-fructose, while its glycosyl transferase enzyme changed sucrose to glucose and fructose. This monosaccharide would fermented to be lactic acid which caused dental enamel demineralization. Dental carries and plaque were the most caused of mouth smell (Brooks *et al*., 2001). Meanwhile *Streptococcus pyogenes* caused pharyngitis which also complicated to pneumonia, peritonitis, meningitis, etc.

Bacteriostatic activity of leaves volatile oil against Streptococcus mutans and Streptococcus pyogenes was tested using well diffusion method with ampicillin as standard and DMSO as a blank. The volatile oils was made at 3 concentrations (10%, 20% and 30%) had bacteriostatic activity (table 2) with growth inhibition diameter area start at 16.065 mm, 17.340 mm, 19.18 mm against *Streptococcus* mutans, while against *Streptococcus pyogenes* start at 21.56 mm, 23.48 mm, 25.24 mm. The standard (ampicillin) had wider inhibition at 35.52 mm and 42.94 mm. *Streptococcus pyogenes* was more sensitive than *Streptococcus mutans*. The bacteriostatic activity was possibly by eugenol and eugenol acetate (80-88%) content in leaves volatile oil (Guenther, 1990) The active compounds of clove leaf volatile oil which showed antibacterial activity against *Strept. mutans* and *Strept. pyogenes* were most probably eugenol (Rf 0.56 at TLC silica gel F254 and toluene-ethyl acetate (93-7) as mobile phase) and eugenol acetate (80-88%). Both were phenolic derivates which caused protein denaturation in the bacterial cells (Lim, 1998). A linear correlation between concentration test and bacteriostatic activity was obtained (r_{count} 0.992 and 0.976 > $r_{table\ (0.05)}$ 0.95).

CONCLUSION

It was possible to develop fresh clove leaves volatile oil as dental and throat care preparations.

ACKNOWLEDGEMENT

Thank to Ms. M. Antolis (Faculty of Pharmacy Widya Mandala Catholic University) for the research work and PHK A-2 (2008) Faculty Pharmacy Widya Mandala Catholic University Surabaya for funding some part of this research.

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SIMULTANEOUS DETERMINATION OF SULFAMETOXAZOLE AND TRIMETOPRIM IN ORAL SUSPENSION WITH BRANDED NAME AND GENERIC NAME BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

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Abstract : The determination of sulfametoxazole and trimetoprim in suspension with branded name and generic name by reversed phase high performance liquid chromatography (HPLC) has been conducted. The HPLC system used a column (4.6 mm x 25 cm) containing VP-ODS as stationary phase; water : acetonitrile : triethylamine (1400 + 400 + 2) V/V as mobile phase; and the condition were flow rate of 2 ml/minute,and wavelength of 254 nm. The result showed that two separated chromatograms of good resolution were obtained. The first chromatogram was sulfametoxazole with retention time of 2.2 minutes and the second was trimetoprim with retention time of 6.5 minutes. The linearity between the peak area versus concentration of sulfametoxazole in the range from 50 to 250 µg/ml was shown by the correlation coefficient, r = 0.9997 with the regression equation, Y = 46709.25327X + 46088.31281. For trimetoprim in the range concentration from 10 to 50 µg/ml gave the correlation coefficient, r = 0.9997 and the regression equation, Y = 5800.13118X + 5576.943053. The requirement of oral suspension in USP 30th edition (2007) namely containing sulfametoxazole and trimetoprim not less than 90.0 % and not more than 110.0 % as described in the label. The result of this research showed that all samples (Trimoxul, Sanprima, Primadex, Cotrimoxazole) analysed met the requirement of USP, except cotrimoxazole sample showed that sulfametoxazole above upper limit (110.0 %), namely 122.54 %. The validation of method showed the result that the methode fulfilled the requirement of validation with percentage recovery of 103.28% for sulfametoxazole; 98.67% for Trimetoprim. Standard deviation (SD) of sulfametoxazole was 1.79% ; Trimetoprim was 1.44% and the relative standard deviation (RSD) of sulfametoxazole was 1.74%; Trimetoprim was 1.46%. Limit of Detection (LOD) of sulfametokxazole was 13.46 µg and trimetoprim was 4.51 µg; Limit of Quantitation (LOQ) of sulfametoxazole was 44.88 µg; trimetoprim was 15.03 µg.
Keywords : *HPLC, Sulfametoxazole, Trimetoprim, Validation*

INTRODUCTION

Cotrimoxsazole represent the combination from Sulfametoxsazole and Trimetoprim with the comparison of 5:1, having the character of bactericide with the broad spectrum if compared to sulfonamide. Cotrimoxazole is a combination antibiotic: Trimethoprim/ sulfamethoxazole (TMP/SMX), Bactrim®, Septrim®. Cotrimoxazole is widely available, inexpensive and safe Cotrimoxazole is used to treat many different types of infection. Cotrimoxazole can also be used to prevent infections, including bacterial pneumonia, some forms of diarrhea, and *Pneumocystis* pneumonia (PCP).

PCP is a common and deadly infection seen in both adults and children with HIV/AIDS. The spectrum of trimetoprim is equal to sulfametoxsazole, but its potency as antibactery 20-100 times stronger than sulfametoxsazole. According to Pharmacopoeia of Indonesia of 4th edition (1995), the determination of sulfametoxsazole and trimetoprim in tablets determined by high performance liquid chromatography (hplc), used the mobile phase as the mixture of water + acetonitrile + triethylamine (1400 + 400 + 2) v / v, using uv detector 254 nm with the column ODS (3,9 mm x 30 cm), the flow rate was 2 ml / minute. According to some literature the determination of sulfametoxazole and

trimetoprim in suspension can be conducted by hplc use the different mobile phase and flow rate. For example in USP XXX (2007), used the mobile phase, column, detector and flow rate same with the condition in Pharmacopoeia of Indonesia of 4th edition (1995). Bergh and Breytenbach (1987) used the mobile phase of acetonitrile + water (25 : 75) v/v contained the ammonium acetate 1% and pH 6.90 ± 0.1; the flow rate of 1 ml/minute and UV detector at 254 nm. Roos and Lau-Cam (1986) used the mobile phase of methanol + acetic acid + triethylamine (20 + 1.5 + 0.5 + 78) v / v with the flow rate of 1.5 ml/minute, UV detector at 254 nm, column 300 mm x 3.9 mm, 10 µm - µ Bondapak C_{18}. High performance liquid chromatography method gives a lot of advantage for example quickly, good resolution, ideal for separation of molecules and ion, easy to sample recovery, usable column repeatedly and can be used automatically. The requirement of cotrimoxsazole's suspension in USP XXXI (2008) namely contain the sulfametoxsazole, $C_{10}H_{11}N_3O_3S$ and trimetoprim, $C_{14}H_{18}N_4O_3$, not less than 90,0 percent and not more than 110,0 percent of the labeled amount. In this research, the researcher used the experimental condition of hplc a little different from the procedure of USP XXX (2007), namely the column VP-ODS (4.6 mm x 25 cm). The researcher conducted also the validation test. As the parameter of validation test were accuration, presision, variation of coefficient, limit of detection and limit of quantitation.

The aim of this research is determination of sulfametoxazole and trimetoprim in generic name and trade name suspension by hplc, to know whether the amount of sulfametoxazole and trimetoprim in generic name and trade name suspension fulfilled the requirement of USP XXX (2007), and to Conduct validation test of HPLC method.

EXPERIMENT

Time and place of research

This research was conducted in laboratory of quantitative pharmaceutical chemistry and Research Laboratory of Faculty of Pharmacy of University of Sumatera Utara in September until November 2009.

Chemicals

Acetonitrile, methanol, trietylamine, acetic acid glacial, sodium hydrokside, were obtained from Merck Chemicals, *aquabidestilata* was used from PT. Ikapharmindo Putramas, Sulfametoxazole (BPFI) and trimetoprim (BPFI) were obtained from PPOM Jakarta, sulfametoxazole and trimetoprim (raw material) used from PT. Mutifa, suspensi sanprima from PT. Sanbe Farma, suspensi trimoxul from PT. Interbat, suspensi primadex from PT. Dexa Medica, suspensi cotrimoxazole from PT. Phyto Kemo Agung Farma.

Apparatus

The apparatus were used in this research were 1 unit of hplc from Shimadzu contain of pump (LC 20 AD), UV/Vis detector (SPD 20 A), column Sim-Pack VP ODS (4.6 x 250 mm), degasser (DGU 20 A5), injector (Rheodyne 7225 i), vacum pump (Gast DO A-PG04-BN), Sonificator (Branson 1510), syringe 100 µl (SGE), filter for mobile phase and sample (membrane of Whatman PTFE 0.45 µm), membrane of cellulosa nitrat 0.45 µm, and membrane of Whatman PTFE 0.2 µm, infra red spectrophotometer (Shimadzu), DRS 8000, analytical balance (Mettler Tolledo), centrifuge, pH meter (Hanna), and other glass ware. The sampling result were obtained of suspensi Trimoxul (PT. Interbat), Sanprima (PT. Sanbe Farma), Primadex (PT. Dexa Medica) and Cotrimoxazole (PT. Glorious Phyto Kemo Farma).

Mobile phase

Mixed 1400 ml water + 400 ml acetonitrile, and 2.0 ml trietylamine in volumetric flask of 2000 ml, let it at room temperature and arrange the pH 5.9 ± 0.1 with addition of

acetic acid glacial solution in water (1 in 100 ml). Dissolved and filled up to volume with water. All solvents were filtered through a 0.45 µm millipore filter before use and degassed in an ultrasonic bath.

Stock and standard solution

Sulfametoxazole BPFI (50 mg) and trimetoprim BPFI (50 mg) were accurately weighed in a 50 ml volumetric flask and dissolved in methanol and filled up to volume with methanol. So that will be obtained the concentration 1000 µg/ml and then filter. The filtrat will be used as stock solution.

Qualitative test for sulfametoxazole and trimetoprim

20 µl of sulfametoxazole stock solution was injected to hplc system and record the retention time. The retention time was 2.2 minutes. 20 µl of trimetoprim stock solution was injected to hplc system and record the retention time. The retention time was 6.5 minute

Quantitative test for sulfametoxazole and trimetoprim

Calibration curve of sulfametoxazole

The stock solution of sulfametoxazole were pipetted 0.5, 1.0, 1.6, 2.0, and 2.5 ml, entered each into volumetrics flask 10 ml, dissolved with mobile phase and filled up to volume with mobile phase to yield a solution with final concentrations of 50, 100, 160, 200 and 250 µg/ml. 20 µl of each filtrat were injected into hplc system, setting the flow rate 2 ml/minute, and uv detector at 254 nm and will be resulted chromatograms. From the area of chromatograms we can obtain a calibration curve and then we can calculate the equation of regression.

Calibration curve of trimetoprim

The stock solution of trimetoprim were pipetted 0.1, 0.2, 0.32, 0.4, and 0.5 ml, entered each into volumetrics flask 10 ml, dissolved with mobile phase and filled up to volume with mobile phase to yield a solution with final concentrations of 10, 20, 32, 40 and 50 µg/ml. 20 µl of each filtrat were injected into hplc system, setting the flow rate 2 ml/minute, and uv detector at 254 nm. From the area of chromatograms we can obtain a calibration curve and then we can calculate the equation of regression. Pipetted 2 ml of suspension sample, entered into 50 ml volumetric flask, then dissolved in 30 ml methanol. Degassing for 10 minute, filled up to volume, shaked and sentrifuged. Pipetted 5 ml supernatant, entered into other 50 ml volumetric flask, filled up to volume with mobile phase. So that will be obtained the concentration 160 µg/ml. Filtered through a 0.20 µm millipore filter. Then injected 20 µl to to hplc system, flow rate 2,0 ml/minute and detected at wavelength 254 nm, then calculated its concentration. Filtered through a 0.20 µm millipore filter. Then injected 20 µl to to hplc system. Calculated its concentration.

Determination of sample

Injected 20 µl of sampel solution into hplc system, flow rate 2,0 ml / minute and detected at wavelength 254 nm. The area of chromatogram (Y) substitude to the equation of regression. The equation is $Y = aX + b$, so that will be obtained the sample concentration (X).

Validation procedure

Pipetted 2 ml suspension of cotrimoxazole, then entered into 50 ml volumetric flask. Added 20 ml of sulfametoxazole stock solution and 4 ml of trimetoprim stock solution, then dissolved with 30 ml methanol and filled up to volume with methanol,

centrifuged for 10 minutes. Pippeted 5 ml of supernatant entered into 50 ml volumetric flask, filled up to volume with mobile phase.

Calculation of LOD and LOQ

Limit of detection (LOD is the smallest amount of analite in sample which can be detected. Limit of Quantitation (LOQ) represent the smallest amount of analite in the sample which can be quantitate.

To calculate to LOD and LOQ:

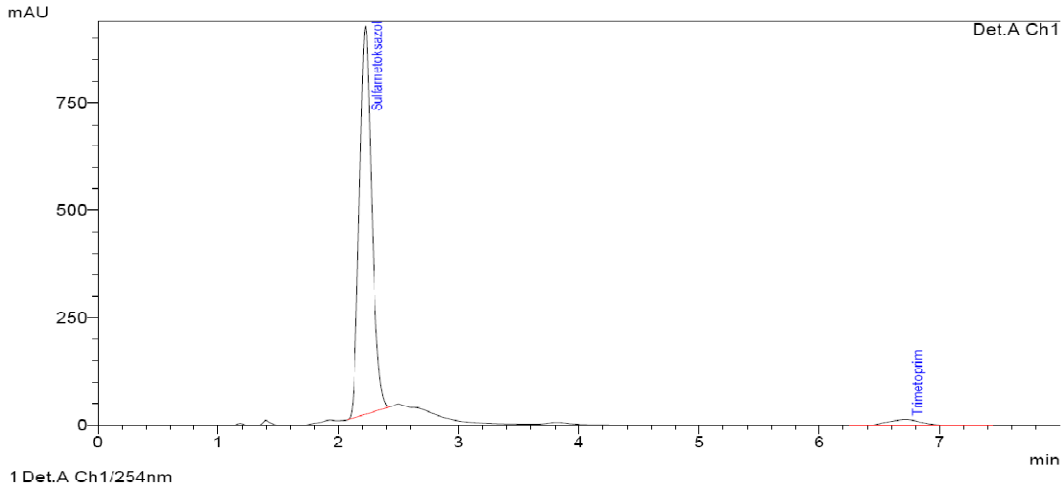
$$SD = \sqrt{\frac{(Y - Yi)^2}{n-1}}$$

$$LOD = \frac{3xSD}{Slope}$$

$$LOQ = \frac{10xSD}{Slope}$$

RESULTS AND DISCUSSION

Cotrimoxazole suspension represents the combination of sulfametoxazole and trimetoprim with the comparison (5:1), where both of this compound have the character of semi polar and soluble in alcohol. So that its determination can be conducted hplc used the mobile phase the mixture of water : acetonitrile : triethyl amine (1400 + 400 + 2) v/v, isocratic system. The procedure in this research as according to the procedure of USP 31st edition (2008). Only the column was different. We used the column of Shimpac VP-ODS (4.6 mm x 25 cm) and uv detector at wavelength 254 nm.

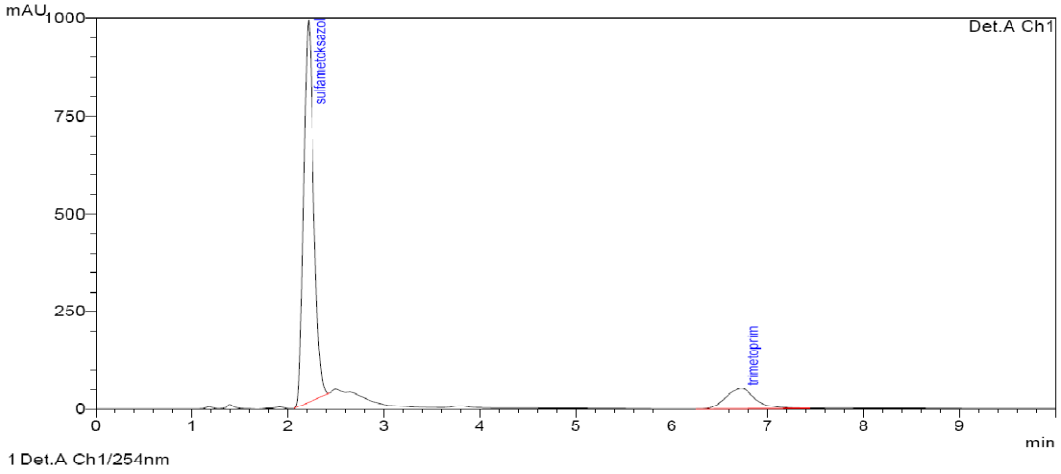


PeakTable

Detector A Ch1 254nm

Peak#	Ret. Time	Resolution	Area	Height	HETP	Theoretical Plate#
1	2.219	0.000	6449057	903884	130.700	1912.773
2	6.716	12.130	301146	14335	100.431	2489.264

Figure 1. Chromatogram of sulfametoxazole (BPFI) and trimetoprim (BPFI) which concentration 160 µg and 32 µg respectively

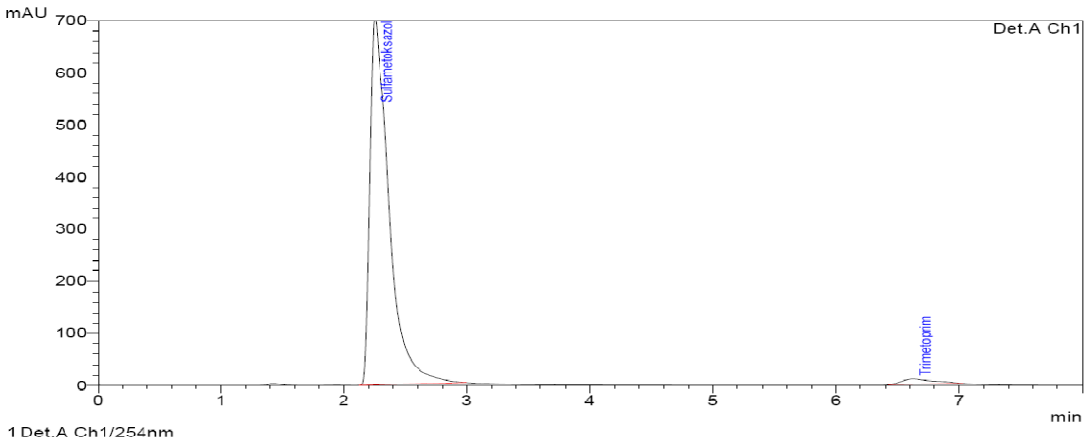


PeakTable

Detector A Ch1 254nm

Peak#	Ret. Time	Resolution	Area	Height	HETP	Theoretical Plate#
1	2.211	0.000	7124359	977136	136.237	1835.039
2	6.721	12.010	1123110	53151	102.557	2437.665

Figure 2. Chromatogram of solution of Sanprima suspension (sample) which added with spike of trimetoprim standard

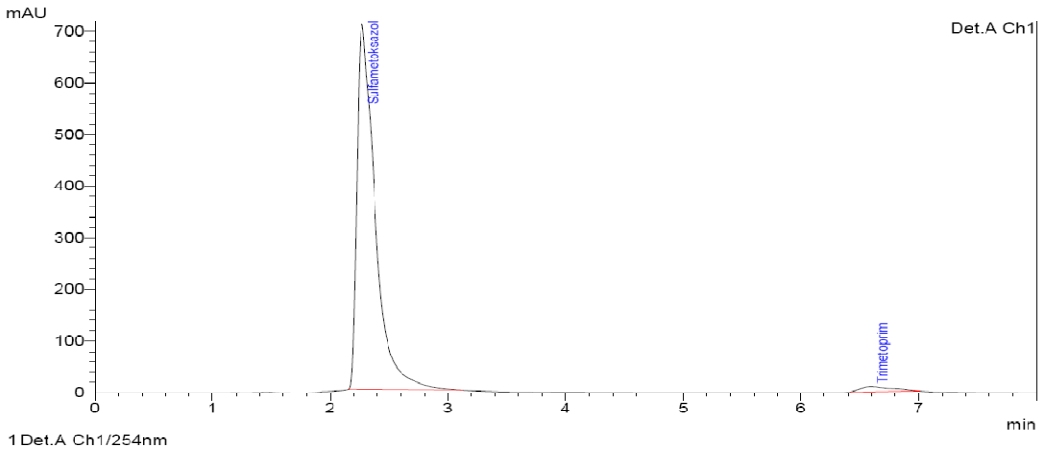


PeakTable

Detector A Ch1 254nm

Peak#	Ret. Time	Resolution	Area	Height	HETP	Theoretical Plate#
1	2.249	0.000	7717087	706097	244.054	1024.364
2	6.621	11.243	198968	10416	87.975	2841.702

Figure 3. Chromatogram of solution of Trimoxul suspension



PeakTable

Detector A Ch1 254nm

Peak#	Ret. Time	Resolution	Area	Height	HETP	Theoretical Plate#
1	2.266	0.000	7609636	706470	234.301	1067.004
2	6.591	11.218	181496	9534	87.655	2852.084

Figure 4. Chromatogram of solution of Primadex suspension (sample)

The quality test used HPLC showed the retentition time 2.2 minutes for sulfametoxazole and 6.5 minutes for trimetoprim. This matter was happened because there is polarity difference between these compound. The non polar compound would be retained longer in the column than the polar compound. That's why its retention time more longer. Sulfametoxazole has the character more polar than trimetoprim according to their retention time. The linierity of calibration curve of sulfametoxazole (BPFI) based on the area in the concentration range from 50 – 250 µg showed the linear corelation with corelation coeficient (r) = 0.9997 and equation of regression, Y = 46709.25327 X + 46088.31281

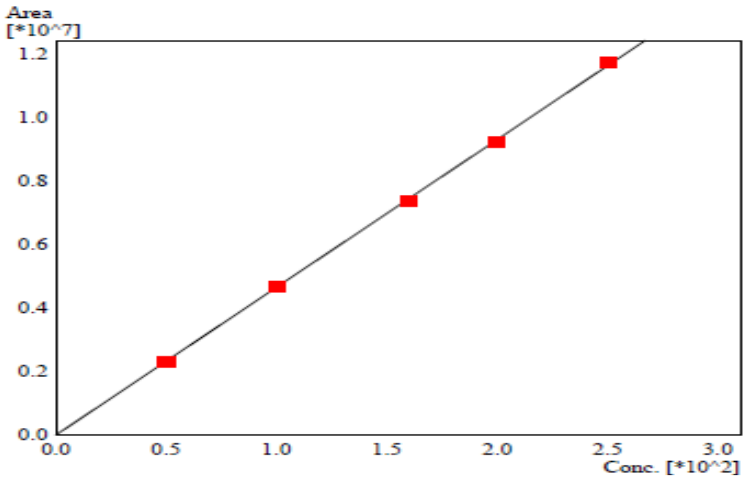


Figure 5. Calibration curve of sulfametoxazole (BPFI)

The linierity of calibration curve of trimetoprim (BPFI) based on the area in the concentration range from 10 – 50 µg showed the linear corelation with corelation coeficient (r) = 0.9997 and equation of regression, Y = 5800.13118X + 5576.943053.

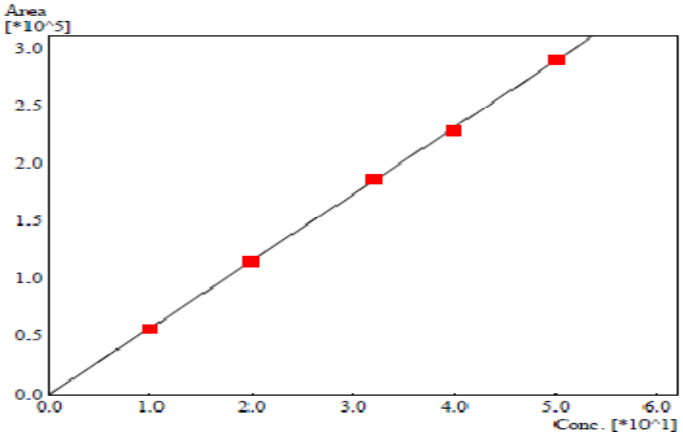


Figure 6. Calibration curve of trimetoprim (BPFI)

Table 1. The result of sample analysis

No	Sample	Concentration of sulfametoxazole	Concentration of trimetoprim
1	Trimoxul (PT.Interbat)	101.74 ± 1.2845	102.71 ± 4.6671
2	Sanprima (PT Sanbe Farma)	102.27 ± 1.6245	102.71 ± 5.0875
3	Primadex (PT Dexa Medica))	100.76 ± 0.5030	99.17 ± 7.4132
4	Cotrimoxazol (PT Phyto Kemo Agung Farma)	122.54 ± 0.5624	107.14 ± 11.3227

Table 1 showed that the concentration of sulfametoxazole and trimetoprim in all sample fulfilled the requirement of 31st USP (2008) that stated : contain not less than 90,0% and not more than 110,0% from the amount labelled, except: the amount of sufametoxazole in cotrimoxazole suspension, namely 123.45% ± 0.56, more than 110.0% from the labelled amount. The validation test was conducted in this research used the standard addition methode. A certain amount of standard was added to the sample (Trimoxul suspension, PT. Interbat) and then conducted the accuration test with the parameter of (% recovery) and presision test with the parameter of Standard Deviation (SD) and Relative Standard Deviation (RSD), LOD, and LOQ.

The accuration test with the parameter of percent recovery conducted by addition 80 µg/mg Sulfametoxazol BPFI, and 16 µg/mg Trimetoprim BPFI. Percent recovery of sulfametoxazole was 103.28% and trimetoprim was 98.67%. This result was acceptable because met the accuration standard. The range of accuration standard was 80 – 110%.
The result of precision test for sulfametoxazole with the parameter of standard deviation (SD) = 1.7929 and relative of standard deviation (RSD) = 1.7359%. The RSD value was permitted ≤ 2%. LOD and LOQ in this research were 13.47 µg /ml and 44.89 µg /ml. The result of precision test for trimetoprim with the parameter of standard deviation (SD) = 1.4405 and relative of standard deviation (RSD) = 1.4599 %. The RSD value was permitted ≤ 2%. LOD and LOQ in this research were 4.51 µg /ml and 15.03 µg /ml. From this result can be concluded that the method used fulfill the standard of validation. So that this method applicable to determine the sulfametoxazole and trimetoprim in suspension.

Table 2. Data of recovery test of sulfametoxsazole and trimetoprim used standard addition methode

Addition of substance	Peak area	% of recovery
Sulfametoxazole	11525560	102.2
	11545207	102.73
	11665932	105.96
	11633103	105.08
	11521556	102.1
	11502947	101.61
Mean (%)		103.28
Standard Deviation (SD)		1.7929
Relative Standard Deviation (RSD) (%)		1.7359
Trimetoprim	284719	96.63
	287376	99.44
	288279	100.44
	285514	97.44
	286429	98.44
	287534	99.63
Mean (%)		98.67
Standard Deviation (SD)		1.4405
Relative Standard Deviation (RSD) (%)		1.4599

CONCLUSION

The result of this research showed that from 4 samples of Sulfametoxazole and Trimetoprim suspension, there was one sample (generic name) not fulfill the requirement of USP 31st edition (2008), namely cotrimoxazole. The result of validation test indicated that the method fulfill the standard of validation test include the accuration and precision test, so that this method is applicable to determine sulfametoxazole and trimetoprim in suspension. Suggested that to be conducted a furthermore research to determine the other sample related to cotrimoxazole by hplc with modification of stationary phase, mobile phase and others

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OPTIMIZATION FORMULA OF *ALOE VERA* L. POWDER EXTRACT EFFERVESCENT GRANULES

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Abstract : *Aloe vera* L. is one of medicinal plants spread throughout Indonesia. Based on researches and experiences empirically, Aloe vera L. has been proven to cure or prevent some diseases. The aims of this research were to make effervescent granules of Aloe vera L. powder extract and looking for optimum formula of effervescent granules of Aloe vera L. powder extract which have granules and dissolving time physical properties which are appropriate with rule and regulations. Optimization techniques used this research was factorial design method with two factors and two levels which are concentration of PVP K-30 with low level 2% and high level 5%, and concentration of lactose monohydrate with low level 5% and high level 10%. The observed responses to determine the optimum granules formula in factorial design are angle of repose, granules friability, and dissolving time. The result showed that PVP K-30, lactose monohydrate, and interaction between PVP K-30 and lactose monohydrate significantly influenced the angle of repose, granules friability, and dissolving time. Based on the Design-Expert optimization program, effervescent granules formula with optimum physical properties of granules to reach for 4.58% of PVP K-30 and 9.08% of lactose monohydrate and the result is angle of repose 36.42°, granules friability 0.93%, and granules dissolving time 1.76 minutes.

Keywords: *Aloe vera* L., PVP K-30, lactose monohydrate, effervescent granules, factorial design

INTRODUCTION

Aloe vera L. is a native plant from Afrika especially Mediterania that belong to family Liliaceae which has about 200 species. Based on researches and experiences empirically, *Aloe vera* L. has been proven to cure or prevent some diseases. In this research *Aloe vera* L. will be formulated as effervescent granules because it has a lot of profit such as good taste, ease of use, and convenient. In efforts looking for optimum formula was used factorial design. Because this method can be used find out factors that influences and the interaction and may be found optimum formula. In addition to that was more efficient than *trial and error* which need creativity of formulator, long of duration, need large cost, and often may be failed.

This study used factorial design 2^2 and purposed to learn concentration variation and interaction both of component parts of granules are PVP K-30 and lactose monohydrate to physical properties of granules (index of compressibility, angle of repose, granules friability) and dissolving time.

METHODOLOGY

Materials

Aloe vera L. powder extract (Natura Laboratoria Prima, East of Java, Indonesia), citric acid, sodium bicarbonate, sodium lauryl sulphate (SLS), lactose, PVP K-30, and aspartame.

Method

Optimization techniques used in this research was factorial design method with two factors and two levels which are concentration of PVP K-30 with low level 2% and high level 5%, and concentration of lactose monohydrate with low level 5% and high level 10%. Method was used is wet granulation, which the process has been done by separate

granulation (acid component and base component were granulated separatedly) then the granules have physical properties, such as moisture content, flowability, angle of repose, *Carr's Index*, granules density, granules friability, and dissolving time. Responses observed by factorial design were angle of repose, granule friability, and dissolving time.

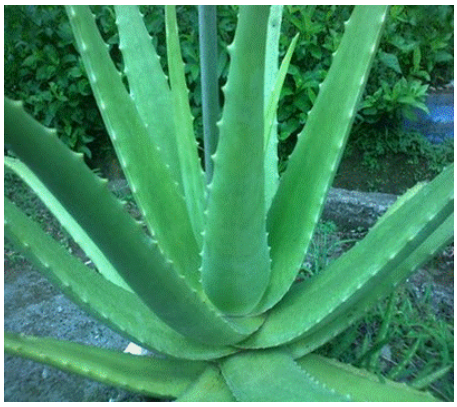

Figure 1. *Aloe vera* L.

Table 1. Formula of *Aloe vera* L. powder extract effervescent granules

Ingredient	Amount (gram) per sachet			
	F I	F II	F III	IV
Aloe vera L. powder extract	1.75	1.75	1.75	1.75
Citric acid	1.47	1.47	1.47	1.47
Sodium bicarbonate	2.10	2.10	2.10	2.10
PVP K-30	0.14	0.35	0.14	0.35
Lactose monohydrate	0.35	0.35	0.70	0.70
Aspartame	0.105	0.105	0.105	0.105
Sodium lauryl sulphate	0.07	0.07	0.07	0.07

RESULT AND DISCUSSION

A. Hasil Identifikasi Aloin secara Kromatografi Lapis Tipis (KLT)

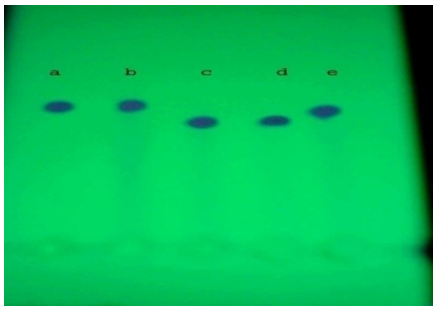

Figure 2. Thin Layer Chromatography of Aloin, purple-colored (λ = 254 nm, mobile phase = Ethyl Acetate : methanol :water = 95 : 4 :1); Rf value = 0,41-0,43 (Norman, 1989)

B. Result of physical properties of granules

The result of physical properties of granules is presented in Table 2.

Table 2. Granules characteristics

Parameter	F I	F II	F III	F IV
Moisture content (percent)	2.52 ± 0.49	3.10 ± 0.21	2.37 ± 0.21	3.19 ± 0.03
Angle of repose (degree)	38.05 ± 0.94	35.95 ± 0.67	36.42 ± 0.06	36.47 ± 0.03
Carr's Index (percent)	11.33 ± 2.31	9.33 ± 1.15	8.67 ± 1.15	7.67 ± 1.53
True density (g/cm^3)	0.54 ± 0.04	0.52 ± 0.01	0.61 ± 0.01	0.53 ± 0.01
Tapped density (g/cm^3)	0.61 ± 0.03	0.57 ± 0.002	0.67 ± 0.01	0.58 ± 0.003
Friability (percent)	1.53 ± 0.35	2.30 ± 0.87	2.73 ± 0.31	0.30 ± 0.1
Dissolving time (minute)	1.59 ± 0.07	1.78 ± 0.11	1.58 ± 0.06	1.79 ± 0.09

C. *Contour plot* of the observed responses

1. Angle of repose

$$Y = 36.72 - 0.52\ X_A - 0.28\ X_B + 0.54\ X_AX_B$$

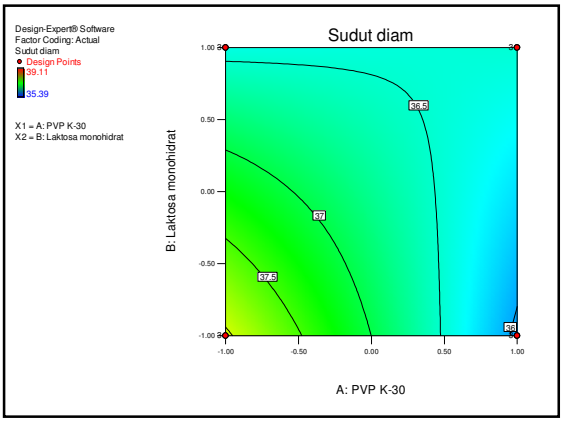

Figure 3. *Contour plot* of angle of repose from *Aloe vera* L. powder extract effervescent granules

Based on analysis of ANAVA, showed that PVP K-30, lactose monohydrate, and the interaction of both influences significantly to angle of repose *Aloe vera* L. powder extract effervescent granules. PVP K-30 and lactose monohydrate gives negative response means decrease angle of repose, but interaction of both gives positive response means will increase angle of repose.

2. Granules friability

$$Y = 1.72 - 0.42\ X_A - 0.20\ X_B - 0.80\ X_AX_B$$

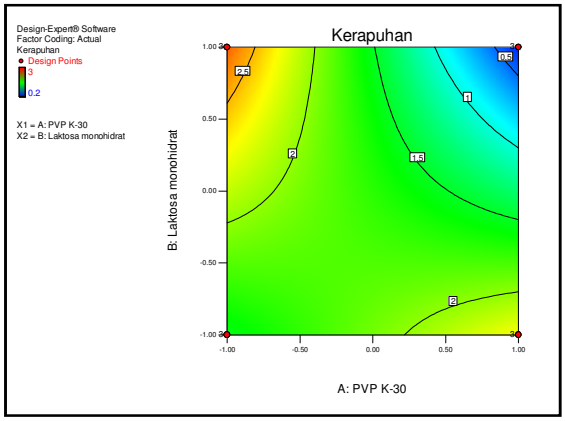

Figure 4. *Contour plot* of friability from *Aloe vera* L. powder extract effervescent granules

Based on analysis of ANAVA, showed that PVP K-30, lactose monohydrate, and the interaction of both influences significantly to friability *Aloe* vera L. powder extract effervescent granules. PVP K-30, lactose monohydrate, and interaction of both gives negative response which will decrease granules friability *Aloe vera* L. powder extract.

3. Dissolving time

$$Y = 101.25 + 5.92\ X_A - 0.083\ X_B + 0.25\ X_A X_B$$

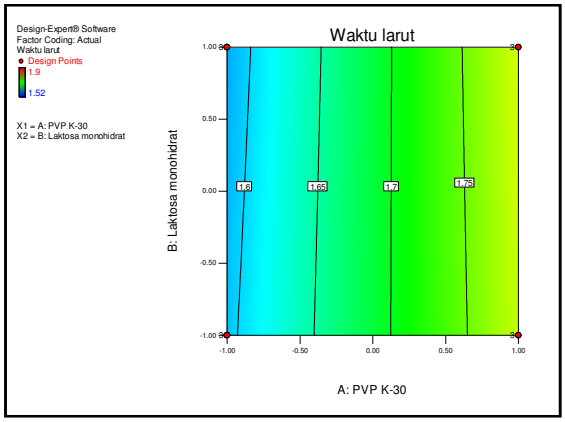

Figure 5. *Contour plot* of dissolving time from *Aloe vera* L. powder extract effervescent granules

Based on analysis of ANAVA, showed that PVP K-30, lactose monohydrate, and the interaction of both influences significantly to dissolving time *Aloe vera* L. powder extract effervescent granules. PVP K-30 more dominant in influences dissolving time *Aloe vera* L. powder extract effervescent granules than interaction between PVP K-30 and lactose monohydrate means will increase dissolving time *Aloe vera* L. powder extract effervescent granules, while lactose monohydrate gives negative response that is decrease dissolving time of granules because lactose monohydrate soluble in water.

Contour plot from each response later overlapping (*superimposed*) until may be found optimum area.

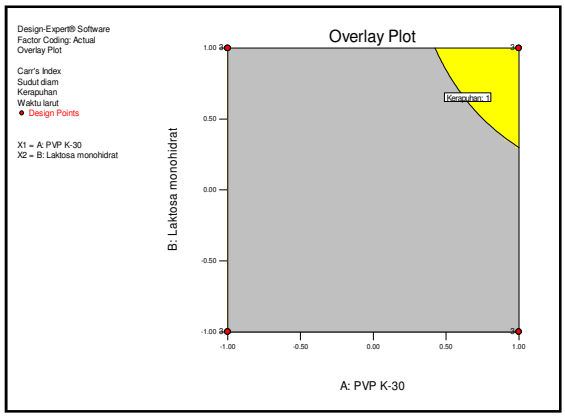

Figure 6. *Superimposed Contour plot* from *Aloe* vera L. powder extract effervescent granules

Yellow-colored area described as prediction optimum area from formula *Aloe vera* L. powder extract effervescent granules with desired responses.

CONCLUSION

- PVP K-30 as a binder, lactose monohydrate as a diluent and the interaction of both

influences significantly to angle of repose, friability, and dissolving time of granules. PVP K-30 can decrease angle of repose and friability of granules but it will increase the dissolving time of granules. Lactose monohydrate will decrease angle of repose, friability, and dissolving time of granules. Interaction between PVP K-30 and lactose monohydrate will increase the angle of repose and dissolving time of granules, but it will decrease friability of granules.

- Optimum formula effervescent granules is the combination of PVP K -30 4.58% and lactose monohydrate 9.08%. Quality the effervescent granules shown that it fullfiled the requirements as a good pharmaceutical preparation with the angle of repose was 36.42%, granules friability was 0.93%, and dissolving time was 1.76 minute.

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MOUTHWASH FORMULATION DEVELOPMENT OF *PIPER BETLE* EXTRACT AND ACTIVITY TEST AGAINTS *STREPTOCOCCUS MUTANS*

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Abstract : *Piper bettle* is Indonesian plant which can be potentially generated to be a safe and efficacious mouthwash for long time use. The objective of this research is simple formulation development of *piper betle* extract and testing the formulation activity against *Streptococcus mutans (s.mutans)* which responsible for dental carries. *Piper betle* extract was produced using *decoctum* method. Minimum Inhibition Concentration (MIC) of this extract was tested againts *s.mutans* using agar diffuse method. After suitable MIC obtained, a mouthwash was prepared by combining extract and suitable excipients. This mouthwash was tested according to MIC against *s.mutans* using Ampicillin tri hydrate as reference. Physical and chemical evaluation was completed regarding to viscosity, pH, organoleptic, density, and inhibition diameter during 28 days at 40^{o}C and humidity of 75%. *Piper betle* extract provided MIC value at concentartion of 20 mg/mL with inhibition diameter of 0.93 cm (testing hole diameter was 0.8 cm). The mouthwash was chemically stable and had inhibition diameter of 12.47 mm as equal as 0.496 ppm of ampicillin tri hydrate. Physically evaluation was shown that mouthwash had viscosity of 1.835 cps, pH of 4.55 and density of 1.0081 gr/ml. On day 7th , colour of the mouthwash was change, posibility due to oxidation of chemical substances in *Piper betle* extract. The mouthwash was able to inhibit S.*mutans* growth during 28 days.

Keywords : *Piper betle* extract, mouthwash, S.*mutans*

INTRODUCTION

Mouthwash is a dosage form that is used on a regular frequency and is used also in the long run because it required a relatively safe and effective preparation of mouthwash manufacture (Lund,1994). Mouthwash from natural materials is one of the alternative answers to produce a mouthwash that is safe and effective. One of the natural ingredients that can potentially be used as a mouthwash is the betel. Betel (*Piper betle Linn*) has been used as a controller of dental caries in region of Southeast Asia since ancient times (Materia medika Indonesia,1989). Betel nut are often used to remove bad breath but the result was not good. Use of betel is traditionally in the form of customs duty on Indonesian "*nyirih"* encourage people to investigate bacteriostatic properties of betel leaf extract (Nalina and Rahim, 2007). The result has been widely publicized that the betel leaf extracts contain ingredients that can work as a bacteriostatic . The existence of these publications are also encouraging people to develop a modern dosage form of betel leaf extract (Sari, Retno; 2006). One form of development is the creation of mouthwash from the betel leaf extract. At presnt time of the experiment, betel leaf aqueous extract will be used as active ingredients. Experiment was only carried out a simple process to make mouthwash from simple natural ingredients. In a further development is expected to use nutritious ingredients of betel leaf that serves as an antimicrobial material for mouthwash preparations (Ali Ntzar, 2010, Jeng, J. H, 2004).

The preparqtion hopely can be made more efficient and gated of other contained materials in the betel leaf extract (Rinaldi**,** 2010).

METHODS

Extraction

The fresh leaves of Piper betle cleaned and chopped into small pieces. Leaf chopping results were weighed and put into the flask. Sliced and soaked the leaves with some water (1:10) and heated to boiling. Warming up to 90^{o}C performed up to 30 minutes, then filtered. Filtrate to cool down wait until then diserbukkan by freeze dry method (Shukmar, 2008)

MIC value determination of betel leaf water extract

MIC determination by agar diffusion method. First of all take time sampling which describes logarithmic phase of growth of *Streptococcus mutans* (*S. mutans*) (Lehner, 1992; Michalek and Mc Ghee, 1982). Then the time of sampling and sampling results are made into the petri dish. Once for compacts made with perforation for punching tool with a diameter of 0.8 cm. Holes are made as much as 6 units. In each hole is inserted Piper betle extract with different concentration. In order for this then incubated for 2 x 24 hours observed diameter servant. MIC concentration is sought where gives concentrations inhibition diameter of 0.1 cm from the tip of the circle hole. If not obtained MIC concentration is necessary to do the test again with a range of concentrations that have been adjusted. Then recording MIC minimum diameter of a circle was saved in the form of inhibition in the number of bacterial colonies per mL.

Activity determination of aqueous extract

Betle leaf tests are performed on the same terms as the condition determining the MIC. In the petri dish as many as 6 units included formulated extract stocks were be drilled, and antibiotics are also known to its potential with 5 different concentrations. The diameter of the inhibition of the extract is converted to the value of the diameter of a known antimicrobial inhibitory potency by using a linear regression equation of the graph relationship between the diameter of inhibition with the concentration of antibiotics and extract logs to determine the activity of the extract (Sharma *et al*., 2009).

Initial and revision formula

Table 1 Initial Preparations Mouthwash Formula of Betle extract

No.	Ingredient	Amount	Function
1	*Piper betle* extract	5 mg/mL	Antimicrobial agent
2	Sorbitol	10%	Viscosity enhancer
3	Mentol	0.5%	*Flavouring agent*
4	Sodium Benzoat	0.1%	Preservatives
5	Aquadest	add to 100%	Diluents

Made revisions to the formula if there are constraints on the original formulation excipients and excipients concentration used by the optimization phase.

Dosage forms Evaluation

Evaluation conducted physical evaluations and the stability of preparations for a month, in a climate chamber with a temperature of 40 ° C and relative humidity 75%. Preparation is taken and evaluated at days 0, 3 7, 14, 21, and 28. Physical evaluation observed from a change of color, pH, and viscosity that occur during storage. Evaluation of chemical stability seen by comparing the MIC values obtained from the initial extract stocks with negative controls in the form of stock matrix.

RESULT AND DISCUSSION

Testing MIC value of betel leaf extract was carried out by testing three different concentrations. MIC value is sought the concentration that gives the diameter of barriers around obstacles with a diameter of 0.1 cm diameter hole. The test starts with using 5 initial concentration 12.5 mg / mL, 25 mg / mL, 50 mg / mL, 75 mg / mL and 100 mg / mL. From these results, five initial concentrations that resistor value is approximately 0.1 cm in diameter was obtained at a concentration of 12.5 mg / mL to 25 mg / mL. For that conducted the test again with 3 different concentrations of 15 mg / mL, 20 mg / mL and 25 mg / mL. From this test result is obtained that the minimum inhibitory concentration values of betel leaf extract eligible extract concentration of 20 mg / mL. For a more clear result can be seen in Table 2.

Table 2. Determination of Minimum Inhibitory Concentration (MIC)

Concentrations	Inhibition diameters
15 mg/mL	0.87 cm
20 mg/mL	**0.93 cm**
25 mg/mL	0.97 cm

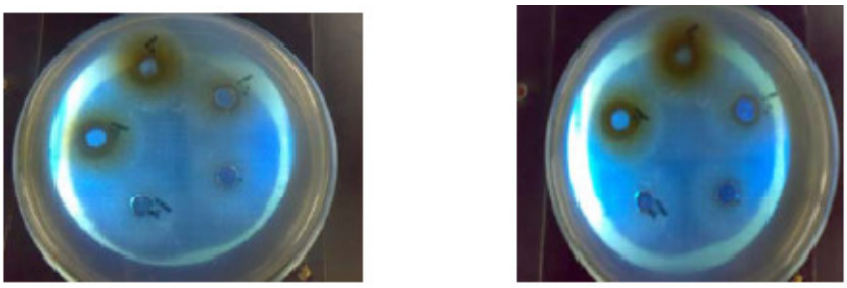

Figure 1 Testing on the minimum inhibitory concentration

Optimization excipients of mouthwash preparations has been done in order to obtain an effective, safe, and efficacious preparation. In this mouthwash formulation excipients such as preservatives, antioxidants, viscosity enhancers, cover up the bad smell of betel, and sweeteners to cover the bitter taste of betel leaf extract were studied. In the process of optimization of antioxidants and preservatives, screening methods were used. A number of extracts with the same volume and concentration were added with a mixture of different preservatives and antioxidants levels. The used preservative was sodium benzoate in the concentration range of 0.1% to 0.5% while that used antioxidants was vitamin C with a used range of concentrations were 0.01% to 0.1% (Handbook of Phamaceutical Exipient, 2005) . Tests carried out for 1 week and the results were found that the combination of 0.2% sodium benzoate and 0.05% vitamin C have yielded physically a stable extract.

In the optimization of saccharin sodium as a sweetener, sorbitol as viscosity enhancers and oleum citri as a cover scent (Handbook of Phamaceutical Exipient, 2005), sampling methods were used to several panelists to include acceptance of patients on doses. Panelists who participated as many as 6 people sell men and 2 women with age range between 16 to 24 years. These test results indicated that the concentration of sodium saccharin optimum concentration was 0.3%, while sorbitol is used as the optimum viscosity enhancers was 10%. Water is saturated with oleum citri used as a cover scent dose. On the use of oleum citri as a cover taste, optimum concentration of 0.2% was used

After doing some optimization stages formula, obtained mouthwash preparations was investigated to test its activity and physical properties. Final formula can be seen in Table 3.

Table 3 Mouthwash Final Formula of betle leaf extract

No.	Ingredient	Amount	Function
1	*Piper betle* extracts	60 mg/mL (3x MIC)	Antimicrobial agent
2	Etanol	10%	Solvent
3	Vitamin C	0.05%	Antioxidant
4	Na-sakarin	0.3%	Sweetening agent
5	Sorbitol	10%	Viscosity enhancer
6	*Oleum citri*	0.2%	*Flavouring agent*
7	Sodium Benzoat	0.2%	Preservatives
8	Aquadest	add to 100%	Diluents

The stability of final mouthwash preparations were tested in climate chamber at a temperature of 45^{o}C and humidity of 75%. In the stability test, the parameters to be tested including organoleptic stability, viscosity, density, pH and antimicrobial effects of such stocks in the period of 28 days. The stability test of preparations was performed in 3 batches and each batch contains 6 bottles of shares with a volume of 25 mL for each bottle. On the 0 day, viscosity, organoleptic dose, pH and antimicrobial effects were measured. The test results days 0, 3, 7, 14, 21, and 28 can be seen in the following table:

Table 4 Mouthwash Stability Testing Processed

Parameters	Time (days)					
	0	**3**	**7**	**14**	**21**	**28**
Viscosity (cP)	1.890	1.780	1.800	1.780	1.780	1.780
Organoleptics	+	+	-	-	-	-
pH	4.50	4.55	4.58	4.56	4.56	4.56
Inhibition diameters (cm)	1.24	1.25	1.25	1.25	1.25	1.23

Description table: + = no physical change of dosage forms (organoleptic)
- = change in physical dosage forms (organoleptic)

The results revealed that stocks of mouthwash has a diameter of stable resistance values during the storage time. This indicates that the mouthwash preparations was stable on inhibitory activity against S. Mutans (Nalina,T; Z.H.A.Rahim, 2006). The color of preparation was change during the stability test. The change is believed to occur due to oxidation reactions of compounds contained in the betel leaf extract. Some efforts to prevent the occurrence of oxidation reactions is the addition of chelating compounds to bind metals that may be contained in the used distilled water. Metal compound is a catalytic oxidation reaction so that the chelating compounds may help in preventing the occurrence of oxidation reactions.

CONCLUSION

The stable mouthwash dosage form has the ability to inhibit S. mutans acivity. The MIC of the mouthwash had the same value for 1 month. Preparation is also experiencing changes in color that might be caused by oxidation reactions of compounds contained in the extract during storage (Rinaldi, 2010).

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SIMULTANEOUS DETERMINATION OF MEFENAMIC ACID, PHENYLBUTAZON, DICLOFENAC SODIUM, PARACETAMOL AND PIROXICAM IN TRADITIONAL MEDICINE BY TLC

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Abstract: A thin layer chromatography (TLC) method with densitometric detection for determination of mefenamic acid, phenylbutazon, diclofenac sodium, paracetamol and piroxicam in traditional medicine was described. Separation was on silica gel TLC plates, using a mixture of toluene : ethyl acetate : acetone : glacial acetic acid (60:40:20:0,1, v/v/v/v) as the mobile phase. Densitometric detection was carried out at 289 nm. The proposed method was linear in the range of 10-30, 8-24, 2-6, 10-30 and 1-3 µg for mefenamic acid, phenylbutazon, diclofenac sodium, paracetamol and piroxicam, respectively. The coefficients variation of mefenamic acid, phenylbutazon, diclofenac sodium, paracetamol and piroxicam were below 2%. In this study, a TLC method, which is simple, rapid and does not require any separation step for each drug, was successfully applied for the quantitative assay of mefenamic acid, phenylbutazon, diclofenac sodium, paracetamol and piroxicam in traditional medicine.

Keywords : mefenamic acid, phenylbutazon, diclofenac sodium, paracetamol, piroxicam and TLC

INTRODUCTION

The use of traditional medicines (medicinal- plant drugs) has recently been increasing as many products, some are illegally manufactured and distributed, are available in the market. There is a belief that the use of medicinal -plant drugs can rapidly alleviate the illnesses and bears no harm to the health (Putiyanan, 2002). However, the contamination with microorganisms and hazardous substances, the adulteration with chemicals or synthetic substances that are unsafe to consumers intentionally or unintentionally- such as methyl alcohol, chloroform, cyanide and heavy metals (lead, arsenic, mercury) can cause serious health problems (Traditional Medicine Recovery Project, 1987). In addition, some products are adulterated with specially-controlled drugs, such as mefenamic acid, phenylbutazon, diclofenac sodium, paracetamol and piroxicam to enhance the therapeutic effects.

Mefenamic acid (M), phenylbutazon (PH), diclofenac sodium (D), paracetamol (PA) and piroxicam (PI) belongs to a group of anti-inflammatory, analgesic and antipyretic drugs. Mefenamic acid (M), is a non-steroidal antiinflammatory drug. It is used as potent analgesic and antiinflamanatory agent in the treatment of osteorthritis, rheumatoid arthritis and other painful musculosketal illnesses (Traditional Mediciner Recovery Project, 1987). Various analytical techniques for the determination of M, PH, D, PA, and PI exist individually or in combination including spectrophotometry (Espinosa-Masnsilla *et al.*, 2005; Dnic *et al.*, 2002; Bogachik *et al.*, 1999; El- Sherif, 2007; Gangwal Sharma, 1996; Toral *et al*., 1996; Bucci *et al*., 1998; Ustun, *et al*., 1992, Vidal, *et al*., 2003), spectrofluorometry (Espinosa-Masnsilla *et al*., 2005; Capitan – Valvey *et al*., 2000; P. C. Ioannou, et. al., 1998; Albero, 1995; Damiani, 1999), luminescence (Arnaud *et al*., 2003; Egorova *et al*., 1999), high performance liquid chromatography (Rouini *et al*., 2004; Sun *et al*., 2003, Mikami *et al*., 2000; Hirai *et al*., 1997; Shafiee *et al*., 2003), FT-Raman spectroscopy (Szostak, 2002), and near infrared spectroscopy (Merckle, *et al*., 1998), and gas chromatography (Anonim, 2002; Giachetti

et al., 1994, Shafiee, *et al*., 2003).

Thin layer chromatography- Densitometry (TLC) can be used for identification and determination mefenamic acid, phenylbutazon, diclofenac sodium, paracetamol and piroxicam in traditional medicines. It has the advantages of being sensitive, selective, rapid, accurate and reproducible. The present paper reports the development and validation of a thin layer chromatography (TLC) method for determination of traditional medicines.

EXPERIMENT

Chemicals

Mefenamic acid, phenylbutazon, diclofenac sodium, paracetamol and piroxicam (Reference Standard), ethyl acetate, methanol, toluena, NH_4OH, Chloroform, Aquadest, glacial acetic acid, aceton, and diethyl ether p.a (E.Merck)

Instruments

Densitometer.—Camag TLC Scanner III, *TLC plates* precoated silica gel F254 aluminium backed TLC plates (E. Merck, Darmstadt, Germany), *TLC chamber*.—Camag twin trough chamber for 20 x 10 cm plates (No. 0225155)

Standard and Sample Solutions

The standard solutions of M, PH, D, PA and PI were prepared in methanol by varying the different concentrations in the ranges of 2-6, 1.6-4.8, 0.4-1.2, 2-6 and 0.16-0.48 mg ml^{-1}, respectively. Sample solutions (4 samples of traditional medicine (A, B, C and D) (70 mg ml^{-1}) were dilution with methanol.

Procedure

Thin-Layer Chromatographic Analysis

Test solutions were applied to the plates use capillary pipe 5 µl, as spots. Ascending chromatography was performed in a twin-trough TLC chamber (Camag) with toluene : ethyl acetate : acetone : glacial acetic acid (60:40:20:0,1, v/v/v/v) as mobile phase.

RESULTS AND DISCUSSION

Method Development

The mobile phase was chosen after several trials with methanol, toluene, ethyl acetate, acetone : glacial acetic acid in various proportions. A mobile phase consisting of with toluene : ethyl acetate : acetone : glacial acetic acid (60:40:20:0,1, v/v/v/v) was selected to achieve maximum separation and sensitivity. The development distance was 10 cm. Under these conditions the R_f values of PA, D, PI, M and PH were 0.28, 0.47, 0.57, 0.75 and 0.88, respectively.

Quantitative analysis of M, PH, D, PA and PI were performed by scanning densitometry with a Camag TLC Scanner-3. The wavelength of maximum absorption (289 nm) was first determined by acquisition of the compound's in-situ UV absorption spectrum (Figure 1) by using the spectrum mode of the scanner. The plates were scanned at 289 nm with the scanner in reflectance–absorbance mode, using the deuterium lamp. Camag CATS-4 software was used for scanner control and data processing. A typical densitogram is shown in Figure 2.

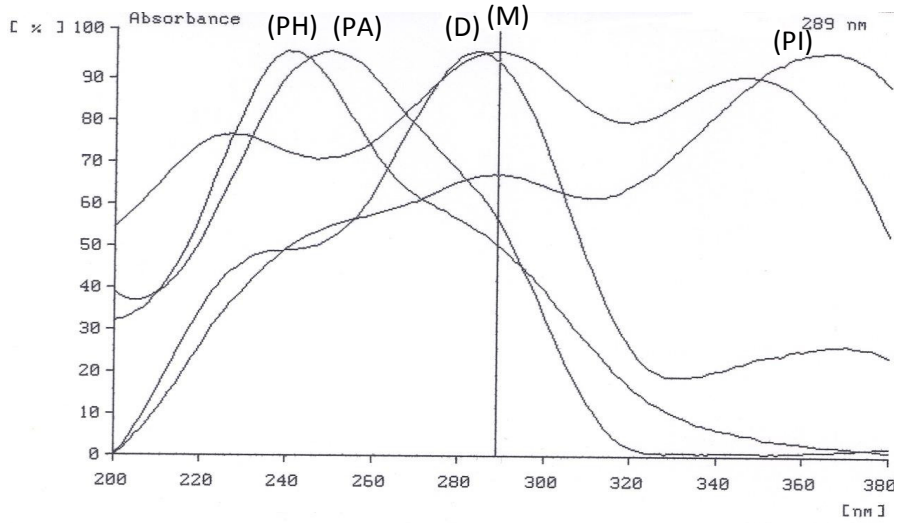

Figure 1. Absorption spectrums of M, PH, D, PA and PI standard from 200 to 380 nm

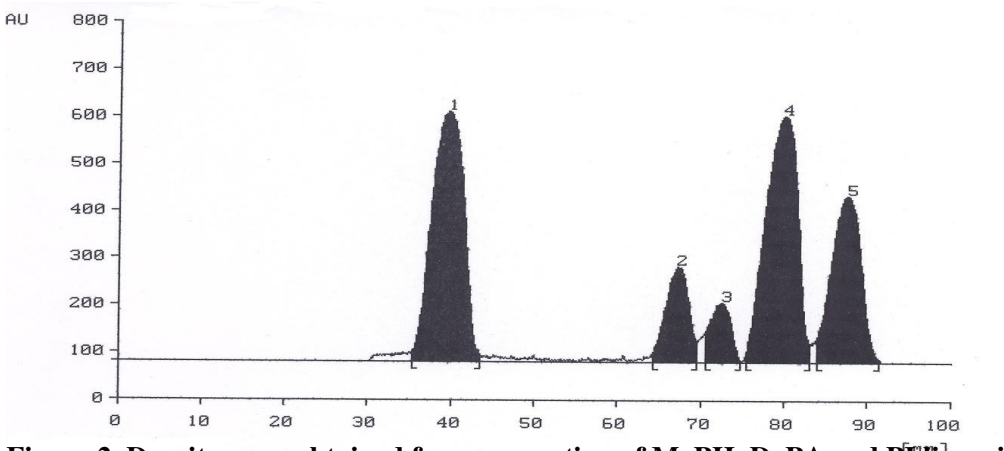

Figure 2. Densitogram obtained from separation of M, PH, D, PA and PI linearity

Table 1 presents the equation of the regression line, correlation coffcient (r^2) for each compound. Excellent linearity was obtained for compounds between the peak areas and concentrations of 10-30 µg with r^2 = 0.9909, 8-24 µg with r^2 = 0.9869, 2-6 µg with r^2 = 0.9915, 10-30 µg with r^2 = 0.9920 and 0.8-2.4 µg with r^2= 0.9915 for M, PH, D, PA and PI, respectively.

Table 1. Linearity Results, Limit of Detection (LOD) and Limit of Quantification (LOQ)

Compound	Equation	r^2	LOD (mg ml^{-1})	LOQ (mg ml^{-1})
Mefenamic Acid	Y= 962.1X + 11321	0.9909	0.1088	0.3627
Phenylbutazon	Y= 631.7X + 3712	0.9869	0.0581	0.1673
Diclofenac Sodium	Y=3073.0X + 5539	0.9915	0.0687	0.2289
Parasetamol	Y= 537.8X + 7735	0.9920	0.1244	0.4147
Piroxicam	Y=3877.0X + 156	0.9915	0.1026	0.3420

X= concentration (µg); Y= area

Limits of Detection and Quantification

Limits of detection (LOD) were established at a signal-to-noise ratio (S/N) of 3. Limits of quantification(LOQ) were established at a signal-to-noise ratio (S/N) of 9. LOD and LOQ were experimentally verified by five concentration of M, PH, D, PA and PI at the LOD and LOQ. The LOD was calculated to be 0.1088.

0.0581, 0.0687, 0.1244, and 0.1026 µg and the LOQ was calculated to be 0.3627, 0.1673, 0.2289, 0.4147, and 0.3420 µg for M, PH, D, PA and PI, respectively (Table 1).

Precision

The precision of the method (within-day variations of replicate determinations) was checked by applied M, PH, D, PA and PI 6 times at the LOQ level. The precision of the method, expressed as the RSD % at the LOQ level, was 0.30, 0.34, 0.47, 0.59, and 0.59% for PI, D, PH, PA and M, respectively.

Accuracy

A standard working solution containing PI, D, PH, PA and M yielding final concentrations of 0.5, 1.38, 5.15, 6.35 and 6.56 µg respectively was prepared. The prepared mixture of standards was applied 6 times as a test sample. From the respective area counts, the concentrations of the M, PH, D, PA and PI were calculated using the detector responses. The accuracy, defined in terms of % recovery of the calculated concentrations from the actual concentrations, is listed in Table 2.

Tabel 2. Accuracy of the Developed Method (n=6)

Compound	Spiked Concentration (µg)	Measured Concentration (µg) Mean ± SD	RSD (%)
Mefenamic Acid	6.56	6.34 ± 0.57	0.59
Phenylbutazone	5.15	5.09 ± 0.47	0.47
Diclofenac Sodium	1.38	1.36 ± 0.34	0.34
Paracetamol	6.35	6.36 ± 0.59	0.59
Piroxicam	0.50	0.49 ± 0.30	0.30

Analysis of Samples Traditional Medicine

1.75 gram of sample (A, B, C and D) was accurately weighed into a 100 mL beaker and dissolved in 10 mL methanol. Then the sample solution was transferred into a 25 mL volumetric flask and adjusted to volume with methanol. The solution was vortexed for 30 s then centrifuged at 4,000 rpm for 10 min. The filtrated supernatant was used for further analysis. For sample B was found piroxicam (1.16%) and phenylbutazone (10.24%), sample D was found paracetamol (2.51%). Samples A and C were not contaminated by undeclared synthetic drugs.

CONCLUSION

The developed method is suitable for the identification and quantification of the ternary combination of mefenamic acid, phenylbutazone, diclofenac, paracetamol, and piroxicam. In samples that were tested for contamination and adulteration, samples B and D the traditional medicines were contaminated paracetamol, phenylbutazone and piroxicam. The proposed method is simple, sensitive, rapid, specific and could be applied for quality and stability monitoring of mefenamic acid, phenylbutazone, diclofenac, paracetamol, and piroxicam.

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NEUROPROTETIVE EFFECTS OF *CENTELLA ASIATICA* TOWARD BDNF (*BRAIN-DERIVED NEUROTROPIC FACTOR*) LEVEL, TNFA, NFKB AND APOPTOSIS ON NEURONAL CELLS CULTURE LPS-INDUCED

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Abstract : Neurodegenerative diseases like Alzheimer's is a most frequently encountered neurodegenerative disease. The patomechanism of neurodegerative still debatable, but suspected by the occurrence of inflammation. Neuroprotective Brain-derived factor (BDNF) are known to have functions to maintain the survival of nerve cells course by various injuries. *Centella asiatica* (CeA) has long been known as neurotonic material, but the mechanism of neuronal cells and glia is not known clearly. The purpose of this research is to determine the effects of CeA ethanolic extract on levels of BDNF, TNFα, NFkB, BCL2 and apoptosis of neuronal cells. Cultures of primary nerve cells derived from rat midbrain sections (*Rattus norvegicus Wistar strain*) aged 0-24 hours. Cells grown in DMEM medium, 10% FBS, glutamine, glucose, penicillin-streptomicin and sodium bicarbonate on plates that have been coated with poly-D-lysine and stored in 5% CO_2 incubator temperature of 37°C. After the cells were confluent cells exposed to CeA extract concentration 50,100 and 200 µg/ml 4 hours and added LPS 10 ng/ml 1 hour before cell harvest. Culture medium was taken for measured of BDNF and TNFα by ELISA and the cells is used for observation of NFkB, BCL2 and apoptosis. Research results showed that CeA reduce significantly the level of NFkB ($p = 0.002$), TNFα ($p = 0.000$) and apoptosis ($p = 0.015$) and increased significantly levels of BDNF ($p = 0.000$), BCL 2 ($p = 0.010$) on the concentration of CeA 200 µg/ml. Results of the above seems caused by madecasoside and asiaticoside activity that serves as an anti-inflammatory agents as well as antioxidants. Not only both these materials, but also CeA contain other antioxidants such as caffeic acid, flaconol, quercetin and others. Various studies have also shown neurostimulant and regeneration effects on dendrite and anti-apoptosis effect. The conclusion of this study was that CeA extract can decrease TNFα, NFkB and apoptosis and increase BDNF, BCL-2 of neuronal cells. The optimum concentration was 200 µg/ml.

Keywords: *Centella asiatica*, BDNF, TNFα, NFkB, BCL2, LPS, neuronal cells, apoptosis.

INTRODUCTION

Neurodegenerative diseases like Alzheimer's and Parkinson's is a neurodegenerative disease most frequently encountered, especially in the age of 40 years. According to WHO, in 2050 estimated the number of people with Alzheimer's will rise by about 100 million people and will continue to increase every year and attacked at a younger age. The cause of this disease still has not been established clearly. However, allegedly due to inflammatory processes that occure in neuronal and glia cells in the brain. Inflammation is a complex process involving many factors including free radicalsand various inflammatory mediators. In addition to causing the death of neuron cells, can also cause inflammation and even loss of dendrite retracts on neuron cells and a decrease in growth factors that result in lost and reduced memory abilities. Inflammatory process causes the issuance of a mediator-inflammatory mediators such as TNFα, various interleukins, which in turn can lead to neuronal loss and death of neuron cells.

BDNF (Brain-derived Neurotropic Factor) is a growth factor belonging to the family that includes neurotropin Nerve growth factor (NGF). Neruotropin can be found in the central nervous system or peripheral. BDNF works on certain nerves in the central

nervous system and peripheral nerves (Acheson *et al.*, 1995). The function of this protein is to sustain the survival of nerve cells and enhance the growth and differentiation of neuronal cells and synapses. BDNF is in active state in the hippocampus, cortex and forebrain which is a vital place for learning, memory and higher thinking. BDNF itself is very important for long-term memory (Huang *et al*., 2001). Neurodegenerative drugs in adition requires a long period of time also caused side effects that are less pleasant. This requires an effective material to overcome neurodegerative but with minimal side effects. One material that has the potential to be developed is *Centella asiatica* (CeA), which has active ingredients include anti-inflammatory (Asiaticoside) and antioxidant (madecasoside) and various other active ingredients may be potentially as neurotonic. This study aims to determine the effect of ethanol extract of CeA for BDNF, TNFα, NFkB, and apoptosis in BCl2 cells LPS-induced neuron.

METHOD

Neuronal Cells Culture

Primary culture of nerve cells derived from rat midbrain sections (*Rattus norvegicus Wistar strain*) aged 0-24 hours. Tues grown in DMEM medium, 10% FBS, glutamine, glucose, penicillin-streptomicin and sodium bicarbonate. Tues grown on plates that have been coated with poly-D-lysine and stored in 5% CO_2 incubator and temperature of 37°C.

Administration of Extract and LPS

After the cells were confluent CeA extract concentration 50, 100 and 200 µg/ml for 4 hours and added LPS 10 ng/ml 1 hour before cell harvest. Culture medium was taken for examination BDNF and TNFα by ELISA and cell method is used for observation of NFkB, and Apoptosis BCL2

ELISA for BDNF and TNFa Level

BDNF and TNFa Level measure with ELISA done as detail procedure in MPBIO

Observation of NFkB, BCL2 dan Apoptosis using Immunocytochemistry

Cell was fixated using methanol absolute and immunocytochemistry done base on BIOCARE MEDICAL procedure.

RESULTS

NFkB Expression

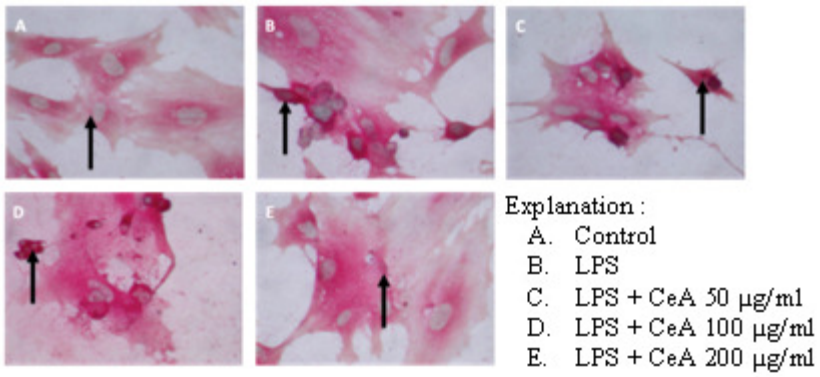

Figure1. NFkB exspression using imunocytochemistry. Black arrow showed NFkB expression in perinuclear area. 400x Magnification

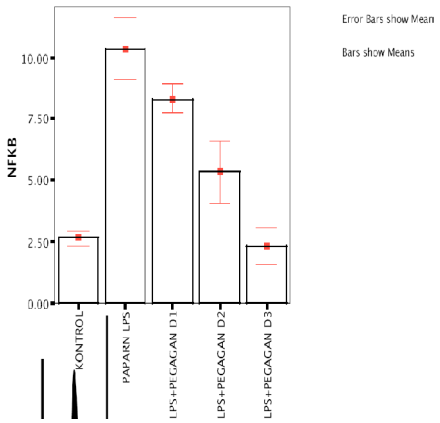

Figure 2 Histogram Quantification of NFkB. Its showed that CeA could decrease NFkB expression. Significant at level p < 0.05.

TNFα Levels

Table 1. Mean of TNFα Level

Treatment	Mean TNFα ± SD	P value*
Control	5.2262 ± 0.70	0.000
LPS	12.1712 ± 1.87	1.000
LPS+P 50 µg/ml	11.6378 ± 1.19	0.930
LPS+P100 µg/ml	8.9602 ± 0.77	0.001
LPS+P200 µg/ml	7.0155 ± 0.86	0.000

*Significant at level p < 0.05

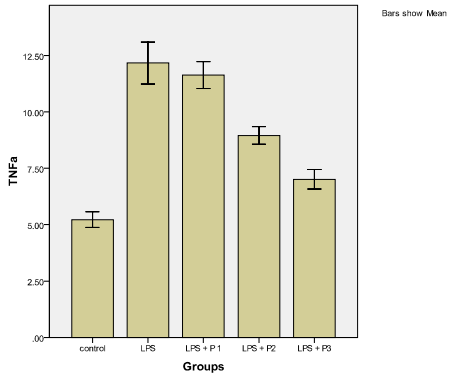

Figure 3. Histogram TNFα Levels. CeA administration decrease TNFα Level.

BDNF

Table 2. Mean of BDNF Levels

Treatment	Mean BDNF ± SD	P value*
Control	11.4370 ± 0.89	0.000
LPS	7,8932 ± 0.57	1.000
LPS+P50 µg/ml	8.4687 ± 0.67	0.684
LPS+ P 100 µg/ml	8.7073 ± 0.70	0.362
LPS+ P 200 µg/ml	11.0660 ± 0.88	0.000

*Significant at level p<0.05

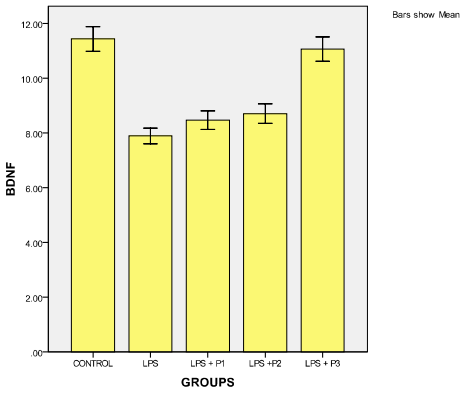

Figure 4. Histogram BDNF Level. CaA increase BDNF level almost the same with normal condition

BCL 2 and Apoptosis Expression

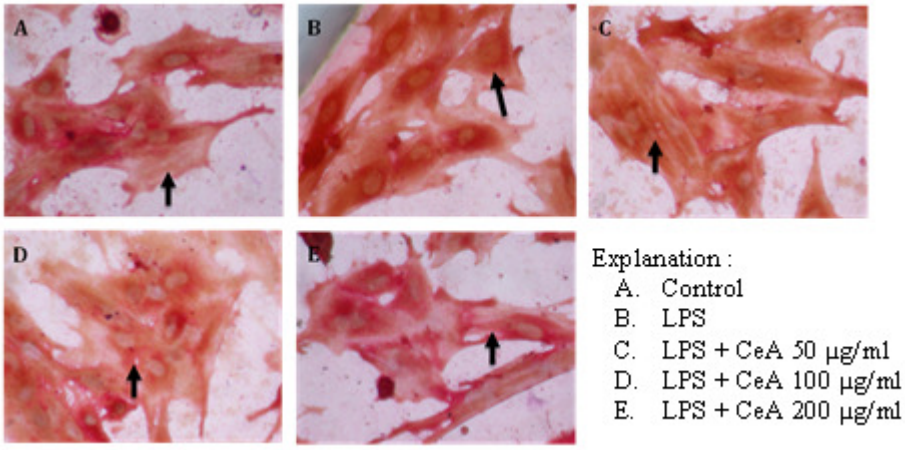

Figure 5. BCL 2 Expression usingImunocytochemistry. Black arrow showed BCL 2 expression in perinuclear. Magnification 400x

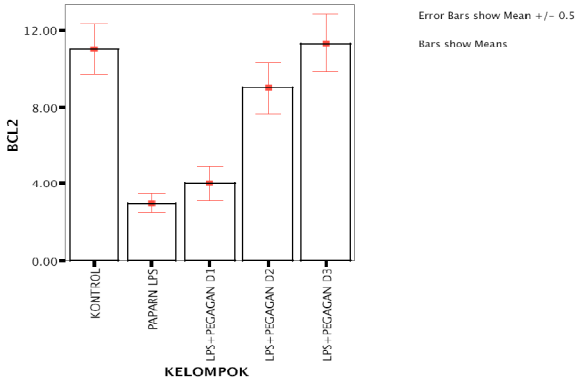

Figure 6. Histogram of BCL2 Expression. Its seems that CeA act as antiapoptotic agent trough intrinsic pathway.

DNA Fragmentation of Neuronal Cells

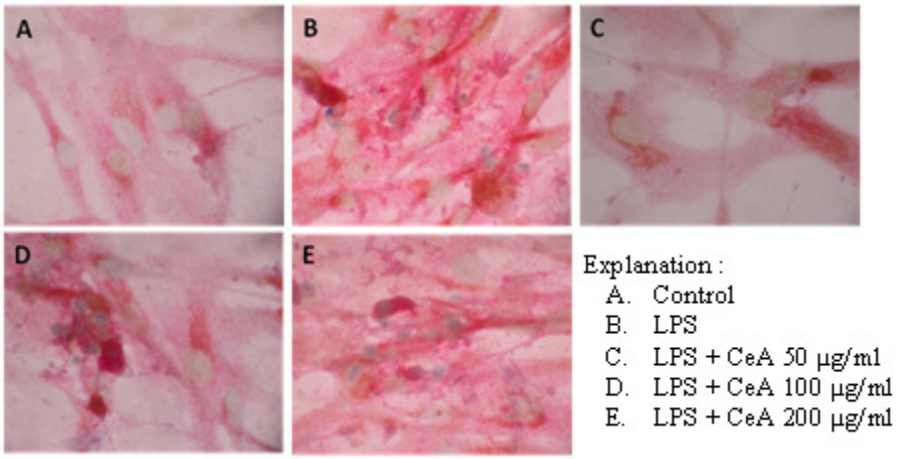

Figure 7. Visualization of Fragmentation DNA using immunocytochemistry. Black arrow showed Fragmented DNA. Magnification 400x

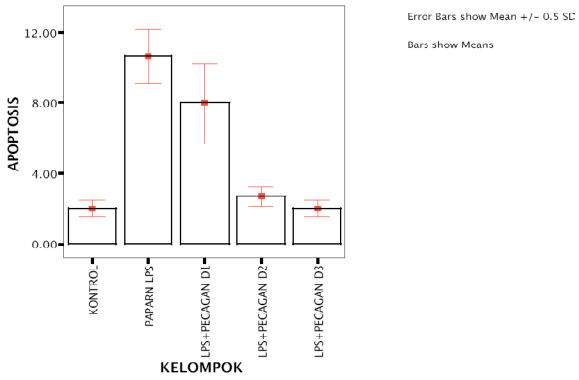

Figure 8. Histogram of Visualization fragmented DNA. Decreasing trends in addition of CeA.

DISCUSSION

NFkB Expression

Immunohistochemical techniques can be used to observe changes in expression and activation of NFkB. LPS 1ug/mL treatment increased the activation of NFkB significantly compared to all groups, indicated by the number of brown color on the cell nucleus, which is a visualization of the reaction between DAB cromogen with SA-HRP in complex secondary antibody biotin-labeled, at the core of glia cells. By performing a calculation of 100 cells, glia cells that have acquired the NFkB activation is significantly increased ($p = 0.003$) on the LPS treatment group. Provision of CeA extract can reduce the expression of NFkB. LPS treatment group with the provision of CeA concentration of 50 mcg / ml lowered the expression of NFkB, although not significantly ($p = 0.674$), but in concentration 100 and 200 µg/ml can reduce significantly the expression of NFkB with $p = 0.045$ and $p=0.002$ respectively.

NF-κB is a transcription factor that plays an important role in inducing a variety of gene regulation in inflammatory response and cell proliferation. NF-κB in normal circumstances be in an inactive state because its bond with the inhibitor κB (IκB) and located in the cytoplasm. NF-kB can be activated by various stimuli such as: cytokines, ROS, LPS, oxidized LDL and COX, which resulted in the release of the bond κB NF-κB thus enters the cell nucleus and regulate various inflammatory mediators, including: TNF-

α, IL - 1, VCAM, and ICAM. (Martin *et al*. 2000; Collins and Cybulsky, 2001). *Centella asiatica* content study further demonstrated that there are various caffeic acid derivatives and flaconol also contains quercetin, kaemferol, catechin, rutin and narigin (Shinomol and Mulidhara, 2008). This is also evidenced by the results of the test antioxidant activity in vitro DPPH method showed antioxidant activity of CeA greater than beta-carotene. Antioxidant components of CeA seems capable of reducing free radicals caused by inflammation, so the expression of NFkB decreases.

TNFα Level

Provision of LPS concentration of 10 ng / ml increasing significantly TNFα level compared to control. TNFα is a specific inflammatory mediators that are released into the cytoplasm which can then penetrate the cell membrane and out into the extra-cellular matrix. Inflammation of nerve cells begins with binding of LPS to LBP which then transfers LPS to CD 14. CD14 is family of glycophosphatidylinositol (GPI) as an LPS receptor which then activates TLR 4. Signal of LPS can induce various protein kinases including protein kinase C (PKC) and several protein tyrosine kinases (PTKs). The relationship between TLRs and mitogen-activared protein kinase (MAKPs) probably derived from IL-1 as TLRs receptor. In addition LPS also increased the activity microglia to release several cytokines such as TNF-α associated with the death of neurons. Tumor Necrosis Factor-α in vivo studies led to complications pathology by increasing the enzyme cyclooxygenase-2 in particular, are changing the arachidonic acid into prostaglandins as mediators of inflammation (Abbas and Lichtman, 2003; Lehnardt et al , 2003).

TNFα is produced in a certain time after the injury. As is well known that LPS can exacerbate disease conditions in the central nervous system inflammation through the certain mechanism.. Lipopholysacharide through its neurotoxin able to activate microglia shown to produce the first TLR in response to the presence injury resulting in brain cell damage of neurons (Lehnardt et al, 2003). Provision of CeA extract can reduce levels of TNFα. Concentration of 50 ug/ml was able to reduce levels of TNFα but not significantly ($p = 0.930$). But, CeA concentrations at 100 and 200 ug/ml can reduce levels of TNFα significantly ($p = 0.001$ and $p = 0000$). This is because the active ingredient in concentrations of 50 ug/ml is not enough to overcome the damage caused by LPS and the concentrations of 100 and 200 appears adequate enough to overcome the injury by LPS. The ability of CeA to encounter inflammation due to LPS is likely caused by an anti-inflammatory ingredients that is madecassoside in CeA which is a triterpenoid. Madecassoside proved to reduce levels of TNFα, IL-6, production of PGE2, COX2 expression and increased up-regulation of anti-inflammatory molecules IL-10 in mice collagen-induced arthritis (Li *et al*., 2009).

BDNF Level

The results showed that the inflammatory condition that is triggered by LPS caused a decrease in BDNF levels significantly ($p = 0.000$). CeA extract increase the BDNF levels almost same compare to control at concentrations 200 µg/ml ($p = 0.912$). BDNF levels increase significantly probably due to the active ingredient in the ethanol extract of CeA that serves as activator of genes coding for BDNF. In addition it is possible also because the antioxidant content (asiaticoside) regulate intracellular calcium levels due to the synthesis of BDNF affected CREB-dependent Ca^{2+}, which is known intracellular Ca^{2+} levels influenced by the stability of the cell membrane. Soumyanath, et al (2005) proved the CeA ethanolic extract in vivo causes rapid regeneration and growth of axon. It was probably caused by the content of Asiatic acid (AA) is present in CeA extracts. Extract of CeA also has growth stimulating properties that could enhance the growth of dendrite of neuron cells (Mohandas *et al*., 2007).

BCL 2 and Apoptosis Expression

Bcl-2 is an integral membrane protein located mainly in the outer mitochondrial membrane. Overexpression of Bcl-2 prevents apoptosis due to a variety of responses. Bcl-2 prevents apoptosis of excess cells in response to various stimuli. Cytosolic cytochrome c is required for the initiation of apoptotic programs, indicating a possible relationship between Bcl-2 and cytochrome c, which is usually located in the mitochondria intermémbran. Cells that had levels of apoptosis are known to have high concentration of cytochrome c in the cytosol. Overexpression of Bcl-2 prevents cytochrome c efflux from mitochondria and initiation of apoptosis. Thus, one role of Bcl-2 that may be in the prevention of apoptosis is to block the release of cytochrome c from mitochondria.

Physiological apoptosis occurs in all living cells. This planned program of death was necessary for the homeostasis of the individual. However, the incidence of apoptosis in neurodegerative becomes dominant so cause neuronal loss. It required an anti-apoptosis of nerve cells that can be prevented neurodegerative disorder.

CeA extracts reduce the occurrence of apoptosis along with increased concentration of the extract. CeA able to reduce amyloid plaques that was confirmed with antioxidant function in vitro could scavenge free radicals, reduce lipid peroxidation and protect DNA damage (Dhanasekaran *et al*., 2009). Content of CeA study further demonstrated that there are various caffeic acid derivatives and flaconol also contains quercetin, kaemferol, catechin, rutin and narigin (Shinomol and Mulidhara, 2008). Gynecologist antioxidants that can protect cell membranes and mitochondrial membranes, so as to protect BCL 2 (contained in the outer membrane of mitochondria) for not releasing caspase. It is understood that later obtained visualization results of DNA fragmentation is a parameter that decreases apoptosis caused of the role of BCL2 as an agent of anti-apoptosis. From the above results it can be concluded that CeA can protect damaged nerve cells due to decreased levels of inflammatory TNFα and NFkB, through antioxidant and anti-inflammatory mechanisms. CeA is also able to reduce apoptosis and increase expression of inflammatory BCl2. In addition CeA also provides protection against cognitive function through increasing levels of BDNF. Optimum concentration gained at 200 µg/ml concentration of *Centella asiatica* extract.

ACKNOWLEDGEMENT

Hibah Strategis Nasional Project (TA 2009) DIRJEN DIKTI.

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POTENCY OF DRAGON BLOOD (*DAEMONOROP DRACO)* AS MEDICINAL PLANT, AND ITS CONSERVATION IN JAMBI PROVINCE-INDONESIA

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Abstract : Dragon blood (*Daemonorop draco)*, is a rattan palm native to Malaysia, India, and Indonesia. In Indonesia, it is called jernang. This species have ecological, sociological and economical value. This study conducted in Jambi Province in 2008. The methods are: observation directly to the field, study literature, interview to local people, and key informant in the study area. This species (*Daemonorop draco*) is used as pigment to color matter for varnishes, and also used as medicinal purpose. Local people around forest in Jambi Province use this plant as medicine for many kinds of disease. They get this traditional knowledge from their ancestor. Besides, It has high and competitive price in international trade, so this species is very worthy for local people. Unfortunately, the existence of this species in nature in threat because over exploitation, and forest fire in 1997 in Jambi destroyed most of jernang area. Then, most of people around the forest exploit this species in the nature without any effort to cultivate them. As a result, it tends to be rare in nature. According to the description above, because this species is threatened in Indonesia's Forest, it's a must to make regulation or efforts to make this species sustainable in nature.

Keywords : Potency; Dragon blood (*Daemonorop draco*); medicinal plantnability, Jambi Province

INTRODUCTION

The blood red resin of the dragon's blood trees have many different applications including their use as pigmen for varnishing, or as medicine. The term dragon's blood is interchangeably used to refer to plants from three quite different families. The bright red resin that is obtained from Dracaenaceae, palm genus Daemonorop, and genus Croton, and all bear red resin (Dransfield, and Manokaran 1993). The red resin was used in ancient time as varnish for wooden furniture, medicine, incense, and dye. Dragon's blood resin is also produced from the rattan palms of the genus Daemonorops of Indonesian island and known as jernang. It is gathered by breaking off the layer of red resin encasing the unripe fruit of rattan. The collected resin is then rolled into solid balls before being sold. This commodity has high economical value, so that it's exploited from the forest community around the forest. Nowadays, the existence of this species become rare.

DISTRIBUTION

Jernang (*Daemonorop draco*) is a rattan palm native to Malaysia, India, and Indonesia, and mostly in Indonesia. In Indonesia, it's located in Jambi, Aceh, and Kalimantan Province.

DESCRIPTION OF JERNANG (*DAEMONOROP DRACO*)

Daemonorop draco is a small palm growing in Indonesia. The long, slender stems of the genus are flexible, and the older trees develop climbing propensities. The leaves have prickly stalks which often grow into long tails and the bark is provided with many hundreds of flattened spines. The berries are about the size of cherry, and pointed. When ripe they are covered with a reddish, resinous substance which is seperated in several ways. The most satisfactory being by steaming or by shaking or rubbing in coarse, canvas

bag. An inferior kind is obtained by boiling the fruits to obtain a decoction after they have undergone the second process (Heyne, 1987).

***Fig* 1.** *Daemonorops draco* (Jernang fruits)

CLASSIFICATION
Kingdom: Plantae
Subkingdom: Tracheobionta
Super Division: Spermatophyta
Class: Liliopsida
Sub Class: Arecidae
Ordo: Arecales
Familie: Arecaceae
Genus: *Daemonorops*
Species: *Daemonorops draco* Wild

CHEMICAL COMPOUND

Chemical compound of jernang resin are 5,7-Dihydroxy-6-methyIflavan, 7-hydroxy-5-methoxyflavan, Dracoflavan A, 5.5',7,7'- Tetrahydroxy-4,8'-biflavan, Dracoflavan BI, Dracoflavan B2, Dracoflavan C, Dracoflavan Cl, Dracoflavan C2, Dracoflavan DI, Dracoflavan D2, Dracooxepine, Dracorubin (digunakan sebagai antiseptic), Nordracorubin, 12-Ursene-3,38-diol (yang digunakan sebagai *antineopalstic agent)* memiliki varian seperti Epiuvaol dan Uvaol (Purwanto *et al*. 2005)

UTILIZATION OF JERNANG RESIN *(Daemonorop draco)* AT KUBU ETHNIC GROUP IN JAMBI PROVINCE

Generally, the usage of rattan is from the stick, but the usage of jernang is from the resin in the skin of the fruit. The main uses of jernang are: varnish of ceramic, rattan, bamboo, and as medicine. As folk medicine 10 to 30 grains were formerly given as an astringent in

diarrhoea, chest pains, post-partum bleeding, internal traumas, and menstrual irregularities, dispel blood stasis, relieve pain of traumatic injuries, contusion, sprains, bruising and stops bleeding, protect decay of ulcer's surface, rheumatism, anti fungal, sedative, and it was considered to be a powerful aphrodisiac and it is often used under the matress as cure for impotency.

ECONOMICAL VALUE of JERNANG

Jernang resin is one of comodity export that it has high value in Jambi Province. The price in the local trade approximately Rp 900.000 – Rp 1.200.000 per Kg.The Demand in international trade is quite stable, mostly importer from China, Hongkong, and Singapore. We can get 3.5 kg jernang resin from 100 kg jernang fruit. The demand of jernang from Indonesia to China is about 400 ton per year, but Indonesia still supplies 27 ton per year. It shows that, it's still prospect to cultivate this speciaes. Profit estimation from one hectare jernang per year is about 35-38 million rupiahs. Jernang could be planted as "tumpang sari system" (intercropping) with another plan, for example rubber plant. So, community around the forest can get benefit both from rubber plant and jernang.

JERNANG DEVELOPMENT PROGRAM

People around forest in Jambi Province exploited this commodity from the forest, and they didn't cultivate this species. The result was this species tend to be rare in the nature (Setyowati, 1999). There were some causes why it's happenned. Firstly, it is because of over exploitation without cultivating them, secondly by forest fire, thirdly: Good jernang resin is produced from half ripe fruits, so the available of the seed is threatened, and finally because of deforestation (Wiriadinata, 1996). To anticipate the lose of jernang (*Daemonorop draco*) in nature some actions have been done. One of them is people in Lamban Sigatal and Sepintun District of Pauh, soralangun Regency start to cultivate this commodity under coordination of Gita Buana NGO.

CONCLUSION AND RECOMMENDATION

According to the description that jernang resin is very potential for social, economical, and ecological aspects, it is very urgent to developt this species as forest plantation to supply the demand of this species as comodity export. Development of this species not only increase the local people's income, but also improve the environment. So, local government should make efforts to make this program as a priority target.

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