

PROCEEDING

Vol. 1 No. 1 October 2011



The International Conference on Bioscience and Biotechnology 2011

*Interfacing Biotechnology, Natural Product Chemistry,
and Tropical Biodiversity for Sustainable Development*

Yogyakarta, October 11th-12th, 2011

Editors:

Prof. Dr. Dr. h.c. Hildebert Wagner
Prof. Geoffrey A. Cordell, Ph.D.
Assoc. Prof. Dr. Mahanem Mat Noor
Dr. Ir. Endang Tri Margawati, M.Agr.Sc.
M. Ja'far Luthfi, Ph.D.
Arifah Khusnuryani, M. Si.

Organized by



Biology Department
Faculty of Science and Technology
State Islamic University Sunan Kalijaga
Yogyakarta - Indonesia

Supported by



UNIVERSITI
KEBANGSAAN
MALAYSIA



International Conference on Bioscience and Biotechnology (ICBB) 2011

October 11th-12th, 2011, Yogyakarta, Indonesia

PROCEEDING



State Islamic University Sunan Kalijaga Yogyakarta
Faculty of Science and Technology
Biology Department

Committees

Steering committee

- Prof. Dr. Musa Asy'arie *Rector of State Islamic University Sunan Kalijaga Yogyakarta*
- Prof. Drs. H. Akh. Minhaji, M.A., Ph.D. *Dean of Faculty of Science and Technology*
- Dra Hj. Khurul Wardati *Vice Dean of Faculty of Science and Technology*
- Arifah Khusnuryani, M.Si. *Head of Biology Department*

Scientific committee

- Dr. Emily Jane McCallum *Institute of Plant Sciences, Zurich, Switzerland*
- Dr. Huahong Wang *Institute of Plant Sciences, Zurich, Switzerland*
- Dr. Agnieszka Mudge *The University of Queensland*
- Prof. Drs. Sutarno, M.Sc., Ph.D. *Universitas Negeri Sebelas Maret*
- Prof. Dr. Ir. Bambang H. Saharjo, M.Agr. *Institut Pertanian Bogor*
- Prof. Dr. Sarjan *Universitas Mataram*
- Prof. Dr. Nyoman Puniawati Soesilo *Gadjah Mada University*
- Dr. Ir. Endang Tri Margawati, M.Agr.Sc *Indonesian Institute of Science*
- Dr. Marini *Malaysian Agricultural Research and Developmental Institute*
- Dr. Kifayah Amar *State Islamic University Sunan Kalijaga Yogyakarta*
- Dr. M. Jafar Luthfi *State Islamic University Sunan Kalijaga Yogyakarta*
- Dr. Sahabudin *Tadulako University*

Organizing committee

- Dr. M. Jafar Luthfi
- Arifah Khusnuryani
- Maizer Said Nahdi
- Anti Damayanti
- Siti Aisah
- Runtut Prih Utami
- Erny Qurotul Aini
- Sulistyawati
- Jumailatus Solihah
- Lela Susilawati
- Ika Nugraheni
- Dias Idha Pramesti
- Najda Rifqiyati
- Dian Noviar
- Widodo

Editorial and Publisher

Biology Department, Faculty of Science and Technology,
State Islamic University Sunan Kalijaga Yogyakarta **Address** Laksda Adisucipto Street No.
1, Yogyakarta, Indonesia Postal Code 55281 **Phone** +62-274-519739 **Fax** +62-274-540971
Official site <http://www.icbb2011.com> **Email** committee@icbb2011.com

Contents

| | |
|------------------------------------|----|
| Preface | i |
| Committees..... | ii |
| Abstract from Keynote Speech | xi |

ORAL PAPER CLUSTER 1

| | |
|---|--------|
| Analysis Interaction of Glucosyltransferase Inhibitor of Caries from Fatty Acid by Molecular Docking Simulation <i>Alfred Pakpahan, Fadilah</i> | A1-5 |
| Effect of Nanocomposite-based Packaging on Postharvest Quality of Water Content-treated Coffee Beans during Storage <i>Erdawati, Riskiono</i> | A6-15 |
| The Modification of Coffee Leaves Beverage (Air Kawa) Processing Through Enzymatic Oxidation <i>P. Darmadji, E.L.D.Permatasari, U. Santoso</i> | A16-21 |
| Utilization of Ligninolytic Enzyme in Biobleaching of Pulp from Empty Fruit Bunches of Oil Palm <i>Happy Widiastuti, Suharyanto, Siswanto</i> | A22-27 |
| Nutritional Profile of Freeze-dried Red Seaweeds from Semporna, Sabah <i>Mansoor Abdul Hamid, Patricia Matanjun & Tiang Ming Chee</i> | A28-33 |
| Comparison of Seed Nutmeg Oleoresin Extraction (Myristica Houtt fragrans) Origin of North Maluku and Maceration Method Using Combined Distillation–Maceration <i>Muhammad Assagaf, Pudji Hastuti, Chusnul Hidayat, Supriyadi, Sri Yuliani</i> | A34-37 |
| Development Process of Frying Distillation in Capturing Flavor that Formed During Deep Frying <i>P. Darmadji, Y. M. Rahmadewi, H. Firdaus, A. Sausania and Supriyanto</i> | A38-43 |
| Effect Roasting of Indonesian Sesame Seed (<i>Sesamum indicum L.</i>) on Odour Profil and Degree of Liking of The Oil <i>Pudji Hastuti, Wahdan, Supriyanto, and Supriyadi</i> | A44-49 |

| | |
|---|----------|
| Antioxidant Activity and Compounds of Indonesian Sesame (<i>Sesamum indicum</i> L.) Oil <i>Pudji Hastuti, Lukita Purnamayati, Siswanti, Supriyanto</i> | A50-54 |
| Application of Liquid Smoke and Smoke Powder for Process Development Instant Seasoning of Indonesian Traditional Food <i>Purnama Darmadji, Mutiara Anindita, and Sri Suparyati</i> | A55-74 |
| A Novel Process to Prepare Chemoselectively Protected N-Phthaloyl- Chitosan without Drying of Solvent and Purging of Water Vapor <i>Radna Nurmasari, Uripto Trisno Santoso, Dewi Umaningrum</i> | A75-78 |
| Encapsulation of Phenolic Compound from Star Fruit with Chitosan Nanoparticle <i>Riskiono Slamet and Erdawati</i> | A79-87 |
| Transition State Analysis of HMM for DNA Exon Controlling Using Bioinformatic Simulation <i>Suhartati Agoes, Alfred Pakpahan, Binti Solihah</i> | A88-92 |
| Synthesis of a Series of Calix[6]arenePolymers from <i>p-ter-butylphenol</i> <i>Susy Yunita Prabawati, Jumina, Sri Juari Santosa, Mustofa</i> | A93-100 |
| Study of Thermal Stability of Riboflavin Synthase of <i>Eremothecium gossypii</i> through Molecular Dynamics Approach <i>Syarifuddin Idrus and Usman S.F. Tambunan</i> | A101-106 |
| Tosylation of N-Phthaloyl-Chitosan without Drying of Solvents and Purging of Water Vapor <i>Uripto Trisno Santoso, Radna Nurmasari, Dewi Umaningrum</i> | A107-111 |
| ORAL PAPER CLUSTER 2 | |
| Associations between Blood Lead Level and Blood Pressure among City Minibus Drivers in Purwokerto City, Indonesia <i>Agung Saprasetya Dwi Laksana, Endo Dardjito</i> | B1-6 |
| Rat Sperm Proteomic Analysis: Effect of the Antifertility Agent <i>Centellaasiatica</i>L. <i>Irfan Yunianto and Mahanem Mat Noor</i> | B7-16 |

| | |
|---|--------|
| Tapak Liman (<i>Elephantopus scaber</i> L) as Immunostimulator and Its Effect on Lymphocyte Differentiation in Mice BALB/C <i>Marmi Kelik</i> | B17-21 |
| Sugar Residues and Their Variations of Distribution on Ovarian Follicles of Timor Deer (<i>Cervus timorensis</i>) <i>N. Rifqiyati</i> | B22-28 |
| Cholesterol Levels, High-Density Lipopolysaccharide and Triglyceride of Civet (<i>Paradoxurus hermaphroditus</i>) <i>Sarmin</i> | B29-33 |
| Preview Kidney Function in Civet (<i>Paradoxurus hermaphroditus</i>): Especially Preview of Urea Nitrogen and Creatinin <i>Sarmin</i> | B34-38 |
| Feeding Ecology of Mentawai langur (<i>Presbytis potenziani</i>) in Siberut, Mentawai Islands <i>Susilo Hadi</i> | B39-43 |
| Green Tea Extract Protects Endothelial Progenitor Cells from Oxidative Damage through Reduction of Intracellular Reactive Oxygen Species Activity <i>Wahyu Widowati, Rahma Micho Widyanto, Dian Ratih Laksmiawati, Winsa Husin, Hana Ratnawati, Indra Bachtiar</i> | B44-55 |
| Potential Cytotoxic on Breast Cancer Cells Line and Antioxidant of Water Extract of <i>Catharanthus roseus</i> [L] G.Don., <i>Dendrothoe petandra</i> L., <i>Curcuma mangga</i> Val., <i>Piper betle</i> L. <i>Wahyu Widowati, Tjandrawati Mozef, Chandra Risdian, Hana Ratnawati, Susy Tjahjani, Ferry Sandra, Lusiana Darsono, Sri Utami Sugeng</i> | B56-64 |
| Preference of <i>Apis cerana</i> to Six Pollen Substitutes <i>R. Widowati, W. Sjamsuridzal, A. Basukriadi, A. Oetari, E. Anwar, V. Enfinali, E.A. Rismawanti, B.A. Luhur</i> | B65 |
| Cytotoxic Activity Prescreen of Leaves of Primate Consumed Plants Subclassis <i>dilleniidae</i> and <i>hamamelididae</i> Using Brine Shrimp Lethality Test <i>A. Zuhrotun, A. Diantini, A. Subarnas, R. Abdullah, M. Thamrin</i> | B66 |
| ORAL PAPER CLUSTER 3 | |
| Tree Species Diversity of Kerinci-Seblat National Park and Its Potentials for Natural Substances-Based Medicines <i>Agus Susatya</i> | C1-8 |

Morphological Variation and Phenetic Relationship of Hyacinth Bean (*Lablab purpureus* (L.) Sweet) in Lombok, West Nusa Tenggara

Ervina Titi Jayanti, Rina S. Kasiamdari, and Budi S. Daryono C9-15

Diversity of Birds in Tepus Village of Gunung Kidul District of Yogyakarta

Faradlina Mufti, Siti Diniarsih, Mas Untung, Joko Setyono, Mustafid Anna, Nurdin Setyobudi C16-23

Detection of Immunoglobulin Geneheavy Chain Binding Protein in *Eimeria tenella* Collected from Yogyakarta Using One Step Reverse Transcriptase PCR

G.Tresnani, J.Prastowo, W.Nurcahyo, B.S.Daryono C24-28

Amplification and Sequencing Growth Hormone Genes in the Nurseri Center for PO Cattle on Balai Besar Inseminasi Buatan (BBIB) Singosari and Unit Perusahaan Aliansi (UPA) Pasuruan

Mariana Rengkuan, Aloysius Duran Corebima, Sutiman Bambang Sumitro, Mohamad Amin C29-34

Isolation, Characterisation and Identification of Sea Urchin-Associated *Bacillus* in Mentigi Beach, West Lombok

Novi Febrianti, Bambang Fajar Suryadi C35-38

Isolation and Identification of *Rhizoctonia* Associated with *Phalaenopsis amabilis* (L.) Blume Roots

Khaterine, Nurbaity Situmorang, Rina Sri Kasiamdari C39-44

The Variation of Diversity of Cave Bats Dweller in Tuban and Menoreh Karstic Area Indonesia

Tatag Bagus Putra Prakarsa, Satino, Muhammad Fajri Rohmad C45

Identification of Pheromone Binding Protein Gene of Yellow Rice Stem Borer *Scirpophaga incertulas* (Walker) (Lepidoptera: Crambidae)

Jazirotul Fitriyati, Rika Raffiudin and I Made Samudra C46

ORAL PAPER CLUSTER 4

Banana (*Musa paradisiaca* L.) and Corn (*Zea mays* L.) Waste as a Biosorbent for Cu Metals from Wastewater of Textile Industry

Aliya Nur Hasanah, Fani Rizkiana, Rachmi Sugiarti, Driyanti Rahayu D1-9

| | |
|--|--------|
| Effects of Chitosan/Montmorillonite Nanocomposites Films on the Growth of Bacteria in Laboratory Media <i>Alvika Metasari</i> | D10-18 |
| Effect of Growth Substances on <i>in vitro</i> Callus Induction and Shoots Elongation of Cashew Nut (<i>Anacardium occidentale</i> L.) <i>Christiani Tumilisar, Rossa Yunita, Febrina Ariyanti L., Avianingtyas</i> | D19-27 |
| Screening and Identification of <i>p,p'</i>-DDT Degrading Bacteria from Agricultural Soil <i>J. Darma Jaya, V. Subhon and S. Maneerat</i> | D28-35 |
| Antibacterial Activity Test and Phytochemical Screening of <i>Smilax celebica</i> Tuber <i>Muhamad Agil, Arifah Khusnuryani</i> | D36-40 |
| Antimicrobial Activity of Leaves, Stems, and Barks of Palasu (<i>Mangifera Caesia</i> Jack) Against Microorganisms Associated with Fish Spoilage <i>Puji Ardinarsih, Risa Nofiani and Afgani Jayuska</i> | D41-49 |
| Effort to Build Environmental Care Attitude with Environmental Education through Integrated Biology Learning Based on Science Process Skills to Support Character Education Development in Indonesia <i>Suciati Sudarisman</i> | D50-59 |
| Comfort Level in the District Sleman <i>Sugiyanto, Sri Utami Zuliana, Winarti</i> | D60-68 |
| The Bacterial Growth and “Crude” β-Galactosidase Characteristics of <i>Klebsiella pneumonia</i> and <i>Lactobacillus bulgaricus</i> <i>Tatik Khusniati, Evindika Tri Padarik and Rini Handayani</i> | D69-74 |
| <i>In Vitro</i> Antagonism Test between <i>Fusarium solani</i> Caused <i>Fusarium Wilt</i> on Orchid <i>Phalenopsis Taida Salu</i> by Using Endophytic Fungi <i>Yuli Setiani and Rina Sri Kasiamdari</i> | D75-80 |
| Improving the Environmental Awareness and Creative Thinking Students Through the Application Problem Based Learning Model <i>Runtut Parih Utami</i> | D81 |

POSTER PAPER

- The Effect of Repeated Exposure of Formalin-Containing Fish Against Liver Cell of Mice Based on the Ratio of SGOT/SGPT**
A. A. Maramis, M. Amin, Sumarno and A. D. Corebima P1-4
- Determination of Rhodamine B in Cosmetics and Food by Using Spectrophotometry UV Visible and TLC**
Aliya Nur Hasanah, Ida Musfiroh, Nyi Mekar Saptarini, Driyanti Rahayu..... P5-12
- Conversion of Sugarcane Bagasse to Bioethanol Using Different Acids Pretreatment and Commercial Yeast**
Anastasia W. Indrianingsih, Vita T. Rosyida, Cici Darsih, Khadijah Jaka P13-18
- Analysis of Cocofeam Microstructure and Mechanical Characteristics from the Mixture of Coconut Fiber and Latex Compound**
I D.K. Anom, Bambang Setiaji, Wega Trisumaryanti, Triyono P19-27
- Genetic Diversity on Goffin's Cockatoo Bird (*Cacatua goffini*) Inferred from Cytochrome B Gene Sequences**
Dwi Astuti..... P28-36
- Genetic Diversity of Stevia (*Stevia rebaudiana* (Bertoni) Bertoni) Based on Molecular Characters**
Dyah Subositi, Rina Sri K. and Budi S. Daryono..... P37-41
- Isolation, Fermentation and Antidiabetic Endophytic Fungi A.AP.3F of the Stem Sambiloto Plant (*Andrographys paniculata* Ness)**
Edward J.Dompeipen, Wahyudi Priyono Suharso, Herry Cahyana, Partomuan Simanjuntak..... P42-48
- Identification of Genetic Markers Associated with Twinning Birth Trait in Cattle**
Endang Tri Margawati, Syahrudin Said and Indriawati..... P49-56
- Effect of Arbuscular Mycorrhizal Fungi and *Trichoderma* Inoculation on Growth of Oil Palm Seedling Inoculated with *Ganoderma* sp.**
H. Widiastuti..... P57-61

| | |
|--|----------|
| Phenotypic and Genotypic Identification of Lactic Acid Bacteria Isolated From an Indonesian Traditional Fermented Fish "Bakasang" | P62-67 |
| <i>Helen J. Lawalata, Langkah Sembiring, Endang S. Rahayu</i> | |
| Antibacterial Activity Assay of Mahkota Dewa (<i>Phaleria macrocarpa</i>) Fruit Against Pathogenic Bacteria | P68-69 |
| <i>Iffa Izza, Lela Susilawati</i> | |
| Development of a Web-based Software for Microbial Culture Collection Data Management | P70-72 |
| <i>Imam Cartealy</i> | |
| The Characteristic of Polyvinyl Alcohol-Carbon from Coconut Shell Carbon | P73-78 |
| <i>Meytij Jeanne Rampe, Bambang Setiaji, Wega Trisunaryanti and Triyono</i> | |
| Flow Cytometric Analysis of MCF-7 Cell Line in Its Treatment with Leaves Extract of <i>Eugenia uniflora</i> L | P79-85 |
| <i>Nita Supriyati, Subagus Wahyuono, Esti Wahyu Widowati</i> | |
| Petroleum Ether, Ethyl Acetate and Methanol Extracts of <i>Pseudocalymma alliceum</i> (Lam.) Sandwith Leaves and Their Antiviral Activities Against Newcastle Disease Virus | P86-88 |
| <i>Nuning Rahmawati and Ratna Asmah Susidarti</i> | |
| The Test Effect of "Genjah Salak" Coconut (<i>Cocos nucifera</i> L) Water on the Heart Rate of Male Wistar Mice | P89-93 |
| <i>Rini Syafriani, Elin Yulinah Sukandar, Tommy Apriantono, Joseph Iskendarso Sigit</i> | |
| Identification on Indonesian Accessions of <i>Curcuma xanthorrhiza</i> Roxb. Using AFLP Markers | P94-101 |
| <i>T. Tajuddin, I. C. Cartealy, A. Safarrida, D. Purwoko, S. Zulaeha and M. Ardiyani</i> | |
| Optimization studies on Alkali Pretreatment of Biomass Waste of Oil Palm Empty Fruit Bunch Fiber for Production of Glucose | P102-107 |
| <i>Yanni Sudiyani, Dyah Styarini, Sudiarmanto, Haznan Abimanyu</i> | |
| Screening of Pediocin Gene Encoding Bacteriocin Isolated from Indigenous Indonesian Traditional Fermented Food | P108 |
| <i>A. Zaenal Mustopa, Linda Sukmarini, Muhamad Ridwan</i> | |

| | |
|--|------|
| <p>Isolation and Cloning <i>Stearoyl-ACP Desaturase</i> (SAD) form Mesokarpoil Palm (<i>Elaeisguineensis</i> Jacq.) var. Tenera <i>DriyaShintia D.A, Imam C. Cartealy, Anna Safarrida, Siti Zulaeha, Agus Masduki, Wahyu Purbowasito</i>.....</p> | P109 |
| <p>TAPS and TTE Buffer as Mobile Phase in DNA Sequencing Using ABI Prism 310 Genetic Analyzer <i>Festy Auliyaur Rahmah, Jumailatus Solihah, Ethik Susiawati Purnomo</i></p> | P110 |
| <p>The Quality of <i>Spermatozoa</i> and Histological Figure of <i>Tubulus Seminiferus</i> of White Rat (<i>Rattus norvegicus</i>, L.) Testis After Supplemented by Green Pea Sprout Juice (<i>Phaseolus radiatus</i>, L.) <i>Imam Fuad Zamzami</i>.....</p> | P111 |
| <p>Application of ISSR Molecular Marker for Detecting Polimorphisme among 21 Accessions of Sambiloto (<i>Andrographis paniculata</i> (Burm.f.) Wallich Ex Ness) from North Sumatera, Jambi and West Nusa Tenggara <i>Juwartina Ida Royani, Dudi Hardianto, Siti Zulaeha, Dwi Rizkyanto, Suparjo, Nurjaya, Wahyu Purbowasito and Bambang Marwoto</i>.....</p> | P112 |
| <p><i>Cytochrome c Oxidase 1</i> (COI) Mitochondria Gene Haplotype Variations of <i>Scirpophaga incertulas</i> <i>Rika Raffiudin, I Made Samudra, Ruth Martha Winnie, Jazirotul Fitriyati, Idham Sakti Harahap</i>.....</p> | P113 |
| <p>Plant Parameters that Contribute to Reduce of Noise Levels <i>Siti Aisah</i></p> | P114 |
| <p>Developing Locally-Made Laboratory Equipment by Modification Using TRIZ Method <i>Taufiq Aji, M. Ja'far Luthfi, Irhason</i>.....</p> | P115 |
| <p>Antibacterial Activity on Singawalang (<i>Petiveria alliace</i>) Leaf to Mycobacterium Tuberculosis Sensitive Strain H37Rv and Multi-Resistant Strains Labkes Eh & Sr <i>Yani Mulyani, Elin Yulinah Sukandar, Ketut Adnyana</i>.....</p> | P116 |
| <p>Effect the Variation Kascing Fertilizer on Growth Plant of Green Mustard (<i>Brassica juncea</i> L.) <i>Yuyuk Wati, Runtut Prih Utami</i>.....</p> | P117 |

Flow cytometric analysis of MCF-7 cell line in its treatment with leaves extract of *Eugenia uniflora* L

Nita Supriyati¹⁾, Subagus Wahyuono²⁾, Esti Wahyu Widowati³⁾

¹ Medicinal Plant and Traditional Medicine Research and Development Office, Tawangmangu, NIRDH

² Faculty of Pharmacy, Gadjah Mada University Yogyakarta

³ Faculty of Science and Technology, UIN Sunan Kalijaga, Yogyakarta

Abstract

The use of natural product in treatment of cancer has attracted more attention since the existing treatments have not yet given any satisfactory result. Dewandaru (*Eugenia uniflora* L), one of promising medicinal plants in Indonesia, was reported to have suppression activity on DNA polymerase of EBV (Eipstein-Bar Virus). This study aimed to reveal the cytotoxicity of *E. uniflora* L leaves extract and examine more deeply whether or not this activity will trigger the apoptosis process of human breast cancer MCF-7.

The extraction process was conducted using petroleum ether, dichloromethane and methanol. Each extract was subjected for MTT assay for cytotoxicity analysis. The prospective compounds were then separated using vacuum column chromatography and preparative thin layer chromatography. MTT method was used to performed cytotoxicity test of each separated fraction on MCF-7 cells. The selected compound which show the most potential activity was analyze to its IC₅₀ value. MCF-7 cells which treated with this toxic compound was analyze for its cell cycle using flow cytometry assay and propidium iodide staining.

The results showed that the IC₅₀ of the toxic compound tested on MCF-7 cells were 10 µg/mL. Flow cytometry analysis showed that this compound has capability in inducing apoptosis. Cell cycle arrest was observed in MCF-7 cells in which cell accumulation occurred in G1 phase.

Key words: *Eugenia uniflora* L, MCF-7, flow cytometry

INTRODUCTION

Cancer is an abnormal growth of cells caused by multiple changes in gene expression leading to disregulate balance of cell proliferation and cell death and ultimately evolving into a population of cells that can invade tissues and metastasize to distant sites, causing significant morbidity and, if untreated, death of the host (Ruddon, 2007). Cancer replaced heart disease as the leading cause of death among men and women aged younger than 85 years in 1999 in the United States. The 3 most commonly diagnosed types of cancer among women in 2011 are breast, lung and bronchus, and colorectal, accounting for about 53% of estimated cancer cases in women. Breast cancer alone is expected to account for 30% (230,480) of all new cancer cases among women (Siegel, 2011)

The ultimate goal of any cancer drug discovery process is discovering and developing effective and non-toxic therapies (Collota, 2008). In this case, cancer treatment using natural products has attracted more attention as the existing treatments did not provide satisfactory results. One of the potential plants is Dewandaru (*Eugenia uniflora* L), also known as the Surinam Cherry

E. uniflora leaves extracts have been showed pronounced anti-inflammatory action (Schapoval, *et al.*, 1994), considerable contractile activity, with a resulting effect on intestinal transit (Gbolade, *et al.*, 1996), endothelium-dependent vasorelaxant effects (Wazlawik *et al.*, 1997) and hypotensive effects (Consolini, *et al.*, 1999; Consolini & Sarubbio, 2002), and inhibit the increase of plasma glucose and triglyceride levels (Arai *et al.*, 1999). Some compounds present in *E. uniflora* leaves extracts have also been shown to inhibit the Epstein–Barr virus, known to be closely associated with nasopharyngeal carcinoma (Lee, *et al.*, 2000), and have antimicrobial (Adebajo, *et al.*, 1989; Holetz *et al.*, 2002) and antifungal activity (Lima, *et al.*, 1993; Souza *et al.*, 2002). However, research on its anticancer activity has not been reported. Therefore, the objective of this research is to study the potential application of *E. uniflora* as anticancer agent, isolate the toxic compound and study its impact on cytotoxicity and apoptosis on MCF-7 breast cancer cells.

MATERIALS AND METHODS

Plant and extracts

Fresh leaves of *E. uniflora* were collected from Tawangmangu, Indonesia and was identified at Medicinal Plant and Traditional Medicine Research and Development Office, Tawangmangu. Their powder was macerated three times using petroleum ether for 24 hours. After filtration the resulting extracts were combined and evaporated to dryness. The residue from petroleum ether was macerated three times using dichloromethane for 24 hours. After filtration the resulting extracts were combined and evaporated to dryness. The residue from dichloromethane was macerated using methanol for 24 hours. Supernatant was evaporated to dryness. Each extracts was tested for cytotoxicity test on MCF-7 cell line.

Fractionation

The toxic extract was partitioned using petroleum ether, petroleum ether/chloroform {(9 :1, 8 : 2, 7 : 3, 6 : 4, 5 : 5, 4 : 6, 3 : 7, 2 : 8, 1 : 9) v/v}, chloroform, chloroform/methanol {(9 :1, 8 : 2, 7 : 3, 6 : 4, 5 : 5, 4 : 6, 3 : 7, 2 : 8, 1 : 9) v/v}, and methanol. The chemical composition of each fraction was monitored on thin layer chromatography. The sub fractions which show similar spots on TLC analysis, were then combined. Each fraction was subjected for cytotoxicity test on MCF-7 cell line.

The toxic fraction were partitioned with petroleum ether, petroleum ether/chloroform {(9 :1, 8 : 2, 7 : 3, 6 : 4, 5 : 5, 4 : 6, 3 : 7, 2 : 8, 1 : 9) v/v}, chloroform, and methanol. The fractions that showed similarity on TLC were combined. Each fraction was subjected for its cytotoxicity on MCF-7 cell line.

Cytotoxicity assay using MTT method

Cell Culture

Human breast cancer cell lines MCF-7 were cultured in Dulbecco's modified Eagle's medium (DMEM, Gibco) supplemented with 10% heat-inactivated fetal bovine serum (FBS) (Gibco) in an incubator with humidified air with 5% CO₂ at 37°C.

Cell viability assay

The viability of the cells was assessed by MTT (3, 4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (Sigma) assay which is based on the reduction of MTT by the mitochondrial dehydrogenase of intact cells to a purple formazan product. Cells were plated onto 96-well plates (2x10³ cells/well) (Iwaki). After 24 h incubation, cells were treated with each extract/fraction with various concentrations for 48 h. Then, MTT solution was added in each well and cells were incubated for 4 h at 37°C and then incubated with 100 µl of soluble

solution at 37°C overnight. The quantity of formazan product was measured by using a spectrophotometric microtiter plate reader (Bio-Rad) at 595 nm wavelength.

Flow cytometry analysis

Apoptosis detection and analysis of cell cycle distribution were performed by flow cytometry. Briefly, cells were incubated for 24 h in a medium without FBS to synchronize the cell cycle. Cells were then treated for 24 and 48 h in the medium containing 10% FBS with isolated compounds solution. Cells were harvested by trypsinization, washed twice with PBS, incubated with 0.125% Triton X-100, and stained with propidium iodide (PI) in PBS containing 0.2 mg/mL RNase A. Stained cells were analyzed using a FACS calibur. For each sample, cells were counted until the count reached 5×10^5 cells. The percentages of cells in the subG1, G1, S, and G2/M phases were determined using the CELLQUEST software.

RESULTS AND DISCUSSION

Extraction and Cytotoxicity Assay

Maceration of powdered leaf of Dewandaru was conducted using petroleum ether, dichloromethane and methanol. The four extracts was then tested on T47D breast cancer cells using concentration series as follows: 500, 250, 125, 62,5 dan 31,25 µg/mL. Each treatment was observed after 48 hours incubation. Figure 1 showed a curved of cell viability versus various concentration extract which is used on the treatment. The results indicate that dichloromethane extracts has the smallest IC₅₀ value of 115 µg/ml, whereas the IC₅₀ for petroleum ether extract and methanol extract was 160 µg/ml and 150 µg/mL respectively.

There were no significant differences among IC₅₀ values of petroleum ether extract, dichloromethane extract and methanol extract. This is due to distribution of toxic compounds in each extract. Extraction process by maceration technique enables the toxic compounds can't extracted perfectly. Dichloromethane extract was then chosen for fractionation because of its smallest IC₅₀ value.

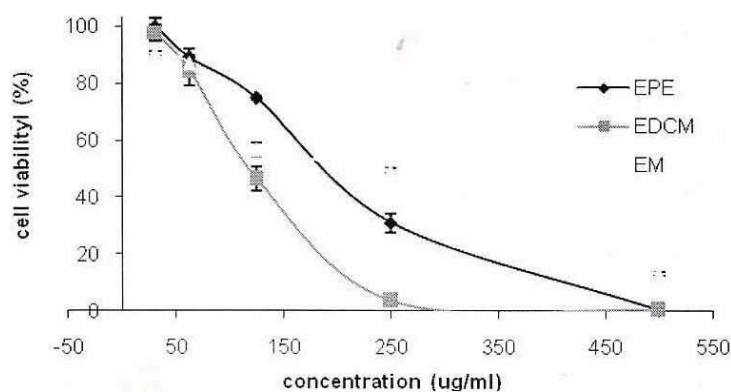


Figure 1. MCF-7 cell viability caused by treatment of petroleum ether extract (EPE), dichloromethane extract (EDCM) and methanol extract (EM) leaves of *E. uniflora* with MTT method

The portion of dichloromethane extract that showed the strongest cytotoxicity activity was partitioned by vacuum liquid chromatography (VLC). The fraction that showed similarity on TLC were combined to give four fractions. Each fractions were tested for cytotoxicity test. Two fraction had cytotoxicity activity (fraction II and III) and the other fraction not toxic.

The most toxic fraction (fraction II) were further were partitioned by VLC using gradient elution. The chemical composition of each fraction was monitored on thin layer chromatography. The fractions that showed similarity on TLC were combined and evaporated given two fraction. Cytotoxicity activity of F2V2 fraction against MCF-7 cells stronger than F2V1 fraction.

F2V2 fraction were further separated by preparatif thin layer chromatography. The TLC spot were scraped into two portion, upper and lower part and diluted in mixture chloroform/methanol 4:1. After filtration each fraction were evaporated and tested for cytotoxic activity. It was found that the lower part of PTLC had cytotoxic activity. The toxic compounds were purified by preparatif TLC given two portion upper and lower part. After 48 treatment of each portion, it was found that the upper part of preparative TLC had cytotoxic activity. Toxic compounds activity of *E. uniflora* against MCF-7 cells showed a linear correlation between the concentration of the test with cell viability as shown in Figure 2.

Cytotoxic activity of toxic compounds *E. uniflora* against MCF-7 cells showed a linear correlation between the concentration of the test with cell viability as shown in Figure 2.

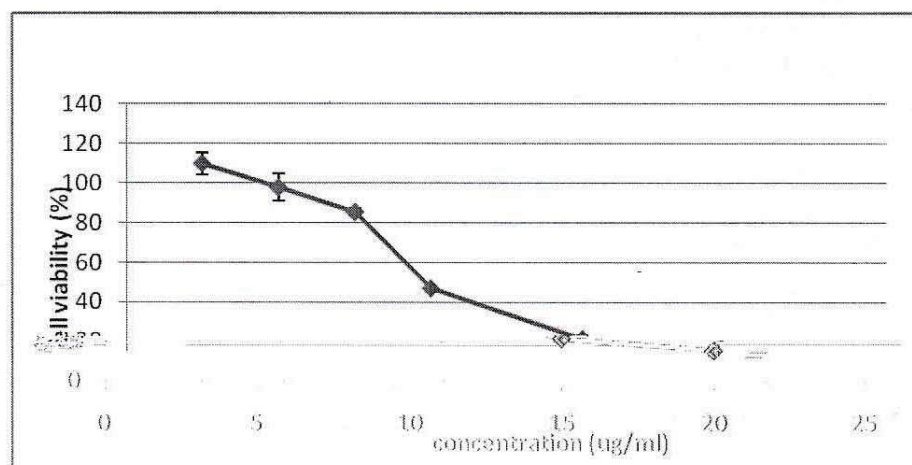


Figure 2. Treatment effects of toxic compounds extracted from *E. uniflora* against MCF-7 cell lines. The cell viability was determined by MTT method.

The calculation showed that IC_{50} of toxic compounds extracted from *E. uniflora* tested on MCF-7 cells is $10 \mu\text{g/mL}$. There were morphological changes in MCF-7 cells due to the treatment of doxorubicin and toxic compounds extracted from *E. uniflora*. The cells look Tues looked round, flat and floats (Figure 3). Sel tampak berbentuk bulat, pipih dan mengapung (gambar 3).

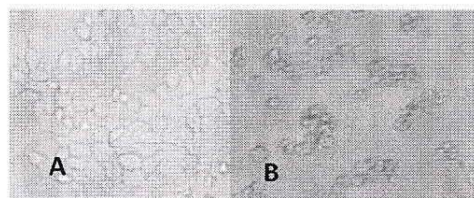


Figure 3. MCF-7 cell morphology (A) without treatment (B) treatment with toxic compounds isolated from *E. uniflora* $15 \mu\text{g/mL}$

Flow cytometry analysis

We analyzed cell cycle distributions using flow cytometry to investigate the effects of toxic compounds extracted from *Eugenia uniflora* L. The distribution of MCF-7 cells in each phase after treatment can be observed in tables and figures below.

Table. 1. The distribution of MCF-7 cells in each phase of cell cycle after treatment using extract of *E. uniflora*. The cell cycle distribution was observed using flow cytometry method

| Treatment | Concentration ($\mu\text{g/ml}$) | Incubation (hour) | Persentase jumlah sel (%) | | | | |
|----------------|------------------------------------|-------------------|---------------------------|-------|-------|-------|------------|
| | | | Sub G1 | G1 | S | G2/M | Polyploidi |
| Control | | 48 | 1.18 | 45,96 | 23.41 | 26,91 | 3,18 |
| | 8 | 24 | 0,98 | 76,05 | 12,08 | 8,33 | 2,90 |
| Toxic compound | 8 | 48 | 16,25 | 59,40 | 8,06 | 15,04 | 1,58 |
| | 6,5 | 24 | 1,00 | 53,63 | 21,86 | 21,42 | 2,60 |
| | 6,5 | 48 | 10,16 | 69,47 | 10,95 | 14,82 | 2,62 |

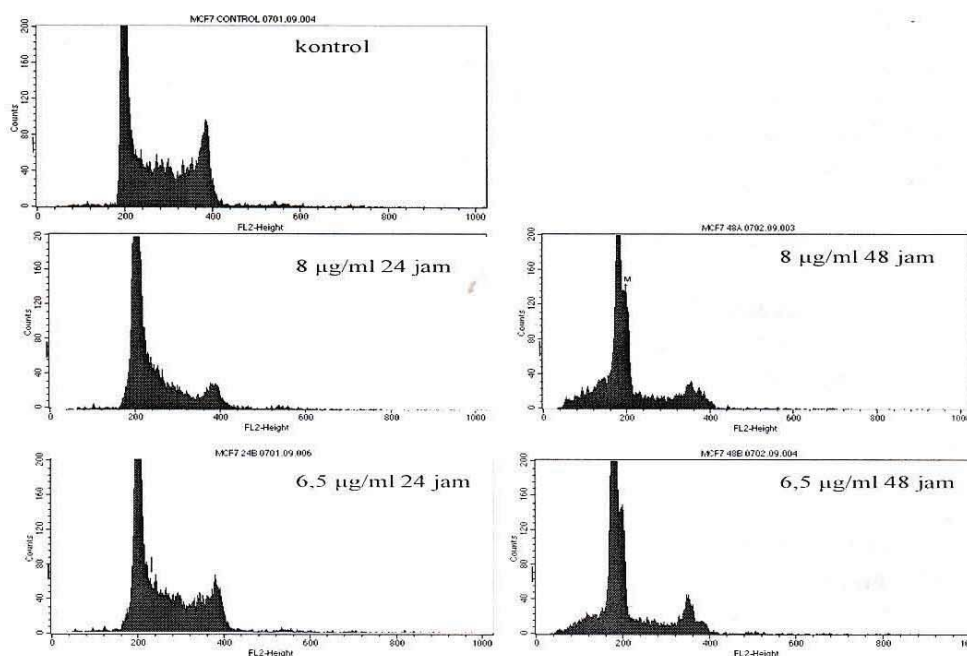


Figure 4. Flow cytometry of MCF-7 cell cancer. Cell were treated with 6.5 and 8 $\mu\text{g/ml}$ of toxic compounds extracted from *E. uniflora*. Incubation process was conducted for 24 and 48 hours.

MCF-7 cell lines were treated with concentrations of toxic compounds of 8 $\mu\text{g/ml}$ and 6.5 $\mu\text{g/ml}$. Incubation process was conducted for 24 and 48 hours. Both concentration were shown to induce the apoptosis process after 48-hour incubation. It was indicated by the increase of the percentage of cells in sub G1 phase. Inhibition of cell cycle which occurs in

G1 phase can reduce the percentage of cell population in S phase. As a result, the cells that enter the phase of G2 / M also reduced. According to the data above, it can be inferred that toxic compounds from *E. uniflora* arrested cell cycle at G1 phase and lead to apoptosis.

In cell cycle analysis using flow cytometry, it can be known that toxic compounds of *E. uniflora* lead to apoptosis. To support these data, the cell was also observed for apoptosis using double staining method. Here we used fluorescent compounds that can bind to DNA / RNA, ethidium bromide-acridine orange (AO-EB), which was subjected against MCF-7 cells. Apoptotic morphology of MCF-7 cells on double staining test results can be examined in Figure 5.

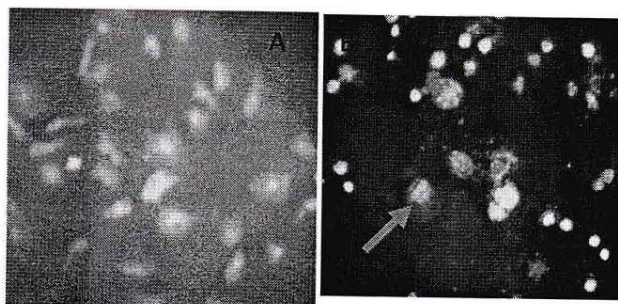


Figure 5. Apoptosis Induction of toxic compounds in the MCF-7 cells with double staining method (ethidium bromide-acridine orange). (A) MCF-7 cells without treatment, (B) MCF-7 cells in treatment with toxic compounds of 9 ug / mL.

The results showed that all apoptosis in control cells exhibit a green fluorescent. It means that there was no cell death. Treatment of MCF-7 using toxic compounds causing some cells be fluorescent which show the loss of cell membrane permeability and indicate the occurrence of apoptosis. Further observations indicate the presence of an enlarged cell nuclei size and the occurrence of multi nucleus. Some cells begin to divide the cell nucleus and form the apoptotic bodies. On one hand, acridine orange can penetrate the membrane of normal cells, binds to the DNA / RNA and cause a bright green fluorescence. On the other hand, ethidium bromide is more easily to penetrate into the decreased permeability of cell membranes and cause orange fluorescence. This method showed the presence of live and dead cells, and cells which undergo apoptosis. One of the characteristics of apoptotic cells is the fragmentation of the cell nucleus that followed by the fragmentation of cells into apoptotic bodies.

CONCLUSIONS

The IC₅₀ values of toxic compounds from *E. uniflora* L tested on MCF-7 cells were 10 µg/ml. Flow cytometry analysis showed that the toxic compounds are capable of inducing apoptosis. Cell cycle arrest was observed in MCF-7 cells in which cell accumulation occurred in G1 phase.

REFERENCES

- Adebajo, A.C., Oloke, K.J., Aladesanmi, A.J., 1989. Antimicrobial activities and microbial transformation of volatile oils of *Eugenia uniflora*. *Fitoterapia* 60, 451–455.
- Arai, I., Amagaya, S., Komatsu, Y., Okada, M., Hayashi, T., Kasai, M., 1999. Improving effects of the extract from *Eugenia uniflora* on hyperglycemia and hypertriglyceridemia in mice. *J. of Ethnopharmacology*, 68, 307–314.

- Colotta, F., 2008, Anticancer Drug Discovery and Development in Colotta, F., Mantovani, A., Targeted Therapies in Cancer : Myth or Reality?. Springer, New York
- Consolini, A.E., Baldini, O.A.N., Amat, A.G. 1999. Pharmacological basis for the empirical use of *Eugenia uniflora* L. (Myrtaceae) as antihypertensive. *Journal of Ethnopharmacology* 66, 33-39.
- Consolini, A. E., & Sarubbio, M. G., 2002, Pharmacological effects of *Eugenia uniflora* (Myrtaceae) aqueous crude extract on rat's heart. *Journal of Ethnopharmacology*, 81, 57-63.
- Gbolade, A. A., Ilesanmi, O. R., & Aladesnmi, A. J.,1996, The Contractile Effects of the Extracts of *Eugenia uniflora* on Isolated Rat Duodenum. *Phytotherapy Research*, 10, 613-615.
- Holetz, F. B., Pessini, G. L., Sanches, N. R., Cortez, D. A. G., Nakamura, C. V., & Filho, B. P. D., 2002, Screening of some plants used in the Brazilian folk medicine for treatment of infectious diseases. *Memorias do Instituto Oswaldo Cruz*, 97, 1027-1031.
- Lee, M., Chiou, J., Yen, K., and Yang, L., 2000, "EBV DNA polymerase Inhibition of tannins from *Eugenia uniflora*". *Cancer Letters*, Volume 154, Number 2, 131-136.
- Lima, E.O., Gompertz, O.F., Giesbrecht, A.M., Paulo, M.Q., 1993. In vitro antifungal activity of essential oils obtained from officinal plants against dermatophytes. *Mycoses* 36, 333-336.
- Ruddon, R.W., 2007, Cancer biology. 4th Ed. Oxford University press. Inc. New York.
- Schapoval, E.E.S., Silveira, S.M., Miranda, M.L., Alice, C.B., Henriques, A.T., 1994. Evaluation of some pharmacological activities of *Eugenia uniflora* L. *J. Ethnopharmacol.* 44, 137-142.
- Siegel, R., Ward, E., Brawley, O., Jemal, A, 2011. Cancer Statistics, 2011, The Impact of Eliminating Socioeconomic and Racial Disparities on Premature Cancer Deaths. *CA Cancer J Clin*, 61, 212-236.
- Souza, L. K. H., Oliveira, C. M. A., Ferri, P. H., Santos, S. C., Junior, J. G. O., Miranda, A. T. B., et al., 2002, Antifungal properties of Brazilian cerrado plants. *Brazilian Journal of Microbiology*, 33, 247-249.
- Wazlawik, E., Da Silva, M.A., Peters, R.R., et al., 1997. Analysis of the role of nitric oxide in the relaxant effect of the crude extract and fractions from *Eugenia uniflora* in the rat thoracic aorta. *J. Pharm. Pharmacol.* 49, 433-437.



the International Conference
on Bioscience and
Biotechnology 2011

Organized by



Biology Department
Faculty of Science and Technology
State Islamic University Sunan Kalijaga
Yogyakarta - Indonesia

Supported by



Certificate of Appreciation

Awarded with thanks to:

Esti Wahyu Widowati

In recognition of his/her significant contribution as:

Poster Presenter

of

*The International Conference
on Bioscience and Biotechnology 2011*

Yogyakarta, October 11th-12th, 2011



Prof. Dr. Akh. Minhaji, M.C., Ph.D.
Dean of Faculty of Science and Technology
State Islamic University Sunan Kalijaga



Dr. M. Jafar Luthfi

Chairperson of the Organizing Committee