SOLID PHASE EXTRACTION AND CHARACTERIZATION OF RUTIN FROM *LABISIA PUMILA* VAR. *ALATA*

NUR NABIHAH BINTI RUZLAN

A thesis submitted in fulfillment of the
requirements for the award of the degree of
Master of Engineering (Bioprocess)

Faculty of Chemical Engineering
Universiti Teknologi Malaysia

MAY 2015

Dedicated to beloved Abah, Ummi, Nenek, Afiq, Arif, Amiruddin, Anis, Adnim, Aminul Hakim and Akmal Salihin.

ACKNOWLEDGEMENTS

In the name of Allah, the Most Gracious and the Most Merciful. All praises to Allah for the strengths and His blessing in completing this thesis.

I take this opportunity to express my profound gratitude and deep regards to my supervisor, Dr. Chua Lee Suan and my co-supervisor, Prof. Dr. Mohd. Roji Sarmidi for their exemplary guidance, monitoring and constant encouragement throughout the course of this thesis. The blessing, help and guidance given by them time to time have contributed to the success of this study.

I also would like to express my deep sense of gratitude to all of the office staffs and technicians of Institute of Bioproduct Development (IBD) for their pleasant support, good cooperation and valuable information and guidance during the period of my laboratory works. A special thanks goes to all my friends; especially my best friends and colleagues, who give me constant encouragement during my study.

Last but not least, my deepest gratitude goes to my beloved parents; Mr. Ruzlan Bin Mat Jusoh and Mrs. Sakinah Binti Hassan, my grandparents; Mr.Hassan and Mrs. Eshah and also to my siblings for their endless love, prayers and encouragement. To those who indirectly contributed in this research, your kindness means a lot to me. Thank you very much.

ABSTRACT

Rutin is one of the glycosylated flavonoids which is widely investigated by researchers from buckwheat samples due to its biological activities, including antioxidant activity. Interestingly, rutin was also found in *L. pumila* var. *Alata* extract, particularly from the leaves of the plants (0.85 mg of rutin/ g of leaves). This study focused on the optimization of methanolic solvent system for rutin elution from the crude extract of *Labisia pumila* var. *Alata* using C_{18} reversed phase solid phase extraction (SPE). The rutin fraction was identified and characterised by using thin layer chromatography (R_f 0.49) and UV-Vis spectrophotometry (285 nm). The presence of rutin was further confirmed and quantified by using a liquid chromatography integrated with tandem mass spectrometer based on rutin ion transition (m/z 609 > 301) in the negative mode of targeted analysis. Methanolic extraction (60% methanol) was found to increase the rutin content (0.14% w/w) in the crude extract. Fractionation of the crude extract using 80% methanol as the solvent system had further increased the rutin content to 1.31% w/w. The fractionation was carried out in a laboratory scale SPE system using C_{18} packed column with the minimum volume of solvent usage (20 mL) with high yield of recovery (76.88%). The rutin fraction (IC_{50} 800 ppm) also exhibited 9.5 times lower scavenging activity than ascorbic acid (IC_{50} 84 ppm) and 6.6 times lower scavenging activity than standard rutin (IC_{50} 122 ppm). However, it showed 1.9 times higher scavenging activity than crude extract (IC_{50} 1500 ppm). A three-layer artificial neural network (ANN) model was developed to predict the yield of rutin based on the polarity of solvent system and volume of eluent. This ANN model produced a good prediction of the experimental data with a correlation coefficient of (R^2) 0.9998 and 1.0 for training and testing data, respectively. As a conclusion, a laboratory scale SPE system for rutin elution from the crude extract of *L. pumila* var. *Alata* has been established in the current study.

ABSTRAK

Rutin ialah salah satu flavonoid terglikosilat yang banyak dijumpai di dalam gandum hitam dan dikaji secara meluas oleh penyelidik-penyelidik kerana ia mempunyai aktiviti biologi yang signifikan, termasuk aktiviti antioksidan. Menariknya, rutin juga boleh dijumpai di dalam ekstrak *L. pumila* var. *Alata*, khususnya daripada daun pokok (0.85 mg rutin/ g daun). Kajian ini dijalankan untuk menentukan sistem pelarut metanol yang optima bagi rutin daripada ekstrak mentah *Labisia pumila* var. *Alata* menggunakan C_{18} fasa berbalik pengekstrakan fasa pepejal (SPE). Pecahan rutin telah dikenalpasti dan disifatkan ciri-cirinya dengan menggunakan kromatografi lapisan nipis (R_f 0.49) dan UV-Vis spektrofotometri (285 nm). Kehadiran rutin seterusnya disahkan dan ditentukan kuantitinya menggunakan kromatografi cecair dengan spektrometer jisim berdasarkan peralihan ion target (m/z 609 > 301) di dalam mod negatif. Pengekstrakan menggunakan metanol (60% metanol) telah meningkatkan kandungan rutin (0.14% w/w) di dalam ekstrak mentah. Pemeringkatan ekstrak mentah menggunakan 80% metanol sebagai sistem pelarut telah meningkatkan lagi kandungan rutin kepada 1.31% w/w. Pemeringkatan telah dijalankan menggunakan sistem SPE skala makmal (1000 mg) dengan C_{18} kolum padat dengan penggunaan isipadu yang minima (20 mL) dan hasil yang tinggi (76.88%). Pecahan rutin (IC_{50} 800 ppm) menunjukkan 9.5 kali aktiviti memerangkap lebih rendah berbanding asid askorbik (IC_{50} 84 ppm) dan 6.6 kali aktiviti memerangkap lebih rendah berbanding standard rutin (IC_{50} 122 ppm). Walau bagaimanapun ia menghasilkan 1.9 kali lebih tinggi aktiviti memerangkap berbanding ekstrak mentah (IC_{50} 1500 ppm). Model rangkaian neural network (ANN) tiga-lapisan telah dibangunkan untuk meramalkan hasil rutin berdasarkan polariti sistem pelarut dan isipadu bahan pengelusi. Model ANN ini menunjukkan ramalan data eksperimen yang tepat dengan pekali korelasi sebanyak 0.9998 and 1.0 untuk data latihan dan ujikaji. Kesimpulannya, sistem SPE pada skala makmal untuk rutin daripada ekstrak mentah *L. pumila* var. *Alata* telah dibangunkan dalam kajian ini.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ABTS	-	2, 2'-azinobis (3-ethylbenzothiazoline-6-sulfonate)
ANN	-	Artificial Neural Network
CAA	-	Cellular Antioxidant Activity
DPPH	-	1, 1-Diphenyl-2-Picrylhydrazyl
DMPD	-	N, N-Dimethyl-p-Phenylenediamine
DNA	-	Deoxyribonucleic Acid
DW	-	Dry Weight
ECL	-	Enhanced Chemiluminescence
ESI	-	Electrospray Ionization
ET	-	Electron Transfer
FRAP	-	Ferric Ion Reducing Antioxidant Parameter
GAE	-	Gallic Acid Equivalent
GC	-	Gas Chromatography
HAT	-	Hydrogen Atom Transfer Reactions
HPLC	-	High Performance Liquid Chromatography
HPTLC	-	High Performance Thin Layer Chromatography
IOU	-	Inhibited Oxygen Uptake
IR	-	Infrared
LC-MS/MS	-	Liquid Chromatography Coupled With Tandem Mass Spectrometry
LDL	-	Low-Density Lipoprotein
LLE	-	Liquid-Liquid Extraction
LPIC	-	Lipid Peroxidation Inhibition Capacity
MARDI	-	Institute Penyelidikan Dan Kemajuan Pertanian Malaysia
MLP	-	Multi-Layer Perception

MS	-	Mass Spectrometry
MSE	-	Mean Squared Error
N	-	Number of Points
NADPH	-	Nicotinamide Adenine Dinucleotidephosphate
NN	-	Neural Network
ORAC	-	Oxygen Radical Absorption Capacity
p-NDA	-	p-Butrisidunethyl Aniline
R	-	Alkyl Group
RE	-	Rutin Equivalent
RNA	-	Ribonucleic Acid
ROS	-	Reactive Oxygen Species
RSM	-	Response Surface Methodology
SASA	-	Scavenging Of Super Oxide Radical Formation By Alkaline
SD	-	Standard Deviation
SLE	-	Solid-Liquid Extraction
SPE	-	Solid Phase Extraction
TEAC	-	Trolox Equivalent Antioxidant Capacity
TLC	-	Thin Layer Chromatography
TOSC	-	Total Oxidant Scavenging Capacity
TRAP	-	Total Radical Trapping Antioxidant Parameter
UPLC	-	Ultra Performance Liquid Chromatography
USA	-	United State of America
UV	-	Ultraviolet
UV-B	-	Ultraviolet B
UV-Vis	-	Ultraviolet-Visible
V	-	Volume
$AlCl_3$	-	Aluminium Chloride
C_0	-	Initial Concentration
C_e	-	Equilibrium Concentration
Fl	-	Flavonoids
H_2O_2	-	Hydrogen Peroxide
MeOH	-	Methanol
q_e	-	Adsorption Capacity

R_f	-	Retention Factor
Y_t	-	Target Output
Y_N	-	Network Output
Y_m	-	Average of Actual Values
•OH	-	Hydroxyl Radical

LIST OF SYMBOLS

A	-	Absorbance
%	-	Percentage
°C	-	Degree of Celsius
µg	-	Microgram
µl	-	Microliter
µm	-	Micrometer
cm	-	Centimeter
Fe	-	Iron
g	-	Gram
g/L	-	Gram per liter
g/mol	-	Gram per mol
h	-	Hour
kg	-	Kilogram
m	-	Meter
mg	-	Milligram
mg/cm^3	-	Milligram per cubic centimeter
mg/g	-	Milligram per gram
mg/L	-	Milligram per liter
MΩ-cm	-	Mega ohm centimeter
m/z	-	Mass per ratio
min	-	Minutes

ml	-	Milliliter
mm	-	Millimeter
nm	-	Nanometer
ppm	-	Part per million
R^2	-	Coefficient of determination
rpm	-	Revolution per minute

LIST OF APPENDICES

CHAPTER 1

INTRODUCTION

1.1 Research Background

Kacip Fatimah or scientifically known as *Labisia pumila* is a herbaceous plant that commonly found in Malaysia, Indochina and Thailand. *L. pumila* is also sometimes referred as Selusoh Fatimah, Rumput Siti Fatimah, Akar Fatimah, Tadah Matahari, Bunga Belangkas Hutan, Pokok Pinggang (Burkill, 1935; Jamia *et al.*; 2003; Rasadah and Zanon; 2003), Mata Pelanduk rimba and Sangkoh (Abdullah *et al.*, 2013). Kacip Fatimah is the second most popular herb in Malaysia as it is known as the queen of plants for women health. This herb is mainly categorized into three types of varieties such as *L. pumila* var. *Alata, L. pumila* var. *Pumila* and *L. pumila* var. *Lanceolata* (Ibrahim *et al*., 2011). Each variety has different characteristics and pharmacological activities.

Herbal plants were reported containing abundant of bioactive compounds from the group of saponins, steroids and flavonoids (Norhaiza *et al.*, 2009). This herb was approved among scientists, herbalists and the pharmacy industry in Malaysia as it gives therapeutical effects and high total contents of phenolic and flavonoids compounds (Jaafar *et al.*, 2012). *Labisia pumila* (*Myrsinaceae*) is a popular herb among Malaysian to contain bioactive compounds such as alkenyl resorcinol, flavonoids and benzoquinones (Jaafar *et al.*, 2008). Recently, researchers

also have verified that Kacip Fatimah has high estrogenic activity and high amount of phenolics (Jamia *et al.*, 2004).

Rutin is one of the polyphenolic compounds found in *L. pumila* var. *Alata*, which is also biologically active compound. Even though rutin is not classified as vitamin, it is recognized as vitamin P (Guo *et al.*, 2007). Rutin exhibits anti-inflammatory, anti-hepatotoxic, antiulcer, anti-allergic and antiviral activities, as well as protection against cardiovascular mortality (Smith *et al.*, 1980; Hertog *et al.*, 1993; Sajeeth *et al.*, 2010). Apart from that, rutin are believed to play an important role in strengthening blood vessels. Moreover, rutin is also used to prevent side effects of cancer treatment called mucositis. Mucositis is a painful condition marked by swelling and ulcer formation in the mouth or lining of the digestive tract (Odetti *et al.*, 2009).

Nowadays, the interest to obtain biologically active compound from natural product is in the increasing trend. It is believed that phytochemicals from plant materials have lesser side effect compared to the chemically synthesized drug. In the present study, rutin was extracted and fractionated from *L. pumila* var. *Alata*. The method of solid phase extraction (SPE) was implemented. The solvent system for rutin elution was optimized based on the polarity between stationary phase of chromatographic column and solvent system. For better understanding the mechanism of rutin elution, a mathematical model based on the polarity of solvent system was used.

Since rutin has unique characteristic in anti-oxidation, DPPH antioxidant assay was carried out to monitor the elution profile of *L. pumila* var. *Alata* fractions from SPE column. As reported earlier by Ibrahim and Jaafar (2011), the antioxidant activity determined by the 1,1- diphenyl-2-picrylhydrazyl (DPPH) assay, as well as the total amount of phenolics and flavonoids were the highest in the leaves of all of *L. pumila* varieties. Hence, DPPH was used to determine the antioxidant quality of extracts and rutin fraction.

1.2 Problem Statement

Rutin is one of the flavonoids highly applied in pharmaceutical and food industries due to its biological and pharmacological properties, particularly the antioxidant activity (Atanassova and Bagdassarian, 2009). The extraction method has extensively been studied by researchers on buckwheat herb as it has considerable amounts of rutin (Kreft *et al.*, 2003; Hinnerburg and Neubert, 2005). However, no specific extraction method for rutin from *L. pumila* var. *Alata* is studied in detail. Hence, this study was conducted to investigate and to characterize rutin content in the leaves of *L. pumila* var. *Alata*. In the previous study, methanolic solvent was reported to be effective for rutin extraction (Paniwnyk *et al.*, 2001). This study is important for large scale rutin extraction since it has significant pharmacological activity.

It was found that mostly phytochemical extraction was based on liquid – liquid extraction using different types of solvents. The choice of solvent for extraction is highly dependent on the chemistry of target compound. In the present study, solid – liquid phase extraction was employed because it is considered as safer, cost effective and environmental friendly method (McDonald, 1995). Many problems associated with liquid-liquid extraction such as incomplete phase separations, less quantitative recovery, more expensive and the disposal of large quantity of organic solvents occur from that extraction have driven the researchers to use solid-liquid extraction (Lucci *et al.*, 2012).

In addition, rutin also has been reported to exhibit anti-oxidation activity to protect human dermal fibroblast from cell damage triggered by ultraviolet irradiation (Choi *et al.*, 2010). Therefore, the antioxidant properties of rutin were also determined by measuring free radical scavenging activity (1,1-diphenyl-2-picrylhydrazyl, DPPH). Previously, anti-oxidation studies were focused on the crude extract of *L. pumila* var. *Alata*. To our knowledge, no antioxidant assay is performed on the plant fraction enriched with rutin. Besides that, rutin was characterized and analysed based on thin layer chromatography (TLC), UV-Vis spectrophotometer and liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) profile.

1.3 Significance of Study

It is expected that the optimization study of rutin fraction from *L. pumila* var. *Alata* crude extract by SPE will affirm good extraction efficiency. SPE method is an increasingly useful sample preparation technique as it is easy to perform, rapid, reliable and can be automated (Bodennec *et al.*, 2000). Moreover, the solvent used and laboratory time also can be reduced. Besides, SPE column is excellent for sample extraction, concentration and clean up.

As the general public becomes more health-conscious, they have also become more aware of the significance of good nutrition. Rutin from natural source is of great interested due to the highly demand in the pharmaceutical industries, as well as food industries. People believe that rutin from herbal and medicinal plants can give beneficial effects to the health due to lesser side effects and its promising health values.

1, 1-diphenyl-2-picrylhydrazyl (DPPH) assay was used to analyze antioxidant activity of rutin fraction. DPPH is preferable due to its cost efficiency method to analyse antioxidant activity. This assay also contributes to good reproducibility of results (Prior *et al.*, 2005).

A mathematical model was used to describe the effect of solvent polarity on the rutin elution. This mathematical model is also very important because it can facilitate the purification process of rutin from the crude extract. This model can be used to predict the yield percent of rutin fraction in the subsequent study.

1.4 Objective

The objective of this research was to establish the optimum polarity of solvent system in solid phase extraction for rutin fraction from the crude extract of *L. pumila* var. *Alata*.

1.5 Scope

The four major scopes in this study include:

i. To determine the optimum solvent polarity and volume of eluent in solid phase extraction for rutin fraction based on solvent selection from *L. pumila* var. *Alata* crude extract.
ii. To characterize and quantify rutin fraction based on Thin Layer Chromatography (TLC), UV-Vis spectrophotometer and Liquid Chromatography Coupled with Tandem Mass Spectrometry (LC-MS/MS) data.
iii. To investigate the antioxidant activity of rutin fraction using DPPH assay.
iv. To evaluate extraction efficiency of rutin elution based on the polarity of solvent system using Artificial Neural Network (ANN).

REFERENCES

Abdullah, N., Chermahini, S. H., Suan, C. L. and Sarmidi, M. R. (2013). *Labisia pumila*: a review on its traditional, phytochemical and biological uses. *World Applied Sciences Journal*. 27 (10), 1297-306.

Afanas'ev, I. B., Dorozhko, I., Brodskii, A. V., Korstyuk, V. A. and Potapovitch, A. (1989). Chelating and free radical scavenging mechanisms of inhibitory action of rutin and quercetin in lipid peroxidation. *Biochemical Pharmacology*. 38(11):1763-9

Agrawal, M., Agrawal, Y., Itankar, P., Patil, A., Vyas, J. and Kelkar, A. (2012). Phytochemical and HPTLC studies of various extracts of *Annona squamosal* (Annonaceae). *International Journal of PharmTech Research*. 4, 364-8.

Ali, Z. and Khan, I. A. (2011). Alkyl phenols and saponins from the roots of *Labisia pumila* (Kacip Fatimah). *Phytochemistry*. 72, 2075-80.

Altiok, E., Baycin, D., Bayraktar, O. and Ulku, S. (2008). Isolation of polyphenols from the extracts of olive leaves (Olea europaea L.) by adsorption on silk fibroin. *Separation and Purification Technology*. 62 (2), 342-8.

Al Duri, B. (1996). Adsorption modelling and mass transfer. In McKay, G. (Ed.), Use of adsorbents for removal of pollutants from wastewaters. *CRC Press, New York.* 133-73.

Amin, A. S., Gouda, A. A. and Youssef, E. H. (2014). Utility of N- bromosuccinimide as an environmental-friendly reagent for sensitive spectrophotometric determination of aripiprazole in tablets. *International Journal of Pharmacy and Pharmaceutical Sciences.* 6 (4), 247-53.

Arceusz, A. and Wesolowski, M. (2013). Quality consistency evaluation of Melissa officinalis L. commercial herbs by HPLC fingerprint and quantitation of selected phenolic acids. *Journal of Pharmaceutical and Biomedical Analysis.* 83, 215-20.

Ashok, P. K. and Saini, B. (2013). HPLC analysis and isolation of rutin from stem bark of *Ginkgo biloba* L. *Journal of Pharmacognosy and Phytochemistry.* 2 (4), 68-71.

Atanassova, M. and Bagdassarian, V. (2009). Rutin content in plant products. *Journal of University Chemical Technology and Metallurgy.* 44(2), 201-3.

Atolani, O. and Olatunji, G. A. (2014). Isolation and evaluation of antiglycation potential of polyalthic acid (furano-terpene) from *Daniella oliveri. Journal of Pharmaceutical Analysis.* Article in Press, Corrected Proof.

Avalaskar, A. N., Itankar, P. R., Joshi, V. S., Agrawal, M. and Vyas, J. (2011). Phytochemical and TLC studies of ethanolic extract of *Sesbania grandiflora* (Fabaceae). *International Journal of PharmTech Research.* 3 (3), 1346-9.

Aviram, M. and Fuhrman, B. (1998). Polyphenolic flavonoids inhibit macrophage-mediated oxidation of LDL and attenuate atherogenesis. *Atehrosclerosis 137* (suppl.). S45- S50.

Avula, B., Wang, Y. H., Ali, Z., Smillie, T. J. and Khan, I. A. (2011). Quantitative determination of triterpene saponins and alkynated-phenolics from *Labisia pumila* using an LC-UV/ELSD method and confirmation by LC-ESI-TOF. *Planta Medica.* 77, 1742-8.

Azmir, J., Zaidul, I. S. M., Rahman, M. M., Sharif, K. M., Mohamed, A., Sahena, F., Jahurul, M. H. A., Ghafoor, A., Norulaini, N. A. N. and Omar, A. K. M. (2013). Techniques for extraction of bioactive compounds from plant materials: a review. *Journal of Food Engineering.* 117 (4), 426-36.

Badarinath, A. V., Rao, K. M., Chetty, C. M. S., Ramkanth, S., Rajan, T. V. S. and Gnanaprakash, K. (2010). A review on *in-vitro* antioxidant methods: comparisions, correlations and considerations. *International Journal of Pharmtech Research.* 2(2), 1276-85.

Balaban, A. T., Oniciu, D. C. and Katritzky, A. R. (2004). Aromaticity as a cornerstoneof heterocyclic chemistry. *Chemical Reviews.* 104, 2777-812.

Balasundram, N., Sundram, K. and Samman, S. (2006). Phenolic compounds in plants and agri-industrial by-products: antioxidant activity, occurance, and potential uses. *Food Chemistry.* 99, 191-203.

Bar, N. and Das S. K. (2012). Gas-non-Newtonian liquid flow through horizontal pipe-gas holdup and pressure drop prediction using multilayer perceptron. *American Journal of Fluid Dynamics.* 2 (3), 7-16.

Benitez, J. M., Castro, J. L. and Requena, I. (1997). Are artificial neural networks black boxes? IEEE *Transactions on Neural Networks*. 8, 1156-64.

Benzie, I. F. F. and Strain, J. J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power". The FRAP assay. *Analytical Biochemistry.* 239, 70-6.

Bodennec, J., Koul, O., Aguado, I., Brichon, G., Zwingelstein, G. and Portoukalian, J. (2000). A procedure for fractionation of sphingolipid classes by solid-phase extraction on aminopropyl cartridges. *Journal of Lipid Research.* 41, 1524-31.

Burkill, I. H. (1935). *A dictionary of the economic products of the Malay Peninsula*, Crown Agent, London, UK.

Buszewski, B., Kawka, S., Suprynowicz, Z. and Wolski, T. (1993). Simultaneous isolation of rutin and esculin from plant material and drugs using solid-phase extraction. *Journal of Pharmaceutical and Biomedical Analysis.* 11 (3), 211-5.

Caltagirone, S., Ranelletti, F. O., Rinelli, A., Maggiano, N., Colasante, A., Musiani, P., Aiello, F.B. and Piantelli, M. (1997). Interaction with type II estrogen binding sites and antiproliferative activity of tamoxifen and quercetin in human non-small-cell lung cancer. *American Journal of Respiratory Cell and Molecular Biology.* 17, 51-9.

Campbell, C. G. (1997). Buckwhet *Fagopyrum esculentum* Moench. *Promoting the conservation and use of underutilized and neglected crops. Germany, Rome, Italy: IPK, IPGRI.* 19.

Cavas, L., Karabay, Z., Alyuruk, H., Dogan, H. and Demir, G. K. (2011). Thomas and artificial neural network models for the fixed-bed adsorption of methylene blue by a beach waste *Posidonia oceanica* (L.) dead leaves. *Chemical Engineering Journal.* 171, 557-65.

Chang, W. S., Lee, Y. J., Lu, F. J. and Chiang, H. C. (1993). Inhibitory effects of flavonoids on xanthine oxidase. *Anticancer Research.* 13, 2165-70.

Choi, H., Kim, D., Kim, D. W., Ngadiran, S. and Sarmidi, M. R. (2010). *Labisia pumila* extract protects skin cells from photoaging caused by UVB irradiation. *Journal of Bioscience and Bioengineering*. 109, 291-6.

Choi, S. U., Ryu, S. Y., Yoon, S. K., Jung, N. P., Park, S. H., Kim, K. ., Choi, E. J. and Lee, C. O. (1999). Effects of flavonoids on the growth and cell cycle of cancer cells. *Anticancer Research.* 19 (6B), 5229-33.

Chua, L. S., Abdul Latiff, N., Lee, S. Y., Lee, C. T., Sarmidi, M. R., Abdul Aziz, R. (2011). Flavonoids and phenolic acids from *Labisia pumila* (Kacip Fatimah). *Food Chemistry.* 127, 1186-92.

Chua, L. S., Lee, S. Z., Abdullah, N. and Sarmidi, M. R. (2012). Review on *Labisia pumila* (Kacip Fatimah): bioactive phytochemicals and skin collagen synthesis promoting herb. *Fitoterapia.* 83, 1322-35.

Chua, L. S. (2013). A review on plant-based rutinextraction methods and its pharmacological activities. *Journal of Ethnopharmacology.* 150, 805-17.

Cieśla, L., Cryszeń, J., Stochmal, A., Oleszek, W., Waksmundzka-Hajnos, M. (2012). Approach to develop a standardized TLC-DPPH• test for assessing free radical scavenging properties of selected phenolic compounds. *Journal of Pharmaceutical and Biomedical Analysis.* 70, 126-35.

Conseil, G., Baubichon-Cortay, H., Dayan, G., Jault, J.M., Barron, D. and De Pietro, A. (1998). Flavonoids: a class of modulators with bifunctional interactions at vicinal ATP- and steroids-binding sites on mouse P-glycoprotein. *Proceeding of the National Academy of Sciences.* 95,9831-6.

Darr, D. and Fridovich, I. (1994). Free radicals in cutaneous biology. *Journal of Investigative Dermatology.* 102, 671-5.

de Groot, H. (1994). Reactive oxygen species in tissue injury. *Hepato-Gastroenterology.* 41, 328-32.

de rijke, E., Out, P., Niessen, W. M. A., Ariese, F., Gooijer, C. and Brinkman, U. A. Th. (2006). Analytical separation and detection methods for flavonoids. *Journal of Chromatography A.* 1112, 31-63.

Dehghani, M., Abadi, M., Ashraf, N., Chamsaz, M. and Shemirani, F. (2012). An overview of liquid phase microextraction approaches combined with UV-Vis spectrophometry. *Talanta.* 99, 1-12.

Dudonné, S., Vitrac, X., Coutiére, P., Woillez, M and Mérillon, J. M. (2009). Comparative study on antioxidant properties and total phenolic content of 30 plant extracts of industrial interest using DPPH, ABTS, FRAP, SOD and ORAC assays. *Journal of Agricultural and Food Chemistry.* 57,1768-74.

Ebrahimzadeh, H., Tavassoli, N., Sadeghi, O. and Amini, M.M. (2012). Optimization of solid-phase extraction using artificial neural networks and response surface methodology in combination with experimental design for determination of gold

by atomic absorption spectrometry in industrial wastewater samples. *Talanta.* 97, 211-7.

Edelsbrunner, P. and Schneider, M. (2013). Modelling for prediction vs. modelling for understanding: commentary on Musso *et al.* (2013). *Frontline Learning Research.* 2, 99-101.

Farid, A. E. (2001). Elucidation of the principles. *Trends in Analytical Chemistry.* 20,649.

Farouk, A. E., Nawi, M. N., and Hassan, S. (2008). Antibacterial peptides from *Eurycome longifolia* (Tongkat Ali) and *Labisia pumila* (Kacip Fatimah) leaves in Malaysia. *Scientia Bruneiana.* 9, 55-63.

Fathiazad, F., Delazar, A., Amiri, R., Sarkez, S. D. (2006). Extraction of flavonoids and quantification of rutin from waste tobacco leaves. Iran. *Journal of Pharmacy Research.* 3, 222-7.

Fazliana, M., Gu, H., Ostenson, C., Yusoff, M. and Nazaimoon, W. M. (2012). *Labisia pumila* extract down-regulates hydroxysteroid (11-beta) dehydrogenase 1 expression and corticosterone levels in ovariectomized rats. *Journal of Natural Medicines.* 66, 257-64.

Floegel, A., Kim, D. O., Chung, S. J., Koo, S. I. and Chun, O. K. (2011). Comparison of ABTS/ DPPH assays to measure antioxidant capacity in popular antioxidant-rich US foods. *Journal of Food Composition and Analysis.* 24 (7), 1043-8.

Gaberscik, A., Germ, M., Skof, A. Drmaz, D. and Trost, T. (2002). UV- B radiation screen and respiratory potential in two aquatic primary producers: *Scenedesmus quadricauda and Ceratophyllum demersum. Verhandlungen Internationale Vereinigung Limnologie*. 27, 422-5.

Gage, T., Douglass, C. and Wender, S. (1951). Identification of flavonoid compounds by filter paper chromatography. *Analytical Chemistry.* 23 (11), 1582-5.

Germ, M., Drmaz, D., Sisko, M. and Gaberscik, A. (2002). Effects of UV- B radiation on green alga Scenedesmus quadricauda: growth rate, UV-B absorbing compounds and potential respiration in phosphorus rich and phosphorus poor medium. *Phyton*. 42, 25-37.

Geyikci, F., Kilic, E., Coruh, S. and Elevli, S. (2012). Modelling of lead adsorption from industrial sludge leachate on red mud by using RSM and ANN. *Chemical Engineering Journal.* 183, 53-9.

Ghiasi, M. and Heravi, M. M. (2011). Quantum mechanical study of antioxidative ability and antioxidative mechanism of rutin (vitamin P) in solution. *Carbohydrate Research.* 346, 739-44.

Gocan, S. and Cimpan, G. (2007). Review of the analysis of medicinal plants by TLC: modern approaches. *Journal of liquid chromatography & related technologies.* 27, 7-9.

Grace, P. A. (1994). Ischaemia-reperfusion injury. *British Journal of Surgery.* 81, 637–47.

Guo, R, Wei, P and Liu W. (2007). Combined antioxidant effects of rutin and vitamin C in triton X-100 micelles. *Journal of Pharmaceutical and Biomedicinal Analysis.* 43, 1580-6.

Gupta, N., Sharma, S. K., Rana, J. C. and Chauhan, R. S. (2011). Expression of flavonoid biosynthesis genes vis-à-vis rutin content variation in different growth stages of Fagopyrum species. *Journal of Plant Physiology.* 168 (17), 2117-23.

Hamad, M. N. (2012). Isolation of rutin from *Ruta graveolens* (Rutaceae) cultivated in Iraq by precipitation and fractional solubilization. *Pharmacie Globale International Journal of Comprehensive Pharmacy.* 3(4), 4-6.

Hanasaki, Y., Ogaw,a S., Fukui, S. (1994). The correlation between active oxy- gens scavenging and antioxidative effects of flavonoids. *Free Radical Biology & Medicine.* 16, 845–50.

Handa, S. S., Khanuja S. P. S., Longo, G. and Rakesh, D. D. (2008). Extraction Technologies for Medicinal. *International Centre for Science and High Technology.* Trieste.

Hawariah, A., Pihie, L., Zakaria, Z.A. and Othman, F. (2012). Antiproliferative and proapoptotic effects of *Labisia pumila* ethanol extract and its active fraction in human melanoma HM3KO cells. *Evidenced-based Complementary and Alternative Medicine.* 2012, 1-12.

Haykin, S. (1994). *Neural Networks: A comprehensive foundation.* New York, USA: Macmillan College Co.

Hertog, M. G. L, Hollman, P. C. H., Katan, M. B. and Klohout, M. (1993). Intake of potentially anticarcinegenic flavonoids and their determinants in adults in The Netherlands. Nutrition and Cancer. 20 21-9.

Hinneburg, L. and Neubert, R. H. (2005). Influence of extraction parameters on the phytochemical characteristics of extract from buckwheat (*Fagopyrum esculentum*) herb. *Journal of Agriculture and Food Chemistry.* 53, 3-7.

Huang, D., Ou, B. and Prior, R. L. (2005). The chemistry behind antioxidants capacity assays. *Journal of Agricultural and Food Chemistry.* 53 (6), 1841-56.

Hussain, M. T., Verma, A. R., Vijayakumar, M., Sharma, A., Mathela, C. S. and Rao, C. V. (2009). Rutin, a natural flavonoid, protects against gastric mucosal damage in experimental animals. *Asian Journal of Traditional Medicines.* 4 (5).

Ibrahim, M. H. and Jaafar, H. Z. E. (2011). Photosynthetic capacity, photochemical efficiency and chlorophyll content of three varieties of *Labisia pumila* Benth. Exposed to open field and greenhouse growing conditions. *Acta Physiol Plant.* 33, 2179-85.

Ibrahim, M. H. and Jaafar, H. Z. E. (2011). The relationship of Nitrogen and C/N ratio with secondary metabolites levels and antioxidant activities in three varities of Malaysian Kacip Fatimah (Labisia pumila Blume). *Molecules.* 16 (7), 5514-26.

Ibrahim, M. H. and Jaafar, H. Z. E. (2012). Reduced photoinhibition under low irradiance enhanced Kacip Fatimah (*Labisia pumila* Benth) secondary metabolites, Phenyl Alanine Lyase and antioxidant activity. *International Journal of Molecular Sciences.* 13, 5290-306.

Iio, M., Ono, Y., Kai, S. and Fukumoto, M. (1986). Effects of flavonoids on xanthine oxidation as well as cytochrome c reduction by milk xanthine oxidase. *Journal of Nutritional Science and Vitaminology.* 32, 635-42.

Jaafar, H. Z. E., Ibrahim, M. H. and Mohamad Fakri, N.F (2012). Impact of soil field water capacity on secondary metabolites, phenylalanine ammonia-lyase (PAL),maliondealdehyde (MDA) and photosynthetic responses of Malaysian Kacip Fatimah (*Labisia pumila* Benth). *Molecules.* 12, 7305-22.

Jaafar, H. Z. E.; Mohamed Haris, N. B.; Rahmat, A. (2008). Accumulation and partitioning of total phenols in two varieties of Labisia pumila Benth under manipulation of greenhouse irradiance. Acta Hort. 797, 387-392.

Jain, N., Jain, R., Swami, H., Pandey, S. and Jain, D. K. (2009). Spectrophotometric method for simultaneous estimation of simvastatin and ezetimibe in bulk drug and its combined dosage form. *International Journal of Pharmacy and Pharmaceutical Sciences.* 1 (1), 170-5.

Jain, S., Dhanotiya, C. and Malviya, N. (2012). Physicochemical characterization and determination of free radical scavenging activity of rutin-phospholipid complex. *International Journal of Pharmaceutical Sciences and Research.* 3 (3), 909-13.

Jamia, A. J., Houghton, P. J., Milligan, S. R. and Ibrahim, J. (2003). The oestrogenic and cytotoxic effects of the extracts of *Labisia pumila var. alata* and *Labisia pumila var. pumila in vitro*. *Sains Kesihatan.* 1, 53-60.

Jamia, A. J., Ibrahim, J., Khairana, H. and Juriyati, H. (2004). Perkembangan penyelidikan dan pembangunan Kacip Fatimah. *New Dimension in Complemetary Health Care: Kuala Lumpur.* 13-9.

Juhascik, M. P. and Jenkins, A. J. (2009). Comparison of liquid/liquid and solid-phase extraction for alkaline drugs. *Journal of Chromatographic Science.* 47, 553-7.

Kadam, P. V., Bhingare, C. L., Nikam, R. Y. and Pawar, S. A. (2013). Development and validation of UV spectrophotometric method for the estimation of Curcumin in cream formulation. *Pharmaceutical Methods.* 4 (2), 43-5.

Kang, J., Lu, X., Zeng, H., Liu, H. and Lu, B. (2002). Investigation on the electrochemistry of rutin and its analytical application. *Analytical Letters.* 35 (4), 677-86.

Karimi, E., Jaafar, H. Z. E. and Ahmad, S. (2011). Phenolics and flavonoids profiling and antioxidant activity of three varieties of Malaysian indigenous medicinal herb Labisia pumila Benth. *Journal of Medicinal Plants Research.* 5 (7), 1200-6.

Karimi, E., Jaafar, H. Z. E. and Ahmad, S. (2011). Phytochemical analysis and antimicrobial activities of methanolic extracts of leaf, stem and root from different varieties of Labisia pumila Benth. *Molecules.* 16, 4438-50.

Karimi, E. and Jaafar, H. Z. E. (2011). HPLC and GC-MS determination of bioactive compounds in microwave obtained extracts of three varieties of *Labisia pumila* Benth. *Molecules.* 16, 6791-805.

Karioti, A., Kitsaki, C. K., Zygouraki, S., Ziobora, M., Djeddi, S., Skaltsa, H. and Liakopoulos, G. (2007). Occurrence of Flavonoids in Ophrys (Orchidaceae) Flower Parts. *Flora.* 203, 602-9.

Katalinic, V., Milos, M., Kulisic, T., and Jukic, M. (2006). Screening of 70 medicinal plant extracts for antioxidant capacity and total phenols. *Food Chemistry*. 94, 550–7.

Khajeh, M. and Barkhordar, A. (2013). Modelling of solid-phase tea waste extraction for the removal of manganese from food samples by using artificial neural network approach. *Food Chemistry.* 141 (5), 712-7.

Khajeh, M. and Dastafkan, K. (2013). Removal of molybdenum using silver nanoparticles from water samples: particle swarm optimization-artificial neural network. *Journal of Industrial and Engineering Chemistry.* 20 (5), 3014-8.

Khajeh, M. Moghaddam, M. G. and Shakeri, M. (2012). Application of artificial neural network in predicting the extraction yield of essential oils of Diplotaenia cachrydifolia by supercritical fluid extraction. *The Journal of Supercritical Fluids.* 69, 91-6.

Khoo, H.E., Azlan, A., Ismail, A. and Abas, F. (2012). Antioxidative properties of defatted dabai pulp and peel prepared by solid phase extraction. *Molecules (Bael, Switzerland).* 17 (8), 9754-73.

Kim, G. N. and Jang, H. D. (2009). Protective mechanism of quercetin and rutin using glutathione metabolism on H_2O_2-induced oxidative stress in Hep G2 cells. *Natural Compounds and Their Role in Apoptic Cell Signalling Pathways.* 537, 530-7.

Kim, K. H., Lee, W. K., Kim, Y. D., Park, H. H., Kwon, B. I. and Lee, J. H. (2005). Optimal recovery of high-purity rutin crystals from the whole plant of *Fagopyrum esculentum* Moench (buckwheat) by extraction, fractionation, and recrystallization. *Bioresource Technology.* 96, 1709-12.

Kongkachuichaya, P., Shitangkoonb, A. and Chinwongamorn, N. (2002). Studies on dyeing of silk yarn with lac dye: effects of mordants and dyeing conditions. *Sci Asia.* 28, 161-6.

Kour, K., Sharma, N., Chandan, B. K., Koul, S., Sangwan, P. L. and Bani, S. (2010). Protective effect of *Labisia pumila* on stress-induced behavioural, biochemical, and immunological alterations. *Planta Medica.* 76, 1497-505.

Kreft, I., Fabjan, N. and Germ, M. (2003). Rutin in buckwheat: protection of plants and its importance for the production of functional food. *Fagopyrum.* 20, 7-11.

Kumar, D., Bhat, Z. A., Kumar, V., Chashoo, I. A., Khan, N. A. and Shah, M. Y. (2010). Pharmacognostical and phytochemical evaluation of *Angelica archangelica* Linn. *International Journal of Drug Development & Research.* 3 (3), 173-88.

Kuntić, V., Filipović, I. and Vujić, Z. (2011). Effects of rutin and hesperidin and their Al (III) and Cu (II) complexes on in vitro plasma coagulation assays. *Molecules*. 16(2), 1378–88.

La-Casa, C., Villegas, I., Alarcon-de-la-Lastra, C., Motilva, V. and Martin-Calero, M. J. (2000). Evidence for protective and antioxidant properties of rutin, a natural flavone, against ethanol induced gastric lesions. *Journal of Ethnopharmacology.* 71, 45-53.

Landberg, R., Sun, Q., Rimm, E. B., Cassidy, A., Scalbert, A., Mantzoros, C. S., Hu, F. B. and vann Dam, R. M. (2011). Selected diety flavonoids are associated with markers of inflammation and endothelial dysfunction in U.S. women. *Journal of Nutrition.* 141 (4), 618-25.

Latiff, A. and Zakri, A. H. (2000). *Protection of traditional knowledge, innovations and practices: the Malaysian experience.* UNCTAD Expert Meeting on Systems and National Experiences for Protecting Traditional Knowledge, Innovations and Practices, Universiti Kebangsaan Malaysia.

Li, H. B., Wong, C. C., Cheng, K. W. and Chen, F. (2008). Antioxidant properties in vitro and total phenolic contents in methanol extracts from medicinal plants. *LWT*. 41, 385–90.

Litridou, M., Linssen, J., Schols, H., Bergmans, M., Posthumus, M., Tsimidou, M. and Boskou, D. (1997). Phenolic compounds in virgin olive oils: fractionation by solid phase extraction and antioxidant activity assessment. *Journal of the Science of Food and Agriculture.* 74, 169-74.

Lucci, P., Pacetti, D., Núñez, O. and Frega, N. G. (2012). *Current trends in sample treatment techniques for environmental and food analysis, chromatography- the most versatile method of chemical analysis.* InTech: Dr. Leonardo Calderon.

Macikova, P., Halouzka, V., Hrbac, J., Bartak, P. and Skopalova, J. (2012). Electrochemical behaviour and determination of rutin on modified carbon paste electroludes. *Scientific World Journal*. 48 (5),1326-31.

Mandal, S., Sivaprasad, P. V., Venugopal, S. and Murthy, K. P. N. (2009). Artificial neural network modeling to evaluate and predict the deformation behavior of stainless steel type AISI 304L during hot torsion. *Applied Soft Computing.* 9 237–44.

Mashita, M. Y., 2005. Labisia pumial (Kacip Fatima) - A plant's profile. Women health and Asian traditional medicine conference I.

Maxwell, S. R., Thomason, H., Sandler, D., Leguen, C., Baxter, M. A. and Thorpe, G. H. (1997). Antioxidant status in patients with uncomplicated insulin-dependent and non-insulin-dependent diabetes mellitus. *European Journal of Clinical Investigation.* 27,484-90.

Melissa, P.S.W., Navaratnam, V. and Yin , C.Y. (2012). Phytoestrogenic property of *Labisia pumila* for use as an estrogen replacement therapy agent. *African Journal of Biotechnology*. 11 (50), 11053-6.

McDonald, P. D. (1995). *A Sample Preparation Primer and Guide to Solid Phase Extraction Methods Development.* (6th ed). Milford, Massachusetts: Waters.

Miliauskas, G. (2006). *Screening, isolation and evaluation of antioxidative compounds from Geranium macrorrhizum, Potentilla fruticosa and Rhaponticum carthamoides.* Doctor Philosophy, Wageningen University.

Miliauskas, G.; Venskutonis, P. R.; Van Beek, T. A. (2004). Screening of radical scavenging activity of some medicinal and aromatic plant extracts. *Food Chemistry.* 85, 231-237.

Mladěnka, P., Zatloukalová, L., Filipskỳ, T. and Hrdina, R. (2010). Cardiovascular effects of flavonoids are not caused only by direct antioxidant activity. *Free Radical Biology & Medicine.* 49(6), 963-75.

Mourabet, M., El Rhilassi, A., Bennani-Ziatni, M. and Taitai, A. (2014). Comparative study of artificial neural network and response surface methodology for modelling and optimization the adsorption capacity of fluoride onto apatitic tricalcium phosphate. *Universal Journal of Applied Mathematics.* 2 (2), 84-91.

Nälsén, C. (2006). *Measurement and evaluation of antioxidant status and relation to oxidative stress in humans*. Doctor of Philosophy, Uppsala Universitet.

Narayana, K. R. A. J. and Krishna, D. R. (2001). Bioflavonoids classification, pharmacological, biochemical effects and therapeutic potential. *Indian Journal of Pharmacology.* 33, 2-16.

Nijveldt, R. J., Nood, E. V., van Hoorn, D. E. C, Boelens, P. G., van Norren, K. and van Leeuwen, P. A. M. (2001). Flavonoids: A Review of Probable Mechanisms of Action and Potential Applications. *The American Journal of Clinical Nutrition.* 74, 418-25.

Norhaiza, M., Maziah, M. and Hakiman, M. (2009). Antioxidative properties of leaf extracts of a popular Malaysian herb, *Labisia pumila*. *Journal of Medicinal Plants Research.* 3 (4), 217-23.

Odetti, P. R., Borgoglio, A., De Pascale, A., Rolandi, R. and Adezati, L. Prevention of diabetes-increased aging effect on rat collagen-linked fluorescence by aminoguanidineand rutin. Diabetes. 39 (1990) 796–801.

Okawa, M., Kinjo, J., Nohara, T. and Ono, M. (2001). DPPH (1, 1-diphenyl-2-picrylhydrazyl) radical scavenging activity of flavonoids obtained from some medicinal plants. *Biological & Pharmaceutical Bulletin.* 24 (10), 1202-5.

Osawa, T. Novel natural antioxidants for utilization in food and biological systems. In Postharvest Biochemistry of plant Food-Materials in the Tropics; Uritani, I., Garcia, V. V., Mendoza, E. M. Eds.; Japan Scientific Societies Press: Tokyo, Japan, 1994; pp 241-251.

Pan, Y., Tiong, K. H., Abd-Rashid, B. A., Ismail, R., Mark, J. W. and Ong, C. E. (2012). Inhibitory effects of cytochrome P450 enzymes CYP2C8, CYP2C9, CYP2C19 and CYP3A4 by Labisia pumila extracts. *Journal of Ethnopharmacology.* 143 (2), 586-91.

Pandey, A., Bani, S., Sangwan, P. and Koul, S. (2010). Selective Th1 upregulation by ethyl acetate fraction of *Labisia pumila*. *Journal of Ethnopharmacology.* 132 (1), 309-15.

Paniwnyk, L., Beaufoy, E., Lorimer, J. P. and Mason, T. J. (2001). The extraction of rutin from flower buds of *Sophora japonica*. *Ultrasonics Sonochemistry*. 8, 299-301.

Pedriali, C. A., Fernandez, A. U., Bernusso, L. C. and Polakiewicz, B. (2008). The synthesis of a water-soluble derivative of rutin as an antiradical agent. *Química Nova.* 31 (8), 2147-51.

Peng, S. X., Branch, T. M. and King, S. L. (2001). Fully automated 96-well liquid-liquid extraction for analysis of biological samples by liquid chromatography with tandem mass spectrometry. *Analytical Chemistry.* 73, 708-14.

Perron, N. R. and Brumaghim, J. L. (2009). A review of the antioxidant mechanisms of polyphenol compounds related to iron binding. *Cell Biochemistry and Biophysics.* 53(2), 75-100.

Pietta, P. G. (2000). Flavonoids as antioxidants. *Journal of Natural Products.* 63, 1035-42.

Pinheiro, P. F. and Justino, G. C. (2010). Structural analysis of flavonoids and related compounds–A review of spectroscopic applications. *Phytochemicals.* 33-57.

Pothiritat, W. and Gritsanapan, W. (2008). Quantitative analysis of total mangostins in *Garcinia Mangostana* fruit rind. *Journal of Health Research.* 22(4),161-6.

Rasadah, M. A. and Zainon, A. S. (2003). Database on ASEAN herbal and medicine plants, ASEAN Publication.

Reverchon, E. and De Marco, I. (2006). Supercritical fluid extraction and fractionation of natural matter. *Journal of Supercritical Fluids.* 38, 146-66.

Reznik, H. and Egger, K. (1961). Benedikts reagens as indicator for phenolische orto-Dihydroxygruppen. *Journal of Analytical Chemistry.* 183-96.

Rice-Evans, C.A., Miller, N.J. and Paganga, G. (1996). Structure-antioxidant activity relationships of flavonoids and phenolic acids: a review. *Free Radical biology and Medicine.* 20, 933-56.

Roberts, E. A. H. and Wood, D. J. (1951). A study of the polyphenols in tea leaf by paper chromatography. *Biochemistry.* 49 (4), 414-22.

Rodriguez-Amaya, D. B. (2010). Quantitative analysis, in vitro assessment of bio-availability and antioxidant activity of food carotenoids—A review. *Journal of Food Composition and Analysis.* 23, 726–40.

Rodriguez, J., Ortuno, C., Benedito, J. and Bon, J. (2013). Optimization of the antioxidant capacity of thymes (Thymus vulgaris L.) extracts: management of the drying process. *Industrial Crops and Products.* 46, 258-63.

Rodriguez, J., Melo, E.C., Mulet, A. and Bon, J. (2013). Optimization of the antioxidant capacity of thymes (Thymus vulgaris L.) extracts: management of the convective drying process assisted by power ultrasound. *Journal of Food Engineering.* 119 (4), 793-9.

Sadrzadeh, M., Mohammadi, T., Ivakpour, J., and Kasiri, N. (2008). Separation of lead ions from wastewater using electrodialysis: Comparing mathematical and neural network modeling. *Chemical Engineering Journal*. 144, 431–41.

Sajeeth, C. I., Manna, P. K., Manavalan, R. and Jolly, C. I. (2010). Quantitative estimation of gallic acid, rutin and quercetin in certain herbal plants by HPTLC method. *Der Chemica Sinica.* 1 (2), 80-5.

Samuagam, L., Akowuah, G. A. and Okechukwu, P. N. (2011). Partial Purification and Antinociceptive Investigation of Extracts of Leaves *Labisia pumila. Asian Journal of Pharmaceutical and Clinical Research.* 4(4), 44-6.

Sandstrom,B. (1991). Induction and rejoining of DNA single strand breaks in relation to cellular growth in human cells exposed to three hydroperoxided at 0◦C and 37◦C. *Free Radical Research Communications.* 15, 79–89.

Sapkale, G. N., Patil, S. M., Surwase, U.S. and Bhatbhage, P. K. (2010). A review supercritical fluid extraction. *International Journal of Chemical Sciences.* 8 (2),729-43.

Sasidharan, S., Chen, Y., Saravanan, D., Sundram, K.M., Latha, Y.L. (2011). Extraction, isolation and characterization of bioactive compounds from plants' extracts. *African Journal of Traditional Complementary and Alternative Medicines*. 8 (1), 1–10.

Sentkowska, A., Biesaga, M. and Pyrzynska, K. (2012). Evaluation of ZIC-HILIC columns for the analysis of flavonols. *Analytical Chemistry.* 9, 49-55.

Shahrim, Z., Baharuddin, P. J. N. M., Yahya, N. A., Muhammad, H., Bakar, R. A.and Ismail, Z. (2006). The *in vivo* rodent micronucleusassay of Kacip Fatimah *(Labisia pumila)* extract. *Tropical Biomedicine.* 23, 214-9.

Shoskes, D. A. (1998). Effect of bioflavonoids quercetin and curcumin on ischemic renal injury: a new class of renoprotective agents. *Trans—plantation.* 66, 147-52.

Shukla, P., Gopalkrishna, B. and Shukla, P. (2012). Isolation of rutin from *Phyllanthus amarus*. *International Journal of Pharmaceutical Sciences and Research.* 3 (4), 1198-201.

Silberman, H. and Thorp, R. H. (1954). The estimation of the component cardiac glycosides in digitalis plant samples; part II . the estimation of the desglucoglycosides and some observations on the production of ultra-violet fluorescence with trichloracetic acid. *Journal of Pharmacy and Pharmacology.* 6(1), 546-51.

Sintayehu, B., Asres, K. and Raghavendra, Y. (2012). Radical scavenging activities of the leaf extracts and a flavonoid glycoside isolated from *Cineraria abyssinica* Sch. Bip. Exa. Rich. *Journal of Applied Pharmaceutical Science.* 2 (4), 44-9.

Smith, C. P. O., Thomas, P., Scurr, J. H. and Dormandy, J. A (1980). causes of various ulceration, a new hypothesis. *British Medical Journal.* 296, 1726-7.

Sokmen, A., Sokmen, M., Daferera, D., Polission, M., Candan, F., Unlu, M. and Akpulat, A. (2004). The in vitro antioxidant and antimicrobial activities of the essential oil and methanol extracts of Achillea biebestein Afan (Asteraceae). *Phytotherapy.* 18, 451-6.

Stevens, J., Crawford, M., Robinson, G. and Roenneburg, L. (2007). Automated post-collection concentration for purified preparation fractions via solid-phase extraction. *Journal of Chromatography A.* 1142, 81-3.

Stone, B. C. (1988). Notes on the genus of *Labisia* Lindl. (Myrsinaceae). *Malayan Nature Journal.* 42, 43-51.

Sunarno, B. (2005). Revision of the genus Labisia (Myrsinaceae). *Blumea: Journal of Plant Taxonomy and Plant Geography*. 50, 579–597.

Tapas, A. R., Sakarkar, D. M. and Kakde, R. B. (2008). Flavonoids as neutraceuticals: a review. *Tropical Journal of Pharmaceutical Research.* 7(3), 1089-99.

Velioglu, M. S. G., Gao, L. and Oomah, B. D. (1998). Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products. *Journal of Agricultural and Food Chemistry*. 46, 4113-7.

Viskupicova, J., Danihelova, M., Ondrejovic, M., Liptaj, T. and Sturdik, E. (2010). Rutin derivatives for antioxidant protection of oil-based foods. *Food Chemistry.* 123, 45-50.

Wagenbreth, D., Hagels, H. and Schilcher, H. (1996). Characterization of buckwheat cultivars and gene bank material for rutin content and growth parameters. *Quedlimburg:ISBMAP*. 95-102.

Wantusiak, P. M., Piszcz, P. and Glód, B. K. (2012). A fast and simple method for the measurement of total antioxidant potential and a fingerprint of antioxidants. *Journal of Chromatographic Science.* 50 (10),909-13.

Williams, K. A., Dasilva, C. S., Bhatta, A., Rawal, B., Liu, M. and Korobkova, A. E. (2012). Determination of the drug-DNA binding modes using fluorescence-based assays. *Analytical Biochemistry.* 422, 66-73.

Wong, C. C., Li, H. B., Cheng, K. W., Chen, F. (2006). A systematic survey of antioxidant activity of 30 Chinese medicinal plants using the ferric reducing antioxidant power assay. *Food Chemistry.* 97, 705-11.

Xi, J., Xue, Y., Xu, Y. and Shen, Y. (2013). Artificial neural network modelling and optimization of ultrahigh pressure extraction of green tea polyphenols. *Food Chemistry.* 141 (1), 320-6.

Xu, H., Li, Y., Tang, H., Liu, C. and Wu, Q. (2010). Determination of rutin with UV-Vis spectrophotometric and laser-induced fluorometric detections using a non-scanning spectrometer. *Analytical Letters.* 43, 893-904.

Yang, J., Guo, J. and Yuan, J. (2008). In vitro antioxidant properties of rutin. *LWT-Food Science and Technology.* 41,1060-6.

Yusoff, M. M. and Iwansyah, A. C. (2011). Comparative evaluation of total phenolics and free radical scavenging activity of aqueous extracts of *Labisia pumila* var. *Alata* from Malaysia and Indonesia. *2nd International Conference on Biotechnology and Food Science.* 7, 4-8.

Zare, H. R., Samimi, R. and Ardakani, M. M. (2009). A comparison of the electrochemical behavior of rutin at an inactivated, activated and multi wall carbon nanotubesmodified glassy carbon electrode. *International Journal of Electrochemical Science.* 4, 730-9.

Zeng, H., Wang, Y., Liu, X., Kong, J. and Nie, C. (2012). Preparation of molecular imprinted polymers using bi-functional monomer and bi-crosslinker for solid-phase extraction of *rutin*. *Talanta.* 93, 172-81.

Zhang, Q. and Ye, M. (2008). Chemical analysis of the Chinese herbal medicine Gan-Cao (licorice). *Journal of Chromatography A.* 1216, 1954-69.

Zhao, K., Yang, L., Wang, X., Bai, Q., Yang, F. and Wang, F. (2012). Preparation of a novel dual-function strong cation exchange/ hydrophobic interaction chromatography stationary phase for protein separation. *Talanta.* 98, 86-94.

Zhang, Y., Shan, X. and Gao, X (2011). Development of a molecularly imprinted membrane for selective separation of flavonoids. *Separation and Purification Technology.* 76, 337-44.

Zhao, Z., Dong, L., Wu, Y. and Lin, F. (2011). Preliminary separation and purification of rutin and quercetin from *Euonymus alatus (Thunb.) Siebold* extracts by macroporous resins. *Food and Bioproducts Processing.* 89 (4), 266-72.

Zheng, W. and Wang, S. Y. (2001). Antioxidant activity and phenolic compounds in selected herbs. *Journal of Agricultural and Food Chemistry.* 49, 5165-70.

Zu, Y., Li, C., Fu, Y. and Zhao, C. (2006). Simultaneous determination of catechin, rutin, quercetin, kaempferol and isorhamnetin in the extract of sea buckthorn (Hippophae rhamnoides L.) leaves by RP-HPLC with DAD. *Journal of Pharmaceutical and Biomedical Analysis.* 41, 714-9.