UNIVERSITI PUTRA MALAYSIA

SOLID-LIQUID EXTRACTION OF GALLIC ACID FROM KACIP FATIMAH (Labisia pumila Benth. & Hook. f.) LEAVES

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By

MOHD AZRIE BIN AWANG

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of Requirements for the Degree of Master of Science

August 2014

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirements for the degree of Master of Science

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August 2014

Chairman : Professor Luqman Chuah Abdullah, PhD
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Labisia Pumila or known as Kacip Fatimah is one of medicinal plants which are rich of bioresource for traditional systems of medicine, food supplements and pharmaceutical intermedtiates. According to scientific researches, the leaf of this plant is proven to posses many beneficial bioactivities and its leaf extract has great potential to be used in developing naturally occurring commercial products in market demand. However, very little research has been carried out with regards to the processing of bioactive extract of *L. pumila* leaves.This research studied a solid-liquid extraction (SLE) process which involved processing of the bioactive extract from *L. pumila* leaves. Experiments were carried out to determine the effects of various operating parameters on the qualitative and quantitative aspects of *L. pumila* leaves. Gallic acid (GA) was selected as the quality indicators of the product because this compound played a major role in the bioactivities including anti-inflammatory, antioxidant activity, anti-microbial, anti-cancer and prevention of osteoporosis. Water was found to be most suitable solvent for extracting gallic acid from *L. pumila* leaves compared to other organic solvents. It gave the highest extraction yield as well as quality extract of GA. It also showed the highest antioxidant and anti-inflammatory. The optimum extraction condition of *L. pumila* leaves were determined as 80°C and the ratio of water to solid is 30:1 (ml:g) based on analysis using Response Surface Methodology (RSM) technique. An equilibrium driven solid liquid extraction (EDSLE) model was developed and successfully applied in describing the process.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan ijazah Master Sains

PENGEKSTRAKAN PEPEJAL-CECAIR ASID GALLIC DARIPADA DAUN KACIP FATIMAH (*Labisia pumila* Benth. & Hook. f.)

Oleh

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Pengerusi : Profesor Luqman Chuah Abdullah, PhD
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Labisia Pumila atau dikenali sebagai Kacip Fatimah merupakan salah satu tumbuhan ubatan yang kaya dengan biosumber untuk sistem perubatan tradisional, makanan tambahan dan farmaseutikal. Menurut kajian saintifik, daun tumbuhan ini terbukti memiliki banyak bioactivity yang bermanfaat dan ekstrak daun mempunyai potensi yang besar untuk digunakan dalam membangunkan produk komersial yang semulajadi di pasaran. Walau bagaimanapun, penyelidikan yang sangat sedikit telah dijalankan berkaitan dengan pemprosesan ekstrak bioaktif terhadap daun *L. pumila*.Kajian ini dijalankan menggunakan kaedah pengekstrakan pepejal-cecair (SLE) bertujuan untuk mengkaji kadar bahan aktif dalam daun *L. pumila*. Kajian dijalankan menggunakan pemboleh ubah yang berbeza bagi menentukan kaitan antara pemboleh ubah tersebut kepada kualiti dan kualitatif aspek bagi daun *L. pumila*. Asid Gallic (GA) telah dipilih sebagai petunjuk kualiti produk kerana bahan aktif ini telah memainkan peranan utama dalam kajian termasuk anti-radang, aktiviti antioksida, anti-mikrob, anti-kanser dan pencegahan osteoporosis. Air merupakan pelarut yang paling sesuai untuk mengekstrak daun *L. pumila* berbanding dengan pelarut yang lain. Ia memberikan kadar pengeluaran yang tertinggi dan juga ekstrak kualiti GA. Ia juga menunjukkan antioksidan dan anti-radang yang tertinggi. Hasil ekstrak yang optimum diperolehi daripada suhu pengekstrakan ialah 80 ° C dan nisbah air kepada pepejal adalah 30:1 (ml:g) berdasarkan analisis Kaedah Respons Permukaan (RSM). Satu model baru yang dinamakan model “equilibrium driven solid liquid extraction” (EDSLE) telah dibangunkan dengan berjaya untuk menerangkan process itu.

ACKNOWLEDGEMENTS

"In The Name of ALLAH, The Most Gracious, The Most Compassionate"

Alhamdulillah, I would like to express the deepest appreciation to my committee chair Professor Dr. Luqman Chuah Abdullah, who has shown the attitude and the substance of a genius: he continually and persuasively conveyed a spirit of adventure in regard to research and scholarship, and an excitement in regard to teaching. Without his supervision and constant help this dissertation would not have been possible.

I would like to thank my committee members, Dr Pin Kar Yong, Associate Professor Dr. Yus Aniza binti Yusof, Dr Chong Gun Hean and Madam Nor Halaliza bt Alias whose work demonstrated to me that concern for global affairs supported by an "engagement" in comparative literature and modern technology, should always transcend academia and provide a quest for our times.

In addition, I would also like to extend my thanks to my colleagues from Herbal Technology Center and Natural Product Division of Forest Research Institute Malaysia (FRIM) especially En Zamree Md Shah and Dr Rasadah Mat Ali for their technical assistance and kind knowledge-sharing.

Last but not least, I want to extend my deepest gratitude to my parents and family members, my friends their encouragement and support.

I certify that a Thesis Examination Committee has met on 18 August 2014 to conduct the final examination of Mohd Azrie bin Awang on his thesis entitled "Solid Liquid Extraction of Gallic Acid from Kacip Fatimah (*Labisia pumila* Benth. & Hook. f.) Leaves" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

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LIST OF ABBREVIATIONS

σ	Coefficient in $D(C_s)$ relation
b_o	Regression coefficient of intercept term
b_1	Linear regression coefficient
b_2	Linear regression coefficient
b_{11}	Squared regression coefficient
b_{22}	Squared regression coefficient
b_{12}	Interaction regression coefficient
C_e	Equilibrium solute concentration in liquid phase (gcm^{-3})
CH_3CN	Acetonitrile
C_L	Solute concentration in liquid phase (gcm^{-3})
$C_{L,exp}$	Solute concentration in liquid phase from experiment result (gcm^{-3})
$C_{L,pre}$	Predicted Solute concentration in liquid phase (gcm^{-3})
C_S	Solute concentration in solid phase (gcm^{-3})
EDSLE	Equilibrium driven solid-liquid extraction
FRIM	Forest Research Institute Malaysia
HPLC	High Performance Liquid Chromatography
GA	Gallic acid

RMSE	Root mean square error
R_{ss}	Ratio of solvent to solid (mlg^{-1})
SEN	Sinensetin
SLE	Solid – liquid extraction
Y	Response of RSM
E_a	Activation energy
k	Extraction constant
R	Universal gas constant
T	Absolute temperature
A	Arrhenius constant

CHAPTER 1

INTRODUCTION

1.1 Herbal industries in Malaysia

World Health Organization (WHO) persistently reported that, in some Asian and African countries, 80% of the world's population depending on herbal medicine while 25% of prescription drugs are derived from plants (WHO, 2008). The World Health Organization estimates four billion people use some form of herbal medicine, and the European market alone is currently worth about RM 25.3 billion, RM 9.9 billion in Japan, RM 15.7 billion at North America and RM 24.3 billion at Asia (Ismail, 2011).The definition of herbal medicine is plant derived material or preparations with therapeutic benefits, it contains raw or processed ingredients from one or more plants (WHO, 2000). The demand for herbs in the production of traditional medicines is ever increasing. Malaysia has a rich tradition of herbal product usage for health, food and beauty. The development in the herbal industry in Malaysia had increased rapidly due to the increase in local and global market demands.

Our local herbal industry has, however, been making progress quietly, and as a market that is growing globally at the rate of about 30% a year, it is well worth a second look. The growing acceptance for the herbal based natural Phyto-medicines globally, the market is likely to grow 8% to 15% annually.

However, by capturing approximately 1% of global market share, it could be easily transferred into a RM 190 billion industry in Malaysia (The Star, 2009). This shows a growing trend of consumers which are moving towards natural products from the source which are believed to cause lesser side effects, compare to the synthetic allopathic drugs. It is with this potential in mind that Malaysia's herbal industry has been identified as one of the agriculture Entry Point Projects (EPPs) under the National Key Economic Areas (NKEAs) in the Economic Transformation Program (ETP). Its target: a gross income of RM3.25bil by 2020.

However, several herb plants in Malaysia have good potential to be developed into global herbal medicines like a *Tongkat Ali* (*Eurycoma lonngifilia*), *Kacip Fatimah* (*Labisia pumila*) as supplement to treat man impotence and hormone replacement therapy for women, respectively. Consumers are becoming more interested to use these natural products which are believed to cause lesser side effects than a product that produced by chemical synthesis.

Herbal products are produced by using certain amount of bioactive extract from medicinal plants. Bioactive extract is referred as extract that possesses therapeutic activities (WHO, 2000). Nowadays, bioactive extracts are used widely in different types of products including herbal medicines, cosmetics, nutritional supplement, beverages, and health care products. These products are more affordable than modern pharmaceuticals.

There are many successful herbal product companies in malaysia such as HPA Industries Sdn. Bhd., Sendayu Tinggi Corporation (M) Sdn. Bhd., Power Root (M) Sdn. Bhd., and so on. Table 1.1 lists some of the most popular medicinal plants and their herbal products that are available in the local market.

Table 1.1: Popular medicinal plants in Malaysia (Pin *et al,* 2009)

Local Name	Scientific Name	Product
Tongkat ali	*Eurycoma longifolia*	**x** Energy drinks **x** Health supplement
Pegaga	*Centella asiatica*	**x** Health supplement **x** Coffee
Kacip Fatimah	*Labicia pumila*	**x** Herbal tea
Misai kucing	*Orthosiphon stamineus*	**x** Herbal tea
Asam keeping	*Garcinia atroviridis*	**x** Weight maintenance supplement
Mas cotek	*Ficus deltoidea*	**x** Herbal tea

1.2 Herbal Processing

Herbal processing is part of a larger industry incorporating nutraceuticals, functional foods, nutritional supplements and herbal medicines (Ramlan *et al*., 2005). In order to produce herbal medicines, standardization, i.e. the process of producing herbal extracts or phytochemicals in which product potency is guaranteed through consistency inactive compound profile and content level, is essential.

By applying the proper process development and effective analysis are the keys to standardization. Process development of herbal processing in Malaysia involves studies of extraction processes such as solid-liquid extraction, micro-assisted extraction, supercritical extraction etc. In order to develop an effective process, optimization in process design methods and collection of physical and chemical data is essential for local herb production.

At present, very little knowledge is available of such parameters such as solubility, partition coefficient and heat transfer coefficients that may be crucial in herbal

processing. In order to build a niche in the herbal products market, Malaysia can undertake product development based on local knowledge in terms of herbal processing, product formulation, form, and usage. Currently, there are two institutions that actively participate in research of herbal processing in Malaysia, namely Chemical Engineering Pilot Plant (CEPP) of University Technology Malaysia (UTM) and Herbal Technology Centre (HTC) of Forest Research Institute of Malaysia (FRIM).

Nowadays, there are several of extraction techniques were developed to increase the yield of bioactive compounds from plants such as ultrasound- assisted extraction, microwave- assisted extraction, supercritical fluid extraction and accelerated solvent extraction. However, solid-liquid extraction is still the most commonly used technique due to its low operating cost and less capital investment as compared to other extraction methods (Tandon and Rane, 2008).

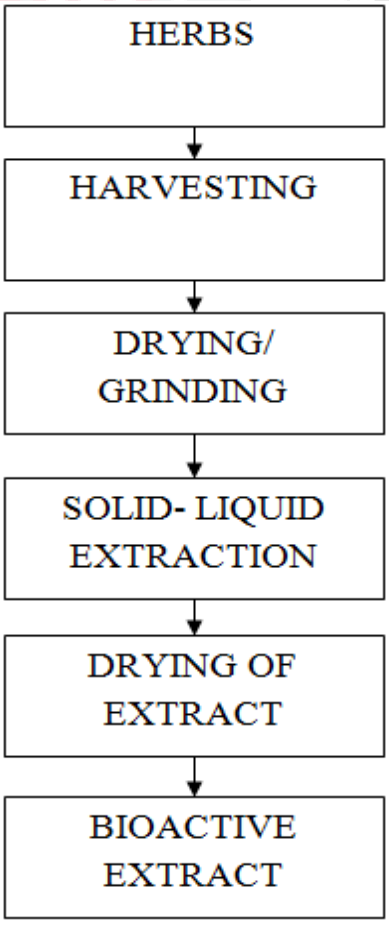

Figure 1.1: Processes involved in production of bioactive extract (Pin *et al.*, 2009)

The processes of extraction and drying of bioactive compounds from herbs are shown in Figure 1.1. According, to Handa. (2008), SLE is referred as a technique to separate active constituents from plant tissue using selected solvents. The extract contains beneficial ingredients of the original plant.

1.3 Kacip Fatimah (*Labisia Pumila*)

Labisia pumila (Myrsinaceae family), as known as Kacip Fatimah in Malaysia is a group of small genus of hardly woody plant. It is a popular herb plants that contain high bioactive compounds (Jaafar *et al*., 2008) and apply for female tonics and health products (Jamal *et al.*, 1998). Figure 1.2 shows the *L. pumila* leaves.

Figure 1.2: *Labisia Pumila* leaves

There are three various types of *L.pumila* species which are *L. pumila* var. *pumila*, *L. pumila* var. *alata* and *L. pumila* var. *lanceolata* (Stone, 1988) and these varieties differentiate from each other by their petiole and leaf characteristic. The *L. pumila* var. *Pumila* has a magnet petiole and ovate leaf blade shape but *L. pumila* var. *alata* ha a winged petiole and red vein and *L. pumila* var. *lanceolata* has a long and non-winged petiole (Stone, 1988). Generally, all these species are usually used to treat the dysentery, flatulence, dysmenorrhoea and gonorrhea (Burkill, 1966 and Rozihawati *et al*., 2003).

The promising traditional applications lead to the chemical and biological studies of this plant throughout modern research. Many researchers discovered that the *L. pumila* leaves possess a lot of beneficial bioactivities that possess anti-inflammatory, antioxidant activity, anti-microbial, anti-cancer properties and prevention of osteoporosis.

1.4 Problem Statements

The extraction of *L. pumila* leaves is one of precious medicinal plants that have million medicinal usages found in Malaysia. According to Ibrahim (2011) bioactive compounds in *L. pumila* leaves consisted mainly of resorcinols, flavonoids and phenolic acids. All these compounds can be built up by micro-manipulation and it is natural antioxidants, which can safely collaborate with free radicals.

Based on previous studies had performed in some research using the methanol as an extraction solvent (Wang *et al*., 2006). Methanol is known to be a poisonous and toxic chemical.

Arrest effects such as severe abdominal, leg, and back pain can follow the inebriating effects of methanol. Other effects such as loss of vision and blindness could also occur after exposure to substantial amounts of methanol.

Few works has been reported on the extraction process of *L. pumila* leaves, especially study on type of extraction solvents used and how it affects the extraction yield and quality. Determination of the suitable solvent for the extraction in order to get the maximum yield of extract as well as their secondary metabolites from *L. pumila* extract.
Therefore, in this project the optimum process parameters to maximize the abundance quantity of bioactive extracts while maintaining their secondary metabolites that produce adorable therapeutic properties were studied.

1.5 Objectives

1. To determine the effects of solvents on extraction yield, quality of extract, anti- inflammatory and antioxidant activity of *L.pumila* leaves extract.
2. To evaluate the optimum extraction parameter for a ratio of solvent to solid and extraction temperature using response surface methodology technique.
3. To simulate the extraction kinetics for the extraction process of *L. pumila* leaves using a mathematical model.

1.6 Scope of Research

In this project will conduct to obtain the optimum physical process for the extraction of *L.pumila* leaves. However, two main processes involved in producing bioactive extracts of *L. pumila* leaves by using solid liquid extraction (SLE) and kinetics study of the extraction process.

The influence of different extraction solvent was evaluated by quantifying the amount of extraction yield, their quality extract as well as bioactivities including anti-inflammatory and antioxidant. The results were used for selection of the most suitable solvent for extraction of *L. pumila* leaves.

A number of physical process conditions influencing the extraction of *L.pumila* leaves including extraction temperature, extraction time and also the ratio of solvent to solid. The influence of two parameters such as extraction temperature and ratio of solid of extracts was evaluated by studying the extraction yields. The qualitative and

quantitative result extraction of *L. pumila* leaves were guiding outline in determining the feasibility extraction temperature and ratio of solvent to solid.

The design of experiments will conduct by designing the experiment using Design Expert® 7.1.5 software. The range of each variable in this process will set based on the literature, especially studies focusing on the extraction of *L. pumila* leaves. Then, the best range of each physical process conditions will apply to the software.In other hand, optimization of experiments was conducted by the suggested of software. Optimum extraction duration was determined by using kinetic data that will show the time that the extraction achieving their equilibrium. The result from this study can be used as a guideline for choosing the feasible extraction parameter for extraction of *L. pumila* leaves.

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