CHARACTERIZATION AND PROCESS OPTIMIZATION OF *Melastoma malabathricum* L. EXTRACT

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ABSTRACT

Melastoma malabathricum L. (MML) or local name as senduduk, have demonstrated the presence of phenolic compound. The phenolic compound in plants has a role to inhibiting the growth of microorganism. Antimicrobial activity in plants has become an important area in the food and beverages industry; therefore natural preservatives will be very potential for more natural and fresh-like foods. In this study, to obtain the phenolic compound from MML was used extraction. Conventional extraction as heating, boiling, or refluxing can be used to extract phenolic, however, the disadvantages are the loss of phenolics due to ionisation, hydrolysis and oxidation during extraction as well as the long extraction time. In this thesis, the potentionals of ultrasonic technology in extraction was investigated. Comparation between ultrasonic-assisted extraction and classical extraction (homogenizer extraction) with incubated waterbath as a control were investigated to provide understandings of influence of ultrasonic irradiation on the production of phenolic compound in MML. Extracts were analyzed for phenolic compound (gallic acid, ellagic acid and total phenols compound). Folin Ciocalteau method was chosen for total phenols determination, while the phenolic acids (gallic acid and ellagic acid) were analyzed by using HPLC. Results showed that ultrasonic-assisted extraction was more effective to produce phenolic compound (gallic acid, ellagic acid and total phenol) from MML extract than using the classical extraction.. Acid-hydrolysis method was found the best method for post-treatment process of MML extract to produce maximum phenolic compound. Some characterization studies also investigated to understandings about nutrient composition, chemical properties and antimicrobial activity of MML extract. MML extract showed inhibitory activity against microorganism, such as *B. cereus, B. subtilis*, *S. typhi* and *E. coli.* The extraction process of MML extract were studied in this thesis, namely solid-to-liquid ratio, extraction temperature and extraction time. Prior to conducting an experimental design approach, in this study was indentification the parameter range in extraction process. The experimental run and optimization were designed using Design Expert Software as suggested by Response Surface Methodology (RSM). The optimum extraction process for highest phenolic compound extracted were obtained at extraction temperature and extraction time of 59.96 °C and 92.55 min, respectively, whereas, the solid loading about 20.07 g. Under this condition, the yield of gallic acid, ellagic acid and total phenol was 1.79 mg/g, 0.16 mg/g and 15.10 mg GAE/g. The results obtained in this study have exposed capability of ultrasonic technology in extraction of phenolic compound. Further works are nevertheless required to provide deeper understanding the mechanisms involved to facilitate the development of an optimum system applicable to the industry.

ABSTRAK

Tesis ini membentangkan penyelidikan tentang penentuan dan pengoptimuman pengaruh dari kondisi ektrak (jumlah sample, suhu ektrak dan masa ektrak) dengan bantuan ultrasonik ektrak air dari kandungan phenol (asid gallik, asid ellagik dan total phenol) dalam upaya untuk menghasilkan *M. malabathricum* L. ektrak yang kaya phenol sehingga untuk mempersiapkan bahan yang dapat langsung dimasukkan ke dalam minuman sebagai tambahan pengawet. Kaedah *Folin Ciocalteau* dipilih untuk penentuan total phenol, manakala asid gallik dan asid ellagik dianalisis menggunakan Kromatografi Cecair Berprestasi Tinggi (HPLC). Satu faktor pada satu masa (OFAT) digunakan untuk menentukan pengaruh dari kondisi ektrak untuk menghasilkan kandungan phenol. Keputusan yang terhasil dari percubaan mendapati kandungan maksimum dari phenol dengan jumlah sample 20 g, suhu ektrak 60 °C dan masa ektrak selama 90 minit, dengan jumlah asid gallik, asid ellagik dan total phenol sebesar 1.72, 0.16 dan 14.78 mg/g. *Response surface methodology* (RSM) digunakan untuk mengoptimumkan kondisi ektrak kandungan phenol dari daun *M. malabathricum* L. Keputusan menunjukkan bahawa parameter jumlah sample mempunyai pengaruh signifikan terhadap kandungan phenol dan masa ektrak mempunyai kesan yang signifikan terhadap nilai asid gallik dan total phenol. Keadaan optimum dikenalpasti sebagai jumlah sample 20,07 g, suhu ektrak 59,96 °C dan masa ektrak sekitar 92,55 minit. Dalam keadaan ini, hasil dari asid gallik, asam ellagik dan total phenol 1,79 mg/g, 0,16 mg/g dan 15,10 mg GAE/g. Nilai percubaan pada pembolehubah respon pada keadaan optimum sesuai dengan baik dengan nilai ramalan.

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LIST OF ABBREVIATIONS AND SYMBOLS

AAS	Atomic absorption spectrometry
ANOVA	Analysis of variance
ATCC	American Type Culture Collection
Ca	Calcium
CCD	Central composite design
CIP	Collection of Institut Pasteur
cm	Centimeter
Cu	Copper
DOE	Design of experiment
DNA	Deoxyribonucleic acid
EA	Ellagic Acid
Eq.	Equation
ET	Ellagitannins
FC	Folin Ciocalteau
FD	Folin Dennis
Fe	Ferrum
FTIR	Fourier Transfer Infrared
g	Gram

GA	Gallic Acid
GC	Gas Chromatography
GC-MS	Gas Chromatography-Mass Spectra
GT	Gallotannins
h	Hour
HCl	Hydrochloric acid
HPLC	High Performance Liquid Chromatography
HTs	Hydrolyzable tannins
H_2O	Water
IFO	Information
KBr	Potassium bromide
Kcal	Kilocalorie
KHz	Kilohertz
L	Litre
M	Molar
M. malabathricum L.	*Melastoma malabatricum* Linn
min	Minute
Mg	Magnesium
mg	Milligram

mg GAE/g	Milligram Gallic Acid Equivalent/gram
ml	Milliliter
mm	Micrometer
Mn	Manganese
Na	Natrium
NADPH	Nicotinamide adenine dinucleotide phosphate
NaOH	Sodium hydroxide
NCIMB	National Collections of Industrial, Marine and Food Bacteria
Nm	Nanometer
OFAT	One-factor-at-a-time
PB	Prussian blue
RSKK	Refik Saydam National Type Culture Collection
RSM	Response Surface Methodology
Std. Dev.	Standard Deviasi
TFA	Trifluoroacetic acid
TLC	Thin Layer Chromatography
TP	Total Phenol
UV	Ultraviolet
w/v	Weight/volume

y	Response
Zn	Zinc
3D	3 Dimensional
º	Degree
%	Percentage
µ	Micro/Micron
µl	Microlitre
µm	Micrometer
% w/w	Percentage weight/weight
β	Beta (Coefficient)

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CHAPTER 1

INTRODUCTION

1.1 Background of Study

Contamination and spoilage from microorganisms is a major problem in the food and beverage industry. One of the ways to inhibit the growth of microorganism in foods is to add chemicals that have antimicrobial activity, commonly called preservatives. Preservatives frequently used are acetic, benzoic, lactic, propionic, sorbic acid, nitrites and sulfites. The uses of chemical preservative are questioned in the food industry because they are suspected of having carcinogens. The use of natural preservatives in food will be of great potential for more natural and fresh-like foods. Therefore, the search for natural products with antimicrobial activity has become an important area in the food and beverage industry.

Antimicrobial activities in plants are partly due to the in phenolic compound, particularly tannins and flavonoids. In previous study that conducted by Zakaria (2007) concluded that the leaves of *Melastoma malabathricum* L. (MML) have demonstrated the presence of phenolic compound. MML is an erect shrub or small tree of 1.5 to 5 m in height, found more or less everywhere throughout Malaysia. *Melastomataceae* spp. belongs to the Family of *Melastomataceae*, Order Mrytales, Class Dicotyledon and Division Angiosperm (Sulaiman et al., 2007). It is commonly called “Straits Rhododendron” and locally known as “Senduduk”.

The studied that persent by Yoshida et al. (1992a) had succeeded in isolating several hydrolysable tannins from the dry leaves of MML. The main tannin was oligomers named nobotanin B, diMMLrs named malabathrins B, malabathrins C and malabathrins D, monomers named 1,4,6-tri-*O*-galloyl-β-D-glucoside, 1,2,4,6-tetra-*O*-galloyl-β-D-glucoside, strictinin, casuarictin, pedunculagin, nobotanin D, pterocarinin and oligomers nobotanin G, nobotanin H, nobotanin J. β-sitosterol, α-amyrin, uvaol, sitosterol-3-*O*-β-Dglucopiranoside, quercetin, quercitrin and rutin are also found in MML by Nuresti et al. (2003).

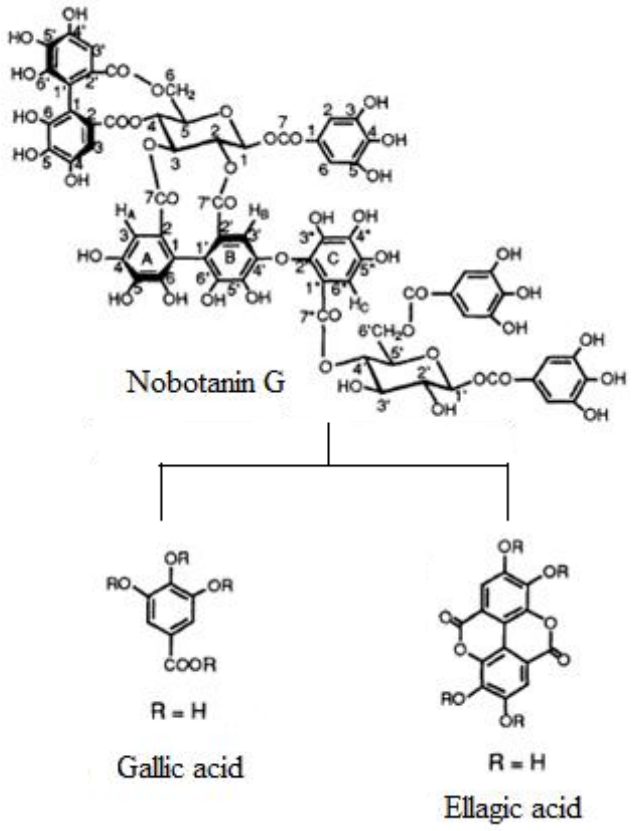

Figure 1.1: Structures of acid hydrolysates from Nobotanin G

Source: Yoshida et al. (1992b)

Acid hydrolysis of nobotanins produces the percentages of gallic acid and ellagic acid (Yoshida et al., 1992b). Figure 1.1 shows the chemical structure of gallic acid and ellagic acid. Thus this study was established to analyze gallic acid, ellagic acid and total phenol in leaves of MML. Gallic acid and ellagic acid were common constituents and known to have antimicrobial activity (Martini et al., 2009; Chanwitheesuk et al., 2007).

1.2 Statement of Problem

Phenolic compound from plants has been extracted using methanol, ethanol, acetone, water, ethyl acetate, propanol, dimethylformamide, and their combinations (Antolovich et al., 2000). However, it is difficult to find satisfactory extraction solvents that are suitable to extract all classes of phenolics. Methanol has been widely used to extract free and simple phenolics in plants, including fruits, vegetables and bitter melons for identification and quantification purposes (Budrat and Shotipruk, 2009; González-Montelongo et al., 2010). However, the use of methanol for extraction is not acceptable for food uses, due to its toxicity to humans. For these reasons, in this study was established using aqueous extraction.

Conventional extraction as heating, boiling, or refluxing can be used to extract phenolic, however, the disadvantages are the loss of phenolics due to ionisation, hydrolysis and oxidation during extraction as well as the long extraction time (Hui et al., 2005). The production of phenolic compound necessitates a search for economically and ecologically feasible extraction technologies. For this purpose, the application of power ultrasound seems to be very promising, as it was concluded from the studies on the extraction of proteins, medicinal compounds, tea solids, etc. (Mason et al., 1996). Recently, ultrasonication has been reported to improve significantly the phenolic compound in *Betula alleghaniensis* Britton, grape skin, *Foluim eucommiae*, *Rosmarinus officinalis* and *Saphora japonica* compare to the control extraction, such as maceration, waterbath incubated, reflux, microwaves and enzym-assisted extraction (Diouf et al., 2009; Corrales et al., 2008; Huang et al., 2009; Albu et al., 2004; Paniwnyk et al., 2001). In this studied was to compare the extractibility of the phenolic compound in MML, using aqueous as a solvent extraction,

by classical procedures (homogenizer) as well as by application of ultrasound, with incubated in waterbath as a control. The further purpose was to investigate the influence of ultrasonic irradiation on the production of phenolic compound in MML.

The MML extract was obtain and fully characterized of the nutrient composition, chemical properties; such as gallic acid and ellagic acid, and antimicrobial activity. The characterization studies to provide understandings of fundamental issues such as nutritional quality, fungtional group and retention time of chemical properties and inhibitation microorganism from MML extract.

The optimization from extraction process (solid loading, extraction temperature and extraction time) to produce the optimum yield of phenolic compound, have became a important area. Classical optimization studies use the one-factor-at-a-time approach, in which only one factor is variable at a time while all others are kept constant. This approach is time-consuming and expensive. In addition, possible interaction effects between variables cannot be evaluated and misleading conclusions may be drawn. The response surface methodology (RSM) can overcome these difficulties, since it allows accounting for possible interaction effects between variables (Khuri and Comell, 1996; Montgomery and Runger, 2003). If adequately used, this powerful tool can provide the optimal conditions that improve a process (Bas and Boyaci, 2007).

1.3 Research Objective

1. To extract the phyto-chemical of MML,
2. To characterize MML extract for the nutrient composition, chemical properties and antimicrobial activity
3. To optimize the extraction process for the production of optimum yield of phenolic compounds.

1.4 Scope of Study

1. The plant materials, leaves of MML with light pink-magenta petals were collected randomly around UMP campus, Gambang, Kuantan, Malaysia.
2. The characterization of MML extract was analyzed for their nutrient composition, chemical properties and antimicrobial activity. The characterization in nutrient composition was included to proximate value of ash compound, protein, fat, total carbohydrate, energy, dietary fiber, cholesterol, mineral compounds and trace element. The chemical properties of MML extract, namely gallic acid and ellagic acid, the characterization was included fungtional goup analyzes using FTIR spectrometer and retention time using HPLC. While the disc diffusion method was used to characterize the antimicrobial activity in MML extracts.
3. There are three extraction techniques was compare from determine the best extraction technique for produce phenolic from MML, namely ultrasonic-assisted extraction, homogenizer extraction and incubated in waterbath. The extraction was used aqueous as a solevent extraction.
4. The method being used to analyze total phenol compound was Folin Ciocalteau method that is by using UV-VIS spectrophotometer. The Folin Ciocalteau method was applied because it is the most recently established procedure for analysing total phenolic compound which has replaced the Folin-Denis reagent method (Singleton and Rossi, 1965). High-performance liquid chromatographic with UV detection at 280 nm was utilized to determine the phenolic acid (gallic acid and ellagic acid compounds). High-performance liquid chromatographic was a simple and rapid analytical method for the determination of phenolic acid (Amakura et al., 2000). The MML extract was tested with hydrolysis method prior analyze phenolic acid using HPLC.
5. Analyzed of optimization extraction process (solid loading, extraction temperature and extraction time) to produce the optimum yield of gallic acid, ellagic acid and total phenol was using Response Surface Methodology (RSM).

1.5 Significance of Study

Nowadays, an increasing awareness of the consumers for the use of synthetic preservative needs research for more efficient antimicrobials with fewer side effects on human health. Phenolic from various natural sources have been reported to have a variety of biological effects, including antimicrobial activities. The most important thing during the produced of phenolic from plants is to extract the plants. Thus extraction technique and extraction process have an important area to produce maximum yield of phenolic from plants.

It is expected the knowledge obtained from this study will affirm good extraction technique for extracted the phenolic compound from MML, also the characterization and optimization in extraction process for development and application in order to produce food grade MML extracts rich in phenols, thus as to prepare ingredients that can be directly be incorporated into flavored waters and/or fruit drinks as additional preservative.

1.6 Organization of Thesis

The organization of this thesis goes as follows:

1. Chapter 2

   In chapter 2 briefly describe the chemistry of major phenolic compound of MML extract such as gallic acid and ellagic acid, also their antimicrobial activity. The chapter also explains the extraction process of phenolic compound and the techniques for determination the total phenol and phenolic acid from MML extract. Previous work related to these techniques and the various applications considered in thesis are reviewed.

2. Chapter 3

   Methodology of extraction, characterization and optimization of MML extract were discussed in this chapter. Included the extraction technique, post-treatment of

extraction, nutrient composition, chemical properties, antimicrobial activity, parameter process range identification and CCD design for optimization were also discussed.

3. Chapter 4

   In chapter 4 describe the results of the best method and post-treatment of extraction phenolic compound from MML extract, characterization of MML extract and optimization of produce the optimum phenolic compound of MML extract, also the validation of empirical model and confirmation run of the optimum MML extract.

4. Chapter 5

   In these chapter summaries the major features of this thesis and proposes some research points that can be investigated for future work.

CHAPTER 2

LITERATURE REVIEW

This chapter has presented a detailed explanation of the topic. The previous works and researches have been provided to relate with this research, thus this research will be more relevant and supported. There are two sections in this chapter, namely chemistry and extraction of MML.

2.1 Chemistry of MML

This section are divided into two sub-sections, there are phenolics compound in leaves of MML and antimicrobial activity of phenolics compound in leaves of MML.

2.1.1 Phenolics Compound in MML

Phenolics are synthesized by plants as secondary metabolites (Balasundram et al., 2006) and occur as tannins or other phenolics (Naczk and Shahidi, 2006). Based on their chemical structures and behavior, tannins can generally be categorized into two large groups; hydrolysable tannins and condensed tannins (Chavan et al., 2001; Okuda et al., 1989).
The major phenolic compounds in the leaves of MML are gallic acid and ellagic acid. There are represent the polyphenolic parts in the molecules of hydrolysable tannins.

Hydrolysable tannins are compounds containing a central core of glucose or another polyol esterified with gallic acid, also called gallotannins, or with hexahydroxydiphenic acid (HHDP), also called ellagitannins. Hydrolysable tannins are hydrolyzed by acids, bases or