SCREENING AND OPTIMISATION STUDY ON ANTIOXIDANT EXTRACTS FROM SELECTED LAMIACEAE PLANTS

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ABSTRACT

This thesis deals with screening of antioxidant activity, phenols content and mineral profiles, optimisation of extraction of antioxidants and optimisation of spray drying of boiling extract from the selected Lamiaceae plants. The objective of this thesis was to select the plant materials of the selected Lamiaceae species that gave the highest antioxidant activity, highest phenols content and best mineral profiles. This thesis presents the optimised levels of experimental variables that gave the highest antioxidant activity-yield. This thesis also presents the optimum levels of the operational variables that influence the spray drying of the liquid extract of the Lamiaceae plant. Fresh *Plecranthus amboinicus* Lour. Indonesia (PALI) samples were randomly collected from Jakarta, Indonesia and samples of *Plecranthus amboinicus* Lour. Malaysia (PALM), *Pogostemon cablin* Benth. (PCB), *Solenostemon scutellariodes* Benth. Red (SSR) and *Solenostemon scutellariodes* Mix Colour (SSMC) were randomly collected from Kuantan, Malaysia. The extracted samples were assessed using 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging assay for determination of antioxidant activity. Folin Ciocalteau method was chosen for total phenols determination, while the essential minerals were analysed by using atomic absorption spectrometer. The 2^2 factorial design of experiment was used in the optimisation of extraction of antioxidants by boiling. The method of Path of Steepest Ascent was used to maximise the antioxidant activity in the spray dried extract. The obtained results indicated that the leaves tissues of PCB samples exhibited the significant ($p<0.05$) highest antioxidant activity (63.18%) and highest phenols content (7.64 mg/g fresh weight) while the highest total minerals content was observed in the PALI leaves (38.41 ppm). For optimisation of extraction of antioxidant by boiling, the theoretical maximum point has the boiling temperature (T) at 116.18°C and boiling time duration (t) at 107.81 minutes which gave an antioxidant activity-yield of 65.153 %. The combination of levels of operational variables where the spray dryer was operated at X_1 (temperature) = 177.65°C and X_2 (concentration of maltodextrin) = 48.65% gave the highest yield of antioxidant activity of spray dried extract. This research suggests a chemical basis for some of the health benefits claimed for in folk medicine and warrant further studies to develop it as a natural functional food.

ABSTRAK

Tesis ini membentangkan penyelidikan tentang saringan aktiviti antioksidan, kandungan phenol dan profil mineral, pengoptimuman ektrak antioksidan dan pengoptimuman pengeringan-semburan ektrak pendidihan daripada tumbuh-tumbuhan Lamiaceae terpilih. Objektif tesis ini adalah untuk memilih bahagian daripada tumbuhan Lamiaceae yang memberi aktiviti antioksidan terbaik, kandungan phenol tertinggi dan profil mineral terbaik. Tesis ini mengenal pasti tahapan pembolehubah-pembolehubah eksperimen yang memberi hasil aktiviti antioksidan yang optimum. Tesis ini juga mencari tahapan optimum untuk pembolehubah operasi yang mempengaruhi pengeringan-semburan untuk ektrak cecair tumbuhan Lamiaceae. Sampel segar *Plecranthus amboinicus* Lour. Indonesia (PALI) didapati secara rawak dari Jakarta, Indonesia dan sampel *Plecranthus amboinicus* Lour. Malaysia (PALM), *Pogostemon cablin* Benth. (PCB), *Solenostemon scutellariodes* Merah (SSR) dan *Solenostemon scutellariodes* Warna Bercampur (SSMC) didapati dari Kuantan, Malaysia. Ekstrak sampel diukur aktiviti antioksidan dengan menggunakan ujian pencarian radikal bebas 1,1-diphenyl-2-picrylhydrazyl (DPPH). Kaedah Folin Ciocalteau dipilih untuk penentuan phenol, manakala mineral surih dianalisis menggunakan spektrofotometer penyerapan atom (AAS). Eksperimen 2^2 Reka Bentuk Faktor (2^2 Factorial Design) diaplikasikan untuk pengoptimuman ekstrak antioksidan. Kaedah Pendakian Paling Curam (the method of Path of Steepest Ascent) diaplikasikan untuk mengoptimum aktiviti antioksidan dalam ekstrak pengeringan-semburan. Keputusan yang terhasil mendapati sampel daun PCB menunjukkan aktiviti antioksidan tertinggi (63.18%) dan kandungan phenol tertinggi (7.64 mg/g berat segar sampel) yang signifikan ($p<0.05$) manakala jumlah kandungan mineral tertinggi didapati dalam daun PALI (38.41 ppm). Untuk pengoptimuman ekstrak antioksidan, suhu didih (T) 116.18°C dan masa didih (t) 107.81 minit merupakan Titik Maksimum Teoritikal (Theoretical Maximum Point) yang memberi hasil aktiviti antioksidan sebanyak 65.153 %. Gabungan tahapan pembolehubah operasi pada $J=3$ yang mana operasi pengeringan-semburan pada X_1 (suhu) = 177.65°C and X_2 (kepekatan maltodekstrin) = 48.65% memberi hasil antioksidan tertinggi dalam serbuk ekstrak-kering berbanding yang lain. Kajian ini mencadangkan potensi faedah kesihatan dalam tumbuhan ini boleh dijadikan asas kimiawi untuk faedah kesihatan yang didakwa dalam perubatan manusia dan memerlukan kajian yang seterusnya untuk dijadikan sebagai makanan berfungsi yang alami.

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LIST OF ABBREVIATIONS

AA	Ascorbic acid
AAS	Atomic absorption spectrometer
ATP	Adenosine-5'-triphosphate
BHA	Butylated hydroxyanisole
BHT	Butylated hydroxytoluene
Ca	Calcium
Cl	Chlorine
Cu	Copper
d.f.	Degree of freedom
DNA	Deoxyribonucleic acid
DOE	Design of Experiment
DPPH	1,1-diphenyl-2-pycrylhydrazyl
Fe	Iron
fw	Fresh weight
g	Gram
GAE	Gallic acid equivalents
H_2SO_4	Sulphuric acid
HCl	Hydrochloric acid
$HClO_4$	Perchloric acid
HIV	Human immunodeficiency
HNO_3	Nitric acid
MeOH	Methanol
Mg	Magnesium
mg	Milligram
ml	Mililiter
Mn	Manganese
n.d.	No date
Na	Sodium
NaCl	Sodium Chloride
Na_2CO_3	Sodium carbonate
nm	nanometer
°C	Degree celcius
p	Probability
PALI	*Plecranthus amboinicus* Lour. Indonesia
PALM	*Plecranthus amboinicus* Lour. Malaysia
PCB	*Pogostemon cablin* Benth
ppm	Part per million
RDA	Recommended Dietary Allowances
S.D	Standard deviation
SSMC	*Solenostemon scutellaroides* Mix Colour
SSR	*Solenostemon scutellaroides* Red
Zn	Zinc
α	alpha
µl	Microliter

CHAPTER 1

INTRODUCTION

This introduction gives the ideas about the rationale of the research formulation. This first chapter discovers the subtopic of background of study, statement of problem, definition of terms, research objectives, research questions, scope of study, and significance of study.

1.1 BACKGROUND OF STUDY

It becomes important for citizens to concern themselves on healthy diet. Nowadays, people are exposed to many types of pollution such as air, water and soil pollution. Such pollutions exposed people to high levels of free radicals. Free radicals are capable of attacking the healthy cells of the body, causing them to lose their structure and function. Cell damage caused by free radicals appears to be a major contributor to aging and to degenerative diseases of aging such as cancer, cardiovascular diseases, cataracts, immune system decline, and brain dysfunction (Percival, 1998).

Fortunately, this damage can be controlled by practising healthy life style including good habitual diet and exercising. Food is the main contributor of our health. Fruits and vegetables that are good sources of micronutrients provide health benefits to human diet and inversely related to cancer risk (Ziegler, 1991). It has been confirmed that fruits and vegetables have protective effects against cellular damage caused by exposure to high levels of free radicals (Ames et al., 1993).

In the olden days, people took traditional medicines that were made from herbs, jungle plants and wild plants. Presently, aborigines are still using wild plants that are found in the jungle to heal themselves from diseases or to avoid certain diseases. It is not surprising that dangerous diseases like malaria do not touch aborigines even though they are exposed to mosquitoes in their settlements. Herbs like *Tongkat Ali* (*Eurycoma longifolia*) and *Kacip Fatimah* (*Labisia pumila*) are commonly used by them. There may be many more herbs in the jungle that have not been revealed by them.

1.2 STATEMENT OF PROBLEM

Lately, due to adverse side effects of modern drugs, many people have resorted to natural products instead of modern drugs. Fundamentally, activities of dangerous free radicals (such as those from pollution) can be lowered using antioxidants. Butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are examples of the most commonly used antioxidants at the present time. However research revealed that these are suspected of being responsible for liver damage and carcinogenesis in laboratory animals (Williams et al., 1999). Hence, more effective antioxidant of natural origin are desired to be developed and utilised (Oktay et al., 2003).

The slogans 'back to nature' and 'being green' have probably influenced people in the world to adopt traditional medicine to cure many diseases. In the higher education institutions, many scientists have worked with new plant foods that have the potential to be effective natural remedies and as well as food supplement. As a result, they come up with new products in the form of drinks, juice, herbal tea, dietary supplements or even pills. In Malaysia, many plant foods that have the potential to be effective natural remedies have been abandoned due to lack of information about their nutritional values. One of these is the Lamiaceae plants. In tropical countries such as India, Vietnam and Indonesia, Lamiaceae is widely used as home remedies to treat and relieve fever, headache, cough, asthma and boil (Che Aniha, 2008). In Malaysia, the use of Lamiaceae plants as remedies was previously unknown and they are used instead as ornamentals. It is a surprise to realize that people grow them without knowing they are rich in nutrient.

This research is therefore aimed to determine antioxidant activities, phenolic contents and mineral profiles in various Lamiaceae plants since these nutrients have significant roles in safeguarding human health. The scope of this study is to compare the Lamiaceae plants from Indonesia which are already used as health food with various Lamiaceae found in Malaysia which up to now have been grown only as ornamentals. This is to confirm the potential of Lamiaceae in Malaysia to be developed as natural functional food. Since not many clinical studies have been conducted using these Malaysian Lamiaceae, it is expected that the knowledge obtained from this study will increase awareness of these traditional plant foods and thus affirm its use in traditional diet among populations.

1.3 DEFINITION OF TERMS

The definition of terms is constructed to guide the reader to familiarise with the terms used. This is the first step to know deeply the topic. The following terms and descriptions will guide you to go through the topic.

1.3.1 *Plecranthus amboinicus* Lour. Indonesia

P. amboinicus Lour. is known locally as *ati-ati hijau* and *bangun-bangun* (Malay) and *torbangun* (Indonesia). This plant has thick leaves and all its parts give off a strong aromatic scent–believed to be similar to the scent of black seed (*jintan hitam*) (Noraida, 2007). If eaten, it will give a thick feeling to the tongue. This plant can grow up to a half metre tall. It has many branches, with fleshy leaves and stems. Leaves are heart-shaped, four to nine cm long, with the margins toothed and rounded. The propagation of this plant is usually by seeds and the rooting of stems. The following figure is the picture of *P. amboinicus* Lour. Indonesia.

Figure 1.1: *P. amboinicus* Lour. Indonesia

1.3.2 *Plecranthus amboinicus* Lour. Malaysia

P. amboinicus Lour. Malaysia is almost similar with *P. amboinicus* Lour. Indonesia but it gives out less aromatic smell compared to *P. amboinicus* Lour. Indonesia. However the smell is still strong compared to the other Lamiaceae. The colour of the leaf is greenish compared to *P. amboinicus* Lour. Indonesia's which is more yellowish. The size of leaf can reach up to eleven cm length and eight cm width with the margins are similar to *P. amboinicus* Lour. Indonesia's which are toothed and rounded. The leaf is egg shaped compared with *P. amboinicus* Lour. Indonesia's which is heart shaped. *P. amboinicus* Lour. Malaysia is shown in the following figure.

Figure 1.2: *P. ambionicus* Lour. Malaysia

1.3.3 *Pogestemon cablin* Benth.

P. cablin Benth. is known as *nilam* (Malay) and patchouli (English). It is a plant that gives pleasantly aromatic scent. *Nilam* is a herb plant that can reach two to three feet in height. It has a hairy stem. Leaves are arranged in opposite pair, oval shape, ten cm lenght and eight cm wide in size. Its leaves surface have green reddish in colour. The following is the picture of *P. cablin* Benth.

Figure 1.3: *P. cablin* Benth.

1.3.4 *Solenostemon scutellariodes* Benth.

S. scutellariodes Benth. is known as *ati-ati* in Malay community. This plant is used as ornamental because of its various colours ranged from violet to green and the mix of pink and green. It is grown as ornamental and as *tutup bumi*. The leaves have a round base and sharp-pointed edges. There are also leaves with swirl in the margins. This plant produced small flowers in bunch shape arranged at the end of long stalk. Its colour is commonly white or blue. Leaves, roots and stems of *ati-ati* contain saponin, while only stems and roots contain flavanoid. The leaves also contain essence oil (Noraida, 2007). In this research, two different *S. scutellariodes* were used which were *S. scutellariodes* Benth. red (SSR) and *S. scutellariodes* Benth. mix colour (SSMC).

Figure 1.4: *S. scutellariodes* Benth. red

Figure 1.5: *S. scutellariodes* Benth. mix colour

1.3.5 Antioxidants

Antioxidants are some of vitamins and some of minerals that occur naturally in foods and also manufactured by our bodies (Packer and Colman, 1999; Norman, 2008). They comprise of important compounds which maintain our health. They function by forming one network that can react to stabilize free radicals which is abundant in human body (Packer and Colman, 1999). Antioxidants inside the human body are molecules that able to fight free radicals and protect normal cells from damage caused by free radicals, which can injure healthy cells and tissues (Norman, 2008; Packer and Colman, 1999).

1.3.6 Phenols

Phenolic compounds are excellent antioxidants by virtue of the electron donating activity of the "acidic" phenolic hydroxyl group. Indeed, many natural and synthetic antioxidants, including α-tocopherol (vitamin E) and butylated hydroxytoluene (BHT), are phenolic compounds.

1.3.7 Minerals

Minerals are inorganic elements required in very small amounts to sustain growth, vitality and well-being. Fruits, vegetables, meat, fish, eggs, cereals and water are natural foods that are abundant with these minerals.

1.4 RESEARCH OBJECTIVES

The objectives of this research were:

1) To analyse the antioxidant activity in different Lamiaceae plants.
2) To analyse the phenol content in different Lamiaceae plants.
3) To analyse the mineral profiles in different Lamiaceae plants.
4) To evaluate antioxidant activity of different Lamiaceae plants in different parts of plants, namely:
    a. roots
    b. stems
    c. petiole
    d. leaves
5) To evaluate phenol content of different Lamiaceae plants in different parts of plants, namely:
    a. roots
    b. stems
    c. petiole
    d. leaves

6) To evaluate mineral profiles of different Lamiaceae plants in different parts of plants, namely:
    a. roots
    b. stems
    c. petiole
    d. leaves
7) To determine the relative amounts of minerals averaged between different Lamiaceae plants.
8) To identify which Lamiaceae plants contain the most of each of the minerals considered.
9) To optimise the extraction of antioxidants from selected Lamiaceae plant.
10) To optimise the spray drying of the liquid extract of the selected Lamiaceae plant obtained by boiling.

1.5 RESEARCH QUESTIONS

The research questions were formulated based on the research objectives. The research questions were as follows:

1. Which Lamiaceae plants gave the highest antioxidant activities?
2. Which Lamiaceae plants gave the highest phenols content?
3. Which Lamiaceae plants gave the best mineral profiles?
4. Which part(s) of which Lamiaceae plant gave the highest antioxidant activities?
5. Which part(s) of which Lamiaceae plant gave the highest phenol content?
6. Which part(s) of which Lamiaceae plant gave the best mineral profiles?
7. What are the relative amounts of minerals in Lamiaceae plants averaged between all types of Lamiaceae plants examined?
8. Which plant contains the most of which mineral?
9. What variables influence the extraction of antioxidants of Lamiaceae plants by boiling, and what are the optimum levels of these variables?
10. What variables influence the spray drying of the liquid extract of Lamiaceae plant, and what are the optimum levels of these variables?

1.6 SCOPE OF STUDY

To achieve the research objectives, the scopes were:

1) The plant materials include samples of five different Lamiaceae plants where the fresh plant of *P. amboinicus* Lour. Indonesia was collected randomly from Jakarta, Indonesia. The others; *P. amboinicus* Lour. Malaysia, *P. cablin* Benth. and *S. scutellariodes* red and *S. scutellariodes* mix colour were collected randomly in Kuantan where these species were grown as ornamentals. The antioxidant actvities, phenols and mineral content in each plant parts which are roots, stems, petioles and leaves from each species were studied and compared.

2) The method used to analyse antioxidant was 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay. DPPH is preferable because it is generally a more cost efficient method to analyze antioxidant activity. The test is easy and needs only a UV-VIS spectrophotometer to perform. The DPPH assays also contribute the good reproducibility (Prior et al., 2005). For phenol determination, the Folin Ciocalteau method was applied because it is the most recently established procedure for analysing total phenolic content which has replaced the Folin-Denis reagent method (Singleton and Rossi, 1965). The atomic absorption spectrometer (AAS) was utilized to determine the mineral profiles because it is a widely used technique for determining the amounts of a large number of metals. In the most common implementation of AAS, an aqueous sample containing the metal analyte is aspirated into an air-acetylene flame, causing evaporation of the solvent and vaporization of the free metal atoms. However, before the determination of minerals take place, the digestion method using nitric acid was selected to oxidise most of the organic matter. The perchloric acid is needed to break down the fatty component and solubilise the plant tissue by destroying its silicate skeleton (Novozamsky et al., 1993).

3) The antioxidant activity was chosen to be studied because it has many benefits to human health (Norman, 2008; Packer and Colman, 1999; Hermani and Rahardjo, n.d). The significance of the antioxidant will be discussed in Chapter 2. Phenols were chosen because they are parts of antioxidants that give many benefits to human health (Grace, 2005). For mineral determination we have chosen only 7 elements which comprise of the major minerals (Na, Ca and Mg) and the minor minerals (Fe, Zn, Mn and Cu). The choice of these seven minerals was due to their significance to the human body (Bastin, n.d; Blake, 2008; Machen, n.d). The discussion of their significance will be detailed later in Chapter 2.

1.7 SIGNIFICANCE OF STUDY

As the general public becomes more health-conscious, they have also become more aware of the significance of good nutrition. Natural food provides the best nutrition as human bodies are adapted for natural food intake. Regrettably, green leafy vegetables and other edible wild plants are considered to be "poor food" and have no extraordinary reputation even though many of these foods have been traditionally known to be health-promoting and have formed a component of the habitual traditional diets of populations of developing countries. As a result, in recent times, they have been largely abandoned and ignored (Hermani and Rahardjo, n.d).

It is expected that the knowledge obtained from this study will affirm good habitual traditional diet among populations of developing countries. Consequently, the poor communities will provide themselves with micronutrients, which are usually deficient in their diets, by consuming vegetables, fruits and nuts while among the affluent, the phytonutrients will help them to combat chronic diseases.