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JUDUL: Effect of extraction and drying methods on antioxidany activity of Limnophila aromatica

IJAZAH: ~~Fedt~~ Teknologi Makan dan Bioprocess

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EFFECT OF EXTRACTION AND DRYING METHODS ON ANTIOXIDANT ACTIVITY OF *Limnophila aromatica*

NG SEAH YOUNG

PERPUSTAKAAN
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THIS THESIS IS SUBMITTED AS A PARTIAL FULFILMENT FOR THE DEGREE OF BACHELOR IN FOOD TECHNOLOGY WITH HONOURS (FOOD TECHNOLOGY AND BIOPROCESSING)

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DECLARATION

I hereby declare that the material in this thesis is my own except for quotations, excerpts, equations, summaries and references, which have been duly acknowledged

16 April 2009

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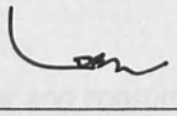

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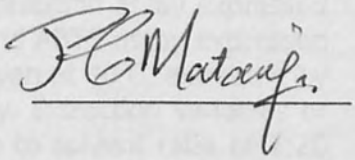

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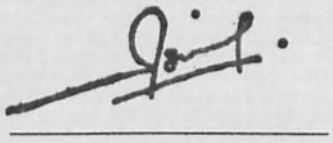

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ABSTRACT

Effect of Extraction and Drying Methods on Antioxidant Activity of *Limnophila aromatica*

The sintata (*Limnophila aromatica*) plant was used locally to treat fever and consumed as vegetable. It was known to contain large amount of flavonoids and phenolic compounds. It was also a promising source of antioxidant activity. Effect of various drying methods, types of extraction solvent, solvent to water ratio, extraction time, temperature and sample to solvent ratio on antioxidant activity were tested. The antioxidant assay used were DPPH and β-carotene bleaching inhibition assay expressed in mg/ml of EC_{50}; FRAP assay expressed in mmol/g of Fe^{2+}; and ABTS assay expressed in TEAC value in μmol/μg. It was found that sample dried in oven at 40°C, extracted by using ethanol as solvent could obtain high antioxidant activity. Extraction variables of 18 hours at 30°C, ethanol to water ratio of 60% and sample to solvent ratio of 1:20 were chosen as mean for optimizing the yield and antioxidant activity by response surface methodology using central composite design. Extract with high antioxidant activity was found extracted by Ethanol to water ratio of 1:71.08, 25 hours of extraction time and sample to solvent ratio of 1:19.98. Under these conditions, the estimated $1/EC_{50}$ value for DPPH assay was 1.997 mg/ml. Estimated Fe^{2+} value for FRAP assay was 3214.51 mmol/mg, TEAC value for ABTS assay was 191.311 μmol/μg, $1/EC_{50}$ of β-carotene bleaching assay was 1.958 mg/ml and extraction yield of 23.694%.

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ABSTRAK

Sintata (Limnophilia aromatica) merupakan suatu tumbuhan yang digunakan oleh orang tempatan untuk mengubati penyakit demam and juga dimakan seperti sayur. Adalah diketahui bahawa tumbuhan ini mengandungi banyak flavonoid dan asid fenolik. Ia juga merupakan satu sumber aktiviti antioxidan yang baik. Kesan cara-cara pengeringan sampel, jenis pelarut digunakan untuk pengekstraktan, nisbah pelarut dengan air untuk pengekstraktan, masa, suhu dan nisbah sampel kepada pelarut untuk pengekstraktan pada aktiviti antioxidan telah dikaji. Cara pengkajian antioxidan yang digunakan ialah DPPH dan β-karoten yang dinyatakan sebagai EC_{50} pada mg/ml; FRAP yang dinyatakan sebagai Fe^{2+} pada mmol/g; dan ABTS yang dinyatakan sebagai nilai TEAC dalam µmol/µg. Didapati bahawa pengeringan sampel dalam oven pada suhu 40°C, dengan menggunakan ketulenan etanol 60% boleh mendapat aktiviti antioxidan yang tinggi. Pembolehubah pengekstraktan pada 18 jam di 30°C, nisbah etanol kepada air pada 60% dan nisbah sampel kepada pelarut pada 1:20 dipilih sebagai min supaya dapat mengoptimumkan hasil extrak dan aktiviti antioxidan dengan menggunakan "response surface methodology" dimana "central composite design" sebagai bentuk eksperimen. Keadaan yang dioptimumkan untuk hasil aktiviti antioxidan yang tinggi ialah pada nisbah pelarut kepada air pada 71.08% etanol, pengekstraktan pada 25 jam dan nisbah sampel kepada pelarut pada 1:19.98. Dalam keadaan sebegini, nilai anggaran $1/EC_{50}$ bagi DPPH ialah 1.997 mg/ml. Anggaran nilai Fe^{2+} bagi FRAP ialah 3214.51 mmol/mg, nilai TEAC bagi ABTS ialah 191.311 µmol/µg, $1/EC_{50}$ bagi β-karoten ialah 1.958 mg/ml dan hasil pengekstraktan ialah 23.694%.

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LIST OF SYMBOL

DPPH	- 2,2-Diphenyl-1-picrylhydrazyl
FRAP	- Ferric Reducing Ability of Plasma
ABTS	- 2,2'-azinobis-(3-ethyl-benzothiazoline-6-sulfonic acid)
TEAC	- Trolox Equivalent Antioxidant Capacity
TPTZ	- 2,4,6-tripyridyl-s-triazine

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CHAPTER 1

INTRODUCTION

The interest in studying antioxidant activity had increased recently due to the increased public awareness on the benefits of antioxidants in disease prevention (Kaefer & Milner., 2008). Antioxidants were enzymes or other organic substances that were capable of counteracting the damaging effects of oxidation in animal tissues (Huang *et al*, 2005). Therefore, antioxidant activity could be simply defined as the ability of the antioxidative compound to inactivate toxic oxygen radicals (Murano, 2003). Antioxidants were not consist of single type of compound, but they exists as various form such as phenolic acids, flavonoids and catechins of phenolic compounds; ascorbic acids, tocopherols, tocotrinols, carotenoids and phytochemicals (Krishnaiah *et al.*, 2007; Shahidi *et al.*, 1992).

Healthy human body could always control the oxidant generated and remain the oxidant-antioxidant balance, which was important in maintaining the cell membrane integrity and functionality, cell proteins and nucleic acids (Knight, 2000). Therefore, problems would only occur if this balance was interrupted due to more oxidant compounds were generated which lead to oxidative stress (Wong *et al.*, 2006). Those extra oxidant compounds would react with the biomolecules in body cells which resulted in cellular injury or death. Illness such as heart diseases, malaria, neurodegenerative diseases, cancer and the aging of body tissues were mainly caused by this oxidative stress (Sian, 2003).

The sources of antioxidants in diet were mainly from plant origins, such as fruits, vegetables and herbs. Various studies were conducted to determine the antioxidant activity in plants to detect the plants which were good source of antioxidant activity (Capecka *et al.*, 2005). Basically within biological systems, antioxidants could come in form of four sources, which the first source was as enzymes such as superoxide

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dismutase; second as large molecules such as albumin; third as small molecules such as ascorbic acid and phenols; and finally as hormones such as melatonin (Prior *et al.*, 2005). The assays of antioxidant activity could also be generalized into hydrogen atom transfer reactions (HAT) and single electron transfer reaction (SET) (Huang *et al.*, 2005). More than one of these antioxidant activity assays should be performed to take into account the various mechanisms of antioxidant action (Frankel & Meyer, 2000)

Herbs were plants which their root, leaves, flower or bark were used for their medical properties. Some of the herbs could be directly consumed while others need to be boiled in water and only the water extract were consumed, with the remaining herb residue disposed; some of the herbs were not for consumption, but for external use (Marwah *et al*, 2007). People from developing countries still use herbs to practice their traditional medical systems which were important for their health care (Mahady, 2001). However in developed country such as the United States of America, they still use traditional medicine and herbs to cure diseases but with slight modifications (Issa *et al.*, 2006). This pattern of usage of herbs was being defined by World Health Organization (WHO) as one of practices in Complementary and Alternative Medicine (CAM) (WHO, 2002). A lot of herbs were found as source of high antimicrobial activity, anti-inflammation, anticarcinogenic, atherosclerosis, antimutagenic, angiogenesis inhibitory activities and antioxidant activities (Cordoso *et al.*, 2006; Kaefer & Milner, 2008; Jayaprakasha *et al.*, 2007). Compounds which could act as antioxidants such as phenolic compounds, ascorbic acid, alpha tocopherol and carotenoids were commonly found in herbs (Yoo *et al.*, 2008).

Herbs often require drying after harvested because they contained high moisture content which was the main factor contributed to the spoilage of highly perishable of herbs (Müller *et al*, 1989). Therefore, drying could improve shelf life, encapsulate original flavour, reduce storage volume and maintain nutritional values of herbs if compared to the fresh herbs (Gunhan *et al*, 2005). The antioxidant activity of the herb was found to be reduced after dried under sun, oven or freeze dried (Chan *et al.*, 2009). The degree of reduction of antioxidant activity in different drying methods was found to vary with different drying temperature and time (Katsube *et al.*, 2008). However, there

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were also studies which showed contradiction that the overall antioxidant properties of certain plants might be enhanced such as tomato (Dewanto *et al.*, 2002), ginseng (Kang *et al.*, 2006) and shiitake mushroom (Choi *et al.*, 2006).

The antioxidant activity of the plant extract were affected by extraction solvents, the pH of the solvent used, extraction time used, temperature of extraction process and particle size of the solid matrix (Chirinos *et al.*, 2007). Common solvents such as acetone, methanol, ethanol, water, hexane, chloroform, butanol and petroleum ether were used to extract antioxidant contained in herbs (Mohsen & Ammar, 2009). However, contradicts were found for the best solvent used because different sample examined would result in different best solvent for antioxidant activity. So, there was no solvent which was best for extraction of all antioxidant compounds (Zhao & Hall, 2008). Besides, to improve the extraction process by reducing the use of solvents and time in extraction, other innovative extraction methods such as supercritical fluid extraction (SFE) (Yi *et al.*, 2008), microwave assisted extraction (MAE) (Morales *et al.*, 2005), accelerated solvent extraction (ASE) and pressurized liquid extraction (PLE) had been introduced (Ong, 2004).

The herb *Limnophila aromatica* was a type of medical herb which could be found in South East Asia and tropical parts of Australia (Food Info, 2009). It was found that *Limnophila aromatica* contains high antioxidant activity (Kukongviriyapan *et al.*, 2007). Chemical compounds from *Limnophila aromatica* was identified with vacuum liquid chromatography and repeated column chromatography and uncommon oxygenated flavonoids was detected as 5,7-diOH- 6,8,4' triOMe flavone, 5-OH- 6,7,8,4'- tetraOMe flavone and 5,7-diOH- 6,4'- diOMe flavone (Bui *et al.*, 2004). There were studies that only compare the effect of drying methods alone (Chan *et al.*, 2009); and also study on the optimization of extraction conditions on herbs (Chirinos *et al.*, 2007). Due to optimization of both drying and extraction methods on herb had not being studied, the main objective of this study was to study the effect of extraction and drying methods on antioxidant activity on *Limnophila aromatica*.

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In pharmaceutical industry, herb often required to be processed into pure extract. In this process, drying and extraction steps had to be done efficiently to reduce the energy consumed and reduce cost of production (Fatouh *et al.*, 2006). Although studies on best extraction method used and best drying method used were available for reference, more studies were required to look into methods that were more applicable to all herbals but not methods for only a specific type of herb. The outcome of this study was to provide drying and extraction parameters to obtain high antioxidant activity for the industry and any possible further research.

The specific objectives of this study were:

1. To determine the effect of drying methods (sun, oven and freeze drying) to the antioxidant activity.
2. To determine the most appropriate solvent and extraction parameters (time, temperate, sample solvent ratio) to extract antioxidant from *Limnophila aromatica*.
3. To optimize the antioxidant extraction method for *Limnophila aromatica* by Response Surface Methodology (RSM).

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CHAPTER 2

LITERATURE REVIEW

2.1 The herbal industry

Herbal medicine could be used as part of the traditional medicinal practices and it had long history since ancient including Traditional Chinese Medicine, Traditional Arab Herbal Medicine, Indian Medicine, Kampo and Ayurveda (Mahady, 2001; Azaizeh *et al.*, 2008). The herbs were often in the form of root, leaves, flower or bark (Marwah *et al*, 2007). The usage of herbs in curing disease could be considered as part of Complementary and Alternative Medicine (CAM) (NCCAM, 2007). Besides that, some of the plants were treated as culinary herb and used as season and to preserve food. The examples of common culinary herbs were cinnamon, garlic, ginger, onion, parsley, pepper and peppermint (Kaefer & Milner, 2008).

Herbs were natural product and their chemical composition varies from one herb to another. Therefore, the effect of herb varies from people to people and there were some different usages of a same herb in different parts of the world (Firenzuoli & Gori, 2007). For example, *Zingiber officinale* or commonly known as ginger was used to treat dyspepsia, flatulence, colic and diarrhea in European countries but it was used to treat cold and influenza in African folk medicines (Kamtchouing *et al.*, 2002; Borrelli *et al.*, 2004). Besides that, the classification methods and theories in using herb also vary between different parts of the world. For example, Traditional Arabic and Islamic herbal medicine were almost same as the modern medicine practiced today (Azaizeh *et al.*, 2008). However, the traditional Chinese medicine follows the concept of *yin* and *yang*, and characterizes herbs into hot, warm, natural, cool and cold (Liao *et al.*, 2007).

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2.1.1 Global herbal industry

World Health Organization (WHO) stated that more than three-quarters of the world population were using traditional medicine which mainly herbs were used for healthcare (WHO, 2001). In year 2002, 75% of the African people still practices traditional medicine and 40% of Chinese people use traditional medicine as health care purposes (Dubey *et al.*, 2004). The percentage of people that had tried traditional medicine at least once were 70% in Canada, 48% in Australia, 42% in United States of America and 38% in Belgium (WHO, 2002).

It was estimated that in 1997, the European market on herbs had reached about $7 billion which German contributed half of the value, which was $3.5 billion. Herb market in France was $1.8 billion; Italy, $700 million; the United Kingdom, $400 million; Spain, $300 million; and Netherlands, about $100 million in 1997. Herbal medicine markets in Asia was $2.3 billion, Japan was $2.1 billion, and the United States of America had traded $3.2 billion in 1997 (Calixto, 2000). The herbal market at 2002 was US$ 23 billion and continued to grow to US$ 40 at 2004 (Kaphle *et al.*, 2006).

High number of the population in Africa still practices traditional medicine, which involves mainly on the use of herbals for curing purposes (Dubey *et al.*, 2004). The herbs used were estimated for about 20,000 tones and created a market of US$ 75 million a year (Mander and Le Breton, 2006). Therefore, it is estimated that there were about 200,000 to 300,000 people along a value chain from collectors, traders, healers and wholesalers who were involved with the trade of medicinal plants (Makunga *et al.*, 2008). Some of the commonly traded African herbs are *Aloe ferox* Mill, *Aspalathus linearis* (Burm.f.) R.Dahlgren, *Hypoxis hemerocallidea* Fisch, *Kigelia africana* (Lam.) Benth, *Leonotis leonurus* (L) R.Br., *Lippia javanica* (Burm.f.) Spreng and *Warburgia salutaris* (G.Bertol) Chiov (Germishuizen et al., 2006).

The herbal usage in United States of America was greatly influenced by traditional Chinese herbal therapy which involves the usage of more than 7000 species

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