EFFECT OF EXTRACTION METHODS ON ESSENTIAL OIL CONTENTS OF *BAECKEA FRUTESCENS* (BERUNGGIS)

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DISSERTATION SUBMITTED IN PARTIAL
FULFILMENT OF THE REQUIREMENTS FOR THE
DEGREE OF BACHELOR OF SCIENCE WITH HONOURS

INDUSTRIAL CHEMISTRY PROGRAMME
SCHOOL OF SCIENCE AND TECHNOLOGY
UNIVERSITY MALAYSIA SABAH

APRIL, 2007

PUMS 99:1

UNIVERSITI MALAYSIA SABAH

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JUDUL: EFFECT OF EXTRACTION METHODS ON ESSENTIAL OIL CONTENTS OF BACKEA FRUTESCENS

Ijazah: DEGREE OF BACHELOR OF SCIENCE WITH HONOURS

SESI PENGAJIAN: 2004/2005

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DECLARATION

I hereby declare that this dissertation is based on my original work, except for quotation and summaries each of which have been fully acknowledged.

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ACKNOWLEDGEMENT

First and foremost I would like to take this opportunity to express my thanks to my supervisor, Dr. How Siew Eng who had dedicated her precious time for guiding and supporting me throughout the whole journey of my research. Not only that, she also personally drove us to Tambunan to collect our sample. Without her patience in teaching me, I would never have finished this dissertation to fulfill the requirement of graduating. Thank you once again.

I would also like to take this opportunity to thank Prof. Dr. Marcus Jopony for his willingness to share his knowledge and reading materials to me as references. Also not forgetting was his support and patience in guiding to understand my work better.

During the time of my research, I was also guided by Pn. Zainab and En. Samudin. As the lab assistant, they had given me a lot of information regarding the lab equipments and instruments. Besides that, I would also like to thank for Cik Noridah Abbas – the lab assistant and Cik Farm Yan Yan from SKTM for their precious time to guide and help me to run the GC-MS for analyzing.

Lastly, I would also like to thank my family for their moral and financial supports; fellow course mates who had helped me to clean the lab when one of my experiments exploded. In addition, they also had also supported and accompanied me from the very beginning until the end of my research. Thank you very much!

ABSTRACT

This study was about extracting essential oils from *Baeckea frutescens* by conventional hydrodistillation (HD) method and ultrasonic-assisted extraction (USE) using ultrasound cleaning bath. The essential oils were analyzed using gas chromatography equipped with mass spectrometry (GC-MS). The weight of essential oils extracted using HD and USE was 1.098% (w/w) and 0.084% (w/w), respectively. Essential oils obtained by HD contained 13 components: 1R-.α.-Pinene; β-Pinene; Limonene; Eucalyptol; 3-Penten-2-ol; Caryophyllene; α-Caryophyllene; 3-Octyne; Cyclohexene 1-methyl-4-1-[1-methylethylidene]-; Hexanedioic acid, bis[2-ethylhexyl] ester; 2,6-Octadienoic acid,3,7-dimethyl-,methyl ester; Cyclohexene,3-methyl-6-[1-methylethenyl]-,[3R-trans]-; and 3-Cyclohexene-1-methanol, .alpha.,.alpha. 4-trimethyl- (α-terpineol). Comparatively, essential oils obtained by USE contained 8 components: 1R-.α.-Pinene; β-Pinene; Eucalyptol (1,8-Cineole); 1,6-Octadien,3,7-dimethyl-; p-menth-1-en-8 ol (α-terpineol); Eicosane; Hexadecane; and 1,2-Benzenedicarboxylic acid,bis[2- methylpropyl] ester. The major components of *Baeckea frutescens,* Eucalyptol (1,8-Cineole) and Pinenes, were found in both extracts. The presence of Hexanedioic acids, bis[2-ethylhexyl] ester, 2,6-Octadienoic acid,3,7-dimethyl-,methyl ester and 1,2-Benzenedicarboxylic acid,bis[2-methylpropyl] ester might possibly contribute to the aroma for the essential oils from *Baeckea frutescens*. The differences in components obtained by the extraction methods may probably be due to the use of drastic heat in HD compared in USE.

KESAN PELBAGAI KAEDAH PENGESTRAKAN KEATAS KANDUNGAN MINYAK PATI DARIPADA BAECKEA FRUTESCENS

ABSTRAK

Kajian ini adalah berkaitan dengan pengestrakan minyak pati daripada Baeckea frutescens *dengan menggunakan penyulingan (HD) dan pengestrakan ultrasonik (USE) dalam takungan ultrasonik. Hasilnya dianalisis dengan kromatografi gas lengkap dengan spektrometri jisim (GC-MS). Sebanyak 1.098% (w/w) dan 0.084% (w/w) minyak pati masing-masing diperolehi menerusi HD dan USE. Minyak pati yang diperolehi secara HD, mengandungi 13 komponen iaitu* 1R-.α.-Pinene, β-Pinene, Limonene, Eucalyptol, 3-Penten-2-ol, Caryophyllene, α-Caryophyllene, 3-Octyne, Cyclohexene 1-methyl-4-1-[1-methylethylidene]-, Hexanedioic acid, bis[2-ethylhexyl] ester, 2,6-Octadienoic acid,3,7-dimethyl-,methyl ester, Cyclohexene,3-methyl-6-[1-methylethenyl]-,[3R-trans]-, *dan* 3-Cyclohexene-1-methanol, .alpha.,.alpha. 4-trimethyl- (α-terpineol). *Minyak pati yang diperolehi secara USE pula mengandungi 8 komponen iaitu* 1R-.α.-Pinene, β-Pinene, Eucalyptol (1,8-Cineole), 1,6-Octadien,3,7-dimethyl-, p-menth-1-en-8 ol (α-terpineol), Eicosane, Hexadecane, *dan* 1,2-Benzenedicarboxylic acid,bis[2- methylpropyl] ester. *Kedua-dua komponen utama* Backea frutescens *iaitu* 1,8-Cienole dan Pinene *didapati hadir dalam kedua-dua ekstraks. Kehadiran* Hexanedioic acid, bis[2-ethylhexyl] ester; 2,6-Octadienoic acid,3,7-dimethyl-,methyl ester *dan* 1,2-Benzenedicarboxylic acid,bis[2-methylpropyl] ester *mungkin menyumbang kepada bau harum minyak pati yang diperolehi. Perbezaan komponen yang diperolehi menerusi kedua-dua kaedah pengestrakan mungkin disebabkan oleh keadaan ekstraksi dalam mana haba yang drastik digunakan dalam HD berbanding USE.*

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LIST OF SYMBOLS AND UNITS

%	Percentage
α	Alpha
β	Beta
γ	Gamma
g	Gram
mL	Mililitre
µL	Microlitre
L	Litre
°C	Degree Celcius
s	Second
min	Minute
mm	Milimetre
kHz	KiloHertz
eV	Electrovolt
ψ	psi
Pk#	Peak number
Ref#	Reference number
PA	Percent Area

LIST OF ABBREVIATIONS

GC	Gas Chromatography
MS	Mass Spectrometry
GC-MS	Gas Chromatography – Mass Spectrometry
HPLC	High Performance Liquid Chromatography
FID	Flame Ionization Detector
HD	Hydrodistillation
USE	Ultrasonic-Assisted Extraction
MAE	Microwave-Assisted Extraction
SFME	Solvent-Free Microwave Extraction
SFE	Supercritical Fluid Extraction
NMR	Nuclear Magnetic Resonance
GSC	Gas-Solid Chromatography
GLC	Gas-Liquid Chromatography
EI	Electron Impact
C_{15}	Carbon chain that contains 15 Carbon atoms
RT	Retention time
Qual	Quality
MW	Molecular weight

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CHAPTER 1

INTRODUCTION

1.1 CONTEXT RELEVANCE OF THIS STUDY

A large proportion of medicines employed in clinical practices today come from plants (herbal) as they are the nature's gift for synthesizing medicinal compounds (Huie, 2002; Teixeira & Fuchs, 2006). Due to low toxicity, high pharmaceutical activity and rare complication, these herbal medicines had played an important role in clinical therapy in many oriental countries for thousands of years (Wen *et al.*, 1993; Cheng *et al.*, 2006). The use of herbs in herbal medicine, including herbal extracts, can be found in the pharmacopoeias of numerous countries (Hostettmann *et al.*, 1995). The herbal plant that will be the focus in this study is *Baeckea frutescens* from the Myrtaceae family.

The medicinal properties of plants can be related in part to the presence of volatile constituents (e.g. essential oil) (Huie, 2002). Essential oil is also known as volatile oil and ethereal oil. It can be defined as concentrated, hydrophobic liquid containing volatile aromatic compounds extracted from plants. The essential oil extracted from the leaves of *Baeckea frutescens* can be used as health tonics and for scenting household preparations.

The essential oil extracted from *Baeckea frutescens* has a fragrance almost similar to "*minyak kayu putih*", a type of medicated oil. Essential oil can be extracted through extraction from plant by hydrodistillation (HD) and various extraction methods (e.g. steam distillation, hydrodistillation, solvent extraction, ultrasonic assisted extraction, microwave assisted extraction, expression Enfleurage and etc).

Sampling techniques (i.e. solvent extraction, steam distillation and hydrodistillation) followed by gas chromatography–mass spectrometry (GC–MS) are used for the analyses of essential oils and used as the tools of quality assessment for herbal medicines (Deng *et al.*, 2005; Fan *et al.*, 2006). Quantitative analysis of active components in traditional herbal medicines is a vital research subject because approximately 60–80% of the world's population still depends on herbal medicines for some aspects of primary health care (Farnsworth *et al.*, 1985; WHO, 2002). However, the aspect of quality control in herbal medicine, inconsistency in quality is an important problem (Liu *et al.*, 2006).

A need for the implementation of more rational sampling techniques to obtain high-quality essential oils is as important since the use of essential oil in the aromatherapy, medication, perfumery, flavor and fragrance industry is massive. Thus, sample preparation becomes the key procedure in modern chemical analysis and also a crucial first step in the analysis of medicinal plants to ensure and provide high-quality herbal products to consumers.

The techniques used so far (e.g. hydrodistillation and Soxhlet) are clearly environmentally aggressive, slow and not suitable both for thermolabile and volatile compounds (Luque de Castro *et al.*, 1999). In a recent study by Huie (2002), had mention the developments and applications of modern sample-preparation techniques for the extraction from medicinal plant or herbal material like solid-phase microextraction (SPME), supercritical-fluid extraction (SFE), pressurized-liquid extraction (PLE), microwave-assisted extraction (MAE), and solid-phase extraction (SPE) and their advantages. In previous studies, numerous comparisons had been made among the mentioned extraction methods based on the yield and the compounds in the essential oil content of various medicinal plants. However, not much are comparing the yield from different extraction method on *Baeckea frutescens*.

1.2 OBJECTIVES

The objectives for this study were:

1. To extract essential oils from *Baeckea frutescens* using various extraction methods.
2. To analyze the content of the essential oils extracted using gas chromatography-mass spectrometry (GC-MS).
3. To compare the content of essential oils extracted with various methods.

1.3 SCOPE OF THE STUDY

This study focused on *Baeckea frutescens* L. a medicinal plant from the Myrtaceae family. This plant sample was collected from Tombotuan, a small village in Tambunan, Sabah during October, 2006. Essential oil was extracted from the leaves of the plant using hydrodistillation (HD), and ultrasonic-assisted extraction (USE). Then, each extract was analyzed by gas chromatography-mass spectrometry (GC-MS) and the components of the essential oil were compared.

CHAPTER 2

LITERATURE REVIEW

2.1 *BAECKEA FRUTESCENS*

Baeckea frutescens L. (Myrtaceae) is a small evergreen tree of the Myrtaceae family which originated from Australia. It was one of the major bush species harvested in Queensland during the year 1997 to 1998.

The common names for *Baeckea frutescens* are weeping baeckea and weeping coast myrtle (Williams, 1979). However, locally it is known as *berunggis* or *rempah gunung* and in Chinese, it is called *gang song* (岗松). *Baeckea frutescens* belongs to the kingdom-*Plantae* (plant), subkingdom-*Tracheobionta* (vascular plant), superdivision-*Spermatophyta* (seed plant), division-*Magnoliophyta* (flowering plant), class-*Magnoliopsida* (dicotyledon), subclass-*Rosidae*, order-*Myrtales*, family-*Myrtaceae* and genus-*Baeckea* L.

2.1.1 Distribution of *Baeckea frutescens*

This plant is widely spread within Australia (Ibrahin Jantan *et al.*, 1998; Satake *et al.*, 1999). It also occurs widely outside Australia extending through South China, New Caledonia, Thailand, Malaya, Sumatra, Borneo, Celebes and New Guinea (Elliot & Jones, 1982; Mabberly, 1990; Wrigley & Fagg, 1996; Bean, 1997; Ibrahim Jantan *et al.*, 1998; Satake *et al.*, 1999; Wilson, 2002). Its beautiful and compact habit encourages this species to grow in a sunny environment, but will be sparser in shade (Elliot & Jones, 1982; Wrigley & Fagg, 1996). In some Asian countries it grows wild in shrub lands on poor sandy or ultra basic soils, high altitudes mountains and midlands (WHO, 1989; Bean, 1997). In peninsular Malaysia, *Baeckea frutescens* are normally being found growing by the sea or on mountain tops especially in Kelantan and Terengganu (Kochummen, 1973).

2.1.2 Morphology of *Baeckea frutescens*

Baeckea frutescens is a small shrub with the height approximately 15 to 20 feet from the ground (Corner, 1952; WHO, 1989). It has plenty of slender branches growing pointing outwards yet straight. The bark is brownish and all parts of the plant are strongly scented (WHO, 1989). Its leaves are needle-like (Photo 2.1) and have an aromatic scent when crushed. It is tiny yet plenty with approximately 0.6 to 1.5 cm in length and 0.005 cm in width (Kochummen, 1973), and are light green in color. It has a plenty of fine seed which can be spread easily. Its flowering period is around April until June but the whole plant is usually being collected from July until October (WHO, 1989). The flower is white in color

and a little brownish in the middle. It is tiny and solitary (WHO, 1989) with the diameter approximately 0.35 cm to 0.5 cm. Its flower can be found growing individually or in pairs. It has five petals which look like bell shape, sub-orbicular and has 7-10 stamens with half ovary. Its fruit is small and capsule-like (Bean, 1997). Photo 2.1 shows the *Baeckea frutescens* with its white flower.

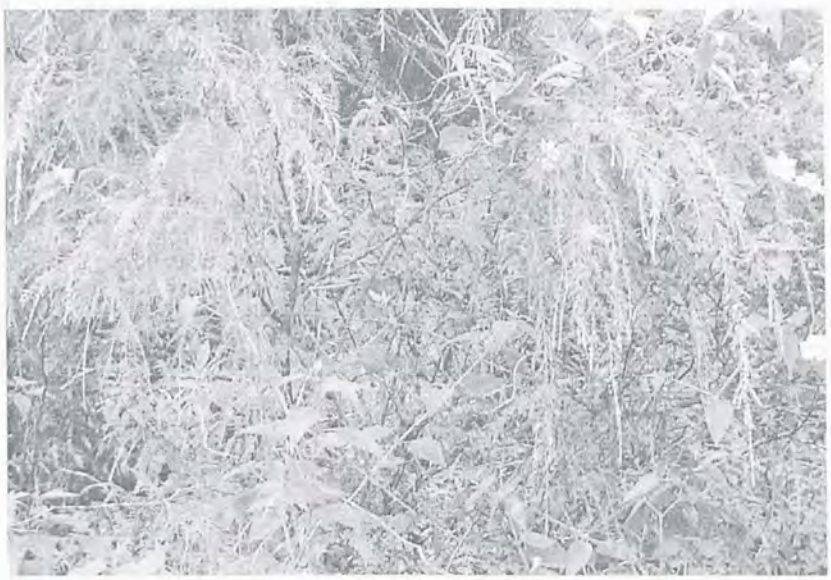

Photo 2.1 The *Baeckea frutescens* with its white flower.

2.1.3 Components in *Baeckea frutescens* Essential Oils

The essential oil of ***Baeckea frutescens*** can be obtained by distillation (WHO, 1989). Previous studies stated that the main components in *Baeckea frutescens* essential oil are pinenes (a type of monoterpenes) and 1, 8-Cineole (also called Eucalyptol) (Ibrahim Jantan *et al.*, 1997).

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