Faculty of Resource Science and Technology

STUDIES ON THE ESSENTIAL OILS FROM *ALPINIA* SPP. AND *BOESENBERGIA* SPP.

Maya Asyikin Binti Mohamad Arif

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STUDIES ON THE ESSENTIAL OILS FROM *ALPINIA* SPP. AND *BOESENBERGIA* SPP.

MAYA ASYIKIN BINTI MOHAMAD ARIF

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DECLARATION

No portion of the work referred in this final year report has been submitted in support of an application for another degree of qualification of this or any other university or institution of higher learning.

Maya Asyikin Mohamad Arif

Program of Resource Chemistry

Faculty of Resource Science and Technology

University Malaysia Sarawak

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LIST OF TABLES

Tables | Page

Table 1.1: The uses of selected species from the genus *Alpinia*. 6

Table 4.1: Percentages and colour of essential oil extracted from different parts of three *Alpinia* spp. 18

Table 4.2: Percentages and colour of essential oil isolated from different parts of three *Boesenbergia* spp. 19

Table 4.3: Chemical composition of the rhizome oils of *Alpinia galanga, Alpinia galabra* and *Alpinia aquatica.* 23

Table 4.4: Chemical composition of the leaves oils of *Alpinia galabra* and *Alpinia aquatica.* 27

Table 4.5: Chemical composition of the rhizome oil, stem oil, flowers oil and leaves oil of *Alpinia galabra.* 30

Table 4.6: Chemical composition of the rhizome oil, leaves oil and fruit oil of *Alpinia aquatica.* 33

Table 4.7: Chemical composition of the rhizome oils of *Boesenbergia rotunda, Boesenbergia pulchella* and *Boesenbergia parva.* 39

LIST OF FIGURES

Figures Page

Figure 2.1: Structure of several compounds isolated from *A. galanga* 7

Figure 2.2: Structure of several compounds isolated from *B. pandurata* 9

Figure 4.1: GC/FID trace of rhizome oil of *Alpinia galanga* 20

Figure 4.2: GC/FID trace of rhizome oil of *Alpinia galabra* 21

Figure 4.3: GC/FID trace of rhizome oil of *Alpinia aquatica* 22

Figure 4.4: GC/FID trace of leaves oil of *Alpinia galabra* 25

Figure 4.5: GC/FID trace of leaves oil of *Alpinia aquatica* 26

Figure 4.6: GC/FID trace of stem oil of *Alpinia galabra* 29

Figure 4.7: GC/FID trace of flower oil of *Alpinia galabra* 30

Figure 4.8: GC/FID trace of fruit oil of *Alpinia galabra* 33

Figure 4.9: GC/FID trace of rhizome oil of *Boesenbergia rotunda* 36

Figure 4.10: GC/FID trace of rhizome oil of *Boesenbergia pulchella* 37

Figure 4.11: GC/FID trace of rhizome oil of *Boesenbergia parva* 37

Figure 4.12: Examples of some monoterpenes and sesquiterpenes that dentified in both *Alpinia* spp. and *Boesenbergia* spp. 42

Figure 4.13: Dendogram of cluster analysis using data from quantitative analysis (*Alpinia* spp.) 43

Figure 4.14: Dendogram of cluster analysis using data from quantitative analysis (*Boesenbergia* spp.) 44

Figure 4.15: Percentage of death of *Artemia salina* as a function of concentration of rhizome oil of *Alpinia galanga, Alpinia galabra* and *Alpinia aquatica* 45

Figure 4.16: Percentage of death of *Artemia salina* as a function of concentration of rhizome oil, leaves oil, stem oil and flower oil of *Alpinia galabra* 46

Figure 4.17: Percentage of death of *Artemia salina* as a function of concentration of rhizome oil, leaves oil and fruit oil of *Alpinia aquatica* 47

Figure 4.18: Percentage of death of *Artemia salina* as a function of concentration of rhizome oil of *Boesenbergia rotunda, Boesenbergia pulchella* and *Boesenbergia parva* 48

ABSTRACT

The essential oils were isolated by hydrodistillation from different parts of *Alpinia* spp. and *Boesenbergia* spp. The oils were then analysed by capillary gas chromatography equipped with either flame ionization detector (GC/FID) or mass spectrometer (GC/MS). Percentage yield of essential oil from *Alpinia* spp. and *Boesenbergia* spp. studied were in the range of 0.56% to 5.86% and 5.70% to 16.33%, respectively. Rhizome oil of *A. galanga, A. galabra* and *A. aquatica* were dominated by β-myrcene (30.71%), aromadendrene (48.72%) and γ-selinene (48.37%), respectively. Stem oil, leaves oil and flower oil of *A. galabra* are comprised mainly of α-gurjunene (52.40%), cis-sabinene hydrate (16.28%) and (Z)-caryophyllene (51.99%), respectively. β-bourbenene (79.6%) and t-muurolol (25.39%) are the major compounds identified in the leaves and fruit oils of *A. aquatica*. Major compounds that were identified in the rhizome oil of *B. rotunda, B. pulchella* and *B. parva* are trans-pinocarveol (38.89%), β-selinene (36.92%) and (+)-β-pinene (3.76%), respectively. Toxicity test on *Artemia salin.* was performed for all the essential oils obtained. The result indicate that all the essential oils have not shown any significance toxicity effect towards *Artemia salina.*

Key words: Essential oil, Zingiberaceae, *Alpinia* spp., *Boesenbergia* spp., Gas chromatography

ABSTRAK

Minyak pati telah dipisahkan menggunakan kaedah penyulingan hidro dari beberapa bahagian tumbuhan *Alpinia* spp. dan *Boesenbergia* spp. Minyak pati tersebut seterusnya dianalisis menggunakan kromatografi gas yang dilengkapkan dengan pengesan pengionan nyalaan (KG/PPN) atau spektrometer jisim (KG/SJ). Peratus hasil minyak pati dari *Alpinia spp.* dan *Boesenbergia spp.* yang dikaji adalah dalam julat 0.56% hingga 5.86% dan 5.70% hingga 16.33%, masing-masingnya. Minyak rizom bagi *A. galanga*, *A. galabra* dan *A. aquatica* adalah didominasi oleh β-mirsena (30.71%), aromadendrena (48.72%) and γ-selinena (48.37%), masing-masingnya. Komponen utama minyak pati daripada batang, daun dan bunga bagi *A. galabra* adalah α-gurjunena (52.40%), sis-sabinena hidrat (16.28%) dan (Z)-kariofilena (51.99%), masing-masingnya. β-bourbenena (79.6%) dan t-muurolol (25.39%) pula adalah komponen utama yang dikenalpasti dalam minyak pati daripada daun dan buah *A. aquatica*. Manakala, komponen utama yang terdapat dalam minyak pati *B. rotunda*, *B. pulchella* dan *B. parva* adalah trans-pinokarveol (38.89%), β-selinena (36.92%) dan (+)-β-pinena (3.76%). Ujian ketoksikan terhadap anak udang, *Artemia salina* telah dilakukan bagi kesemua minyak pati yang telah diperoleh. Keputusan menunjukkan kesemua minyak pati yang dikaji tidak menunjukan kesan ketoksikan yang ketara terhadap *Artemia salina*.

Kata kunci: Minyak pati, Zingiberaceae, *Alpinia* spp., *Boesenbergia* spp., Kromatografi gas

TABLE OF CONTENTS

DECLARATION	ii
ACKNOWLEDGEMENT	iii
LIST OF TABLES	iv
LIST OF FIGURES	v
ABSTRACT	vii
ABSTRAK	viii
CHAPTER 1: INTRODUCTION	1
CHAPTER 2: LITERATURE REVIEW	
2.1 Zingiberaceae family	3
2.2 *Alpinia* spp.	3
2.3 *Boesenbergia* spp.	8
2.4 Extraction methods	10
CHAPTER 3: MATERIAL AND METHODS	
3.1 Plant materials	13
3.2 Extraction of essential oils	13
3.3 Instrumental analysis of essential oils	14
3.4 Cluster analysis	16
3.5 Bioassay	16
CHAPTER 4: RESULT AND DISCUSSION	
4.1 Percentages of essential oils	18
4.2 Chemical components in essential oils	20
4.3 Cluster analysis	43
4.4 Bioassay	45

CHAPTER 5: CONCLUSION 50

REFERENCES 51

CHAPTER 1

INTRODUCTION

The genus *Alpinia* and *Boesenbergia* belong to the Zingiberaceae family. This family consists of not less than 50 genera and 1300 species, distributed throughout the tropics particularly in Southeast Asia (Vaniiaiiya *et al.*, 2003). In Peninsular Malaysia, there are at least 22 genera and 150 species recorded (Sirat and Jamil, 1999). *Alpinia* and *Boesenbergia* are among the genera which have many species being used as ingredients in traditional medicines and flavouring (Sirat and Jamil, 1999).

Alpinia is a large, polymorphic genus, comprising over 250 species, which occurs throughout South and Southeast India and the Solomon Islands, Fiji, Samba and Australia (Valkenburg and Bunyapraphatsara, 2001). *Alpinia* species are characterized by a wide range of volatile compounds and have been subjected for numerous phytochemical studies (Fujita *et al.*, 1994; De Pooter *et al.*, 1995; Kuster *et al.*, 1999). According to Valkenburg and Bunyapraphatsara (2001), the rhizomes of medicinally used *Alpinia* are taken orally for indigestion, stomach-ache and diarrhoea and externally applied for rheumatism, wounds, sores and ringworms (Valkenburg and Bunyapraphatsara, 2001).

Major compounds present in the *Alpinia galanga* leaves essential oil are 1,8-cineole, camphor, β-pinene, (E)-methyl cinnamate, bornyl acetate and guaiol, which can be found in their leaves. Meanwhile, in its rhizome, compounds such as 1,8-cineole, camphor, β-pinene, (E)-methyl cinnamate, α-fenchyl acetate and guaiol have been reported (Jirovetz *et al.* (2003).

Boesenbergia spp. are small herbaceous plants with short and fleshy rhizomes. Many *Boesenbergia* spp. can be used as food and spices, and for traditional medicine such as for colic disorder problem (Tuchinda *et al.*, 2002; Vaniiaiiya *et al.*, 2003). Several compounds have been isolated from *Boesenbergia pandurata* such as camphor (16.1 to 32.1 %), geraniol (16.2 to 26.0 %), (E)-β-ocimene (19.0 to 23.7 %), 1,8-cineole (7.5 to 13.9 %), camphene (5.4 to 6.0 %) and also methyl cinnamate (2.2 to 5.8 %) (Jantan *et al.*, 2001). Besides the essential oils, other compounds such as flavones, pinostrobin, alpinetin, pinocembrin, chalcones and dihydrochalcones have also been isolated from *B. pandurata* (Trakoontivakorn *et al.*, 2001).

Chemometrics method, which is used to analyze the differences in each species of *Alpinia* spp. and *Boesenbergia* spp. is adopted from Hibert (1997) and Otto (1999). By using cluster analysis, compounds can be classified according to their proximity in the variable space. The first step of a typical aggregation method is to find the two compounds closest together and place them by a cluster with coordinates midway between them. This step will be repeated until the entire set is clustered. In the essential oil literature, the most commonly used pattern recognition method is the hierarchical clustering.

The purpose of this study is to extract the essential oils from the *Alpinia* spp. and *Boesenbergia* spp. using hydrodistillation method. The essential oils isolated from these samples was identified and characterized by gas chromatography and gas chromatography-mass spectrophotometry. The biological activity of these oils especially toxicity on brine shrimps, *A. salina* was also be studied.

CHAPTER 2

LITERATURE REVIEW

2.1 Zingiberaceae family

The plant family Zingiberaceae with about 1300 species and 50 genera can be found throughout the world mainly in the tropic (Vaniiaiiya *et al.*, 2003). Its center of distribution lies in Southeast Asia, where more than half of the species occurs, with many small endemic genera numbers (Larsen *et al.*, 1996). For example, the tribe Hedychieae in the family Zingiberaceae consists of 20 genera (Vaniiaiiya *et al.*, 2003).

The Zingiberaceae family is characterized by its aromatic properties since they are rich in essential oils, which are very important in the cosmetic and pharmaceuticals industry. In Malaysia, there are about 30 to 40 species of Zingiberaceae which have long been used as traditional medicine. A variety of diseases and ailments such as rheumatism, high blood pressure, sinus and indigestion can be cured by some species in Zingiberaceae family (Vaniiaiiya *et al.*, 2003).

2.2 *Alpinia* spp.

Alpinia is a large, polymorphic genus comprising over 250 species and belongs to the tribe Alpiniae, which also includes *Amomum, Elettaria* and *Riedelia* (Valkenburg and Bunyapraphatsara, 2001). *Alpinia* is a large genus, and there have been several attempts at a subgeneric classification. The most recent classification divides the genus into 2 subgenera, which are *Alpinia* and *Diaremalpinia*. This classification is based mainly on the character of the labellum, whether it is petaloid or non-petaloid (Valkenburg and Bunyapraphatsara, 2001). Subgenus *Alpinia* occurs throughout the area of distribution of the genus but has a centre of diversity in continental Asia, while subgenus *Dieramalpinia*, has a centre of diversity in New Guinea and the Mollucas (Valkenburg and Bunyapraphatsara, 2001).

From the ecological aspect, *Alpinia* normally prefers humid, shady conditions and not too high temperatures, normally between 27 to 30 °C during daytime and 17 to 18 °C at night (Valkenburg and Bunyapraphatsara, 2001). They often occur in secondary vegetation, bamboo and teak forest. While near villages, they usually grow in the open since they require rich soils (Valkenburg and Bunyapraphatsara, 2001).

Alpinia is a genus in which many complex compounds are found in the aerial parts and in the rhizomes (Valkenburg and Bunyapraphatsara, 2001). Several species contain diaryheptanoid, where the structure resembles to that of curcuminoid and possesses potent anti-inflammatory properties (Valkenburg and Bunyapraphatsara, 2001). The crude water extract showed significant *in-vivo* activity against experimentally induced acute ulcers especially stress ulcer, Shay's ulcers, aspirin-induced gastric ulcers, mepirizole-induced, duodenal lesions and experimentally induced chronic ulcers, especially acetic-acid induced ulcers and thermocautery ulcers (Valkenburg and Bunyapraphatsara, 2001).

The essential oil from fresh and dry rhizomes of *A. galanga* showed *in-vitro* and *in-vivo* antibacterial, antifungal, anti protozoal and expectorant activities (Valkenburg and Bunyapraphatsara, 2001). The water, alcohol and ether extract of the rhizome has strong antibacterial properties against *Bacillus subtilis, Escherichia coli, Staphylococcus aureus* (several strains), *Aeromonas hydrophila, Pseudomonas aeruginosa* and *Klebsiella pneumoniae* (Valkenburg and Bunyapraphatsara, 2001). The alcohol and chloroform extracts also showed antifungal activity against *Candida albicans*, *Cryptococcus neoformans*, *Epidermophyton floccosum*, *Microsporum gypseum* and *Trichophyton rubrum* (Valkenburg and Bunyapraphatsara, 2001).

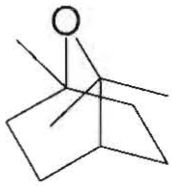
The essential oils from all parts of *A. zerumbet* (from Egypt), also exhibited significant antimicrobial activity against certain gram-positive bacteria such as *Bacillus subtilis, Mycobacterium phlei, Sarcina lutea and Staphylococcus aureus* and gram-negative bacteria, such as *Escherichia coli* and *Pseudomonas aeroginosa*. Geraniol and isothymol from the essential oils of the rhizome possess high antimicrobial activity against plant pathogenic fungus (Valkenburg and Bunyapraphatsara, 2001).

Alpinia spp. has a lot of uses and application, especially in traditional medicines. *Alpinia* spp. can cure diseases and ailments such as diarrhoea, rheumatism, jaundice, bronchitis and others. This can be taken orally or externally applied to cure diseases and ailments. The uses and application of *Alpinia* spp. can be develop to produce more effective drugs in treating chronic diseases like cancers, diabetes and others. Table 1.1 simplified the uses of selected species from the genus *Alpinia*.

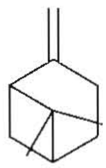
Table 1.1: The uses of selected species from the genus *Alpinia* (Valkenburg and Bunyapraphatsara, 2001).

Species	Uses and application
Alpinia conchigera Griffith	The rhizomes can be used in the treatment of bronchitis, jaundice, headache and vertigo, as well as for ringworms, indigestion and abscesses.
Alpinia elegans (C.Presl) K. Schum.	The rhizome can be taken for haemoptysis and soaked in the water for headache. The leaves, pounded with a little salt, can be rubbed on paralyzed parts of a patient.
Alpinia galanga (L.) Wild	The rhizomes are used for the treatment of skin disease, respiratory disease, for intestinal problems, mouth and stomach cancers, and also as an expectorant. The seeds are prescribed for diarrhoea, vomiting and herpes.
Alpinia malaccensis (Burm.f.) Roscoe	The pounded rhizome can be used to cure wounds and sores. It can also be chewed together with betel nut to make the voice strong and clear and the decoction can be used for bathing feverish people.
Alpinia officinarum Hance	The rhizomes are widely used for dyspepsia, flatulence, vomiting, gastralgia, colic disorder, diarrhoea, fever and malaria. The seeds can also be used in the treatment of heartburn, cholera, toothache, ague and colds.

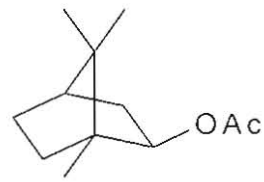
The major compounds that have been isolated from *Alpinia galanga* are such as 1,8-cineole, β-pinene, (E)-methyl cinnamate, bornyl acetate, guaiol, α-terpineol, α-fenchyl acetate, borneol and elemol (Jirovetz *et al.*, 2003). These compounds give aromatic properties to *Alpinia galanga*.



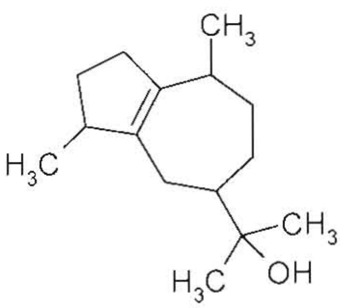
1,8-cineole



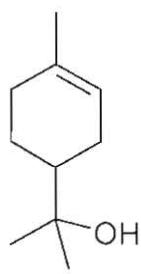
β-pinene



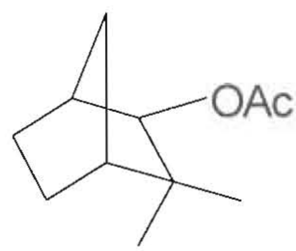
Bornyl acetate



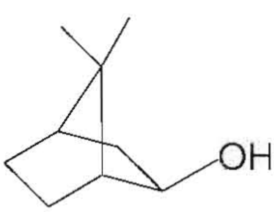
Guaiol



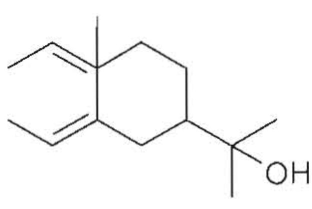
α-terpineol



α-fenchyl acetate



Borneol



Elemol

Figure 2.1: Structure of several compounds isolated from *A. galanga*

2.3 *Boesenbergia* spp.

Boesenbergia spp. are small herbaceous plants with short and fleshy or slender rhizomes (Vaniiaiiya *et al.*, 2003). Many species of *Boesenbergia* can be used as food and spices, and for traditional medicine such as treatment of colic disorder (Tuchinda *et al.*, 2002; Vaniiaiiya *et al.*, 2003;). The extract of the rhizomes from several *Boesenbergia* spp. such as *Boesenbergia curtisii* and *Boesenbergia plicata* exhibited antitumor and anti mutagenic activity (Trakoontivakorn *et al.*, 2001).

One of the most widely used species is *Boesenbergia pandurata,* which has a strong odor and dominating flavor. Some species of *Boesenbergia* have several uses especially in the traditional medicinal practices. For example, *Boesenbergia rotunda* (L.) Mansf. have effects like anti inflammatory for the oral Mucus membranes, relief of bacterial dysentery, interities, stomachache, apositia, anti flatulence and meteorism. The infusion of the root is also believed to promote appetite and prolong life. Meanwhile, the infusion of *Boesenbergia pandurata* (Roxb.) Schltr can relieve flatulence, indigestion and diarrhoea. It can also provides energy and work as vermicide to stimulate nerves.

Based on the previous study, major compounds that have been isolated from *Boesenbergia pandurata* are 1,8-cineole, geraniol, (E)- β-ocimene, camphene, camphor and methyl cinnamate (Jantan *et al.*, 2001). Besides essential oils, other compounds such as flavones (pinostrobin, alpinetin, pinocembrin), chalcones (cardamonin) and dihyrochalcones have also been isolated from *Boesenbergia pandurata* (Trakoontivakorn *et al.*, 2001).

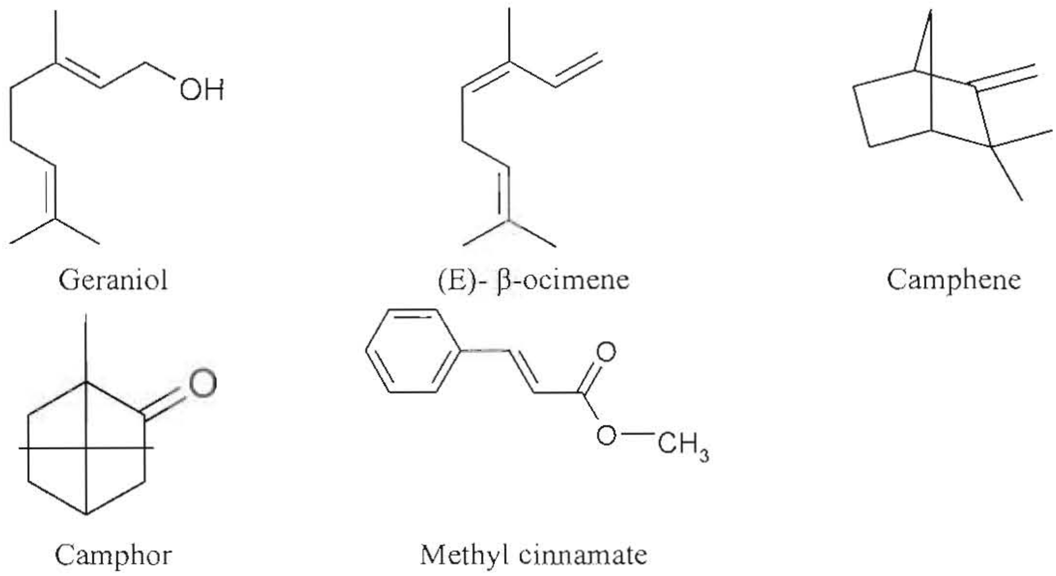


Figure 2.2 : Structure of several compounds isolated from *B. pandurata*

These compounds have their own properties and usages in many fields. For example, 1,8-cineole is active against stored-product beetles and also show insecticidal activity toward the Psyllid *H. cubana* (Nakatsu *et al.,* 2000). Whereas, in the consumer household products, a solution that contained 1,8-cineole, as the active ingredients, can be used to give encapsulated materials suitable for the manufacture of these odour-absorbing, antibacterial, tickicidal fibres (Nakatsu *et al.,* 2000).

Another compound, which is geraniol is one of the acyclic terpenoids that showed strong inhibitory effect on tyrosine activity. The tyrosinase inhibitory effect of geraniol was also shown by the inhibition of melanin production in melanoma cells, which is about 30 % inhibition activity (Nakatsu *et al.*, 2000).

2.4 Extraction methods

There are various extraction methods that can be employed for the isolation of the essential oils. The methods of the extraction of essential oils from plants will give significant effects to the chemical constituents and composition of the essential oils. Therefore, to concentrate the targeted biologically active compound into the essential oils, the most appropriate and convenient method should be selected. The commonly used extraction methods are such as cold press extraction, steam distillation, solvent extraction, and simultaneous distillation-solvent extraction (SDE) and supercritical fluid extraction (SFE).

2.4.1 Cold press extraction

This extraction technique is the simplest, least harmful and the best method to maintain the integrity of the essential oils, since it does not require very high temperature to isolate the compounds in the essential oils. This method has most recently been used to isolate oxygen-containing species, which tend to rearrange or degrade when heat is applied (Nakatsu *et al.*, 2000). Nevertheless, even when this method is used certain chemical species are difficult to isolate. For example, meranzim, isolated from orange peel contains a very reactive epoxide group and can easily be converted to other material in slightly acidic media (Nakatsu *et al.*, 2000).

2.4.2 Steam-distillation

This method is commonly used for industrial scale extractions but also has widespread use in laboratory studies. To yield a condensate, the extraction can be done by passing the steam through a vessel containing sample-mixture (Milner *et al.*, 1997). In this technique, the pH of the liquid phase is usually not controlled. Therefore, the composition of the oil in the condensate may be pH dependent (Milner *et al.*, 1997). In steam distillation, the plant material is subjected to temperatures up to 100 ^{0}C, but the degree of liquid or condensate that controls the plants varies substantially between the extremes of steam distillation (Milner *et al.*, 1997).

2.4.3 Solvent extraction

Solvent extraction method can be done by simply soaking the plant material in organic solvents at ambient temperatures or below or by boiling solvent in a Soxhlet-type apparatus (Milner *et al.*, 1997). The advantage of this method over steam-distillation method is that the lower temperature is used during the extraction process. While in the Soxhlet-type extraction, the boiling point of the solvent usually is below 60 ^{0}C and the temperature is typically 5 ^{0}C to 25 ^{0}C when the plant material is simply soaked in the organic solvent (Milner *et al.*, 1997).

2.4.4 Simultaneous distillation-solvent extraction (SDE)

This technique involves the combination of steam distillation and solvent extraction. This can be done to obtain a more complete oil balance. In this technique, Likens-Nickerson type apparatus is used to isolate the oil by removing it from the substrate and minimizing oil contact with the hot water. This technique can be modified by introducing vacuum condition to the system, which allow for the use of low operating temperature (20 ^{0}C to 40 ^{0}C). This technique is more effective in eliminating some of the most commonly observed artefacts.

2.4.5 Supercritical fluid extraction (SFE)

Supercritical fluid extraction (SFE) is a solvent extraction process that uses a supercritical fluid as the extraction solvent. Supercritical fluids have solvent strength that approach those of liquid solvents and thus will dissolve many substances. Supercritical fluids are able to rapidly transfer dissolved solutes through materials since they have diffusion coefficients that are close to those of gases. This method is faster than liquid extraction and the supercritical fluid solvents are easily removed by reducing the pressure to remove the extracted analytes.

These fluids can make possible separation of multi component mixtures by capitalising on differences in component volatilities and differences in the specific interaction between the mixture components and the supercritical fluid solvents or the solubility (Milner *et al.*, 1997). In general, the essential oil yields from liquid and supercritical carbon dioxide extractions are higher than the corresponding extraction with steam-distillation because supercritical fluid extraction can produce essential oils that have organoleptic properties that more closely resemble those of the plants from which they were extracted (Milner *et al.*, 1997).

CHAPTER 3

MATERIALS AND METHODS

3.1 Plant material

Fresh leaves, rhizomes, flowers, fruits and stems of *Alpinia* spp. and *Boesenbergia* spp. has been collected from the forest around Universiti Malaysia Sarawak, Niah and Simunjan areas. The essential oils from these samples have been isolated using hydrodistillation techniques and herbarium specimens were prepared for the purpose of identification.

3.2 Extraction of essential oil

This method is adopted from Datta (1987). First, the essential oils were extracted using hydrodistillation method in a Clevenger-type apparatus. To perform this extraction, about 100 grams of fresh grounded rhizomes was mixed with 1.5 L of distilled water in a 2 L flat bottom flask, which was assembled to the clevenger trap and connected to the condenser. Hydrodistillation was carried out for 6 hours. The flask was heated to maintain the distillation rates of two drops per second. The oils were separated and dried over anhydrous sodium sulphate and stored at temperature of 4 ^{0}C to 5 ^{0}C. The extraction was repeated for two to three times and the average percentages was calculated.