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ESSENTIAL OIL FROM FIVE *ALPINIA* SPECIES OF SARAWAK

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DECLARATION

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

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Essential Oil from Five *Alpinia* Species of Sarawak

ABSTRACT

Essential oils from five species of *Alpinia* were investigated for their chemical composition and biological activity against *Artemia salina*, *Coptotermes* sp and fungi species; *Trametes Versicolor*, *Gloeophyllum Trabeum* and *Chaetomium*. Essential oil was extracted by using hydrodistillation method and subsequently analyzed by using gas chromatography/flame ionization detector (GC/FID). The percentages of essential oil obtained from five *Alpinia* species ranged from 0.37% (v/w) to 2.56% (v/w). Generally, the highest value of 2.56% (v/w) was obtained from rhizome of *A. latilabris,* while the lowest yield obtained from stem of *A. latilabris* with the percentage of 0.37% (v/w). Leaves of *A. amentaceae* were rich in β-carene (87.33%) and ocimene (5.90%) while ethylpyrazine (50.67%) and nonane (35.10%) was the major compound in rhizome oil. The most abundant compound in *A. aquatica* (Campus) was β-bourbonene (16.83%) and hexanithiol (12.58%), from leaves oil and 2-dodecenal (38.26%) from rhizome oil. The major compound was found in the leaves of *A. nieuenhuizii* was β-pinene (36.35%) and 2-pentanol (22.02%) in rhizome oil. In the *A. latilabris* the major compound found in leaves oil was wine lactone (16.66%) and dimethyl pyrazine (16.43%), while in stem oil was ethylpyrazine (50.32%) and dimethyl pyrazine (26.22%) was found in rhizome oil. A. aquatica (Lawas) was found to be rich in 2-pentanol (58.99%) for leaves oil, furfuryl mercaptan (39.66%) for stem oil and 2-dodecenol (17.30%) for rhizome oil. The bioassay tests against *A. salina* for *Alpinia* sp. have not shown any biological activity. Termicidal test on *Coptotermes* sp showed that the leaves oil of *A. aquatica (*Lawas) gave significant value with the LC_{50} of 0.75%, while the rhizome oil of *A. aquatica (*Lawas) showed no significant value with the LC_{50} of 1.50%. The antifungal test on *Alpinia* sp. showed no biological activity.

Key words: *Alpinia*, gas chromatography/flame ionization detector, essential oils, hydrodistillation, brine shrimp.

Minyak Pati Daripada Lima Spesies *Alpinia* Sarawak

ABSTRAK

Minyak pati daripada lima spesies Alpinia telah dikaji komposisi kimia dan aktiviti biologinya terhadap Artemia salina, Coptotermes sp dan spesis-spesis kulat; Trametes versicolor, Gloeophyllum Trabeum dan Chaetomium. Minyak pati telah diekstrak melalui kaedah penyulingan hidro dan komposisinya telah di analisis dengan menggunakan gas kromatografi/pengesan ion nyalaan. Peratusan minyak pati yang didapati daripada lima spesies Alpinia ini adalah dalam julat antara 0.37% hingga 2.56%. Secara umumnya, peratusan tertinggi telah diperoleh daripada akar A. latilabris sebanyak 2.56%, sementara nilai terendah diperoleh daripada batang A. latilabris dengan peratusan 0.37%. Daun A. amentaceae kaya dengan β-karena (87.33%) dan osimena (5.90%), sementara etil pirazina (50.67%) dan nonana (35.10%) merupakan komposisi utama yang terdapat dalam minyak akar. Kelimpahan tertinggi dalam A. aquatica (Kampus) adalah β-borbonena (16.83%) dan heksanitiol (12.58%), yang didapati daripada daun, manakala 2-dodecenal (38.26%) didapati daripada akar. Komposisi utama yang terdapat daripada daun A. nieuenhuizii adalah β-pinena (36.35%) dan 2-pentanol (22.02%) daripada akar. Komposisi utama yang terdapat dalam A. latilabris adalah wain lakton (16.66%) dan dimetil pirazina (16.43%), sementara komposisi utama batang adalah etilpirazina (50.32%) dan dimetil pirazina (26.22%) ditemui dalam akar. Ujian bioassei terhadap A. salina yang telah dijalankan ke atas spesies Alpinia tidak menunjukkan sebarang aktiviti biologi. Ujian aktiviti terhadap Coptotermes sp memberikan aktiviti terkuat ke atas minyak A. aquatica (Lawas), dengan nilai LC_{50} *0.75%, sementara bahagian akar A. aquatica (Lawas) hanya memberikan nilai* LC_{50} *1.50%. Ujian anti-kulat ke atas spesies Alpinia tidak menunjukkan sebarang aktiviti biologi.*

Kata kunci: *Alpinia sp, penyulingan hidro, kromatografi gas/pengesan ion nyalaan, minyak pati, ujian ketoksikan.*

CHAPTER ONE

INTRODUCTION

1.1 Introduction

The *Zingiberaceae* are part of the order *Zingiberales*, which form an isolated group among the monocotyledons (Larsen *et al*., 1999). It can be found throughout tropical and subtropical region with the main distribution in Asia. The center of diversity of *Zingiberaceae* with at least 20 genera and 160 species occurring in Borneo and 23 genera with 200 species has been recorded in Peninsular Malaysia (Holttum, 1950). Plants in the *Zingiberaceae* family have been used in traditional medicine to cure variety of diseases such as rheumatism, high blood pressure, sinus and indigestion. *Alpinia* belong to this family with *Alpinia* being the largest genus in the family, followed by *Amomum* as the second largest genus in the family of *Zingiberaceae* (Larsen *et al*., 1999). There are several species of *Alpinia* which can be found in Malaysia and only some of the species will be discussed in this study.

Essential oils are volatile compounds which are freely soluble in organic solvent such as alcohol, ether and vegetables and mineral oils and are usually assumed to be that result of distillation or a steam-stripping process (Wilson *et al*., 2000). In Asia, the essential oils from rhizomes and leaves are usually used as a perfume in cosmetics, hair washes, powders and to protect the clothing against insects (Ian *et al*., 2000). Furthermore, essential oils can be used to flavor many foods including sweets, candies, cookies, snacks and chewing gum. Besides that, essential oil is used in aromatherapy. Another important property of essential oil which lead to profitable potential include antimicrobial effects that can defense mechanism in plants against microbial pathogens (Ian *et al.*, 2000).

Essential oils components composed of various kinds of molecules such as terpenoids, phenolics, aromatics, cyclic and acyclic compounds, acetonides, and sulfur- and nitrogen-containing compounds depending on the plant and extraction method used. The main components of essential oil are monoterpenes (C_{10}) and sesquiterpenes (C_{15}) (Parker, 1993). This chemical composition and aroma of essential oils give many valuable psychological and physical therapeutic benefits, which can be achieved through inhalation methods and application of the diluted oil to the skin.

Gas chromatography has proved to be one of the most widely used methods for separation and analysis of organic compounds. It can be used to separate many complex mixtures such as that in gasoline. The sample which will be used in a Gas Chromatographic separation must either be a gas or being capable of being converted to a gas at the temperature of the column. Generally, GC cannot be used for analysis of inorganic salts because inorganic salts cannot be converted to gases at the temperature at which GC columns are operated. As with the several forms of liquid chromatography, GC provides both qualitative and quantitative analytical information (Braun, 1987).

1.2 Objectives of the Project

The objectives of this study are:

i. To extract essential oil from several species of *Alpinia* found locally by hydrodistillation method.
ii. To determine the percentages yield and chemical composition of essential oils in *Alpinia* by using gas chromatography/flame ionization detector (GC/FID).
iii. To study the biological activities of the essential oils in *Alpinia* through bioassay test.
iv. To carry out statistical analysis on the essential oils in order to find out whether it has a significance value for chemotaxonomy purposes.

CHAPTER TWO

LITERATURE REVIEW

2.1 Zingiberaceae family

Zingiberaceae is one of the largest plant families which can be used as essential oil. The plant family *Zingiberaceae* has about 1300 species and 50 genera. It can be found throughout the world mainly in the tropic (Vaniiaiiya *et al*., 2003). The center of distribution located in Southeast Asia, where more than half of the species occurs, with many small endemic genera numbers (Larsen *et al*., 1999). For example, the tribe *Hedychieae* in the family *Zingiberaceae* consists of 20 genera (Vaniiaiiya *et al*., 2003).

According to Larsen *et al* (1999), he stated that there are about 1000 out of 1200 species of *Zingiberaceae* that occur in tropical Asia and known as the Malesian region, which consists of Malaysia, Indonesia, Brunei, Singapore, Philippines and Papua New Guinea, is the richest area of *Zingiberaceae* can be obtained with 24 genera and about 600 species been recorded. In Peninsular Malaysia, there are about 18 genera with more than 160 species of *Zingiberaceae* can be found.

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. There are about 30 to 40 species of *Zingiberaceae* which have been used as traditional medicine in Malaysia. Various diseases can be treated by some species in *Zingiberaceae* such as rheumatism, high blood pressure, sinus and indigestion (Vaniiaiiya *et al*., 2003).

Usually the part been used by the scientist in order to obtain essential oil from plant family of *Zingiberaceae* are rhizomes, leaves and stems. Rhizomes of ginger plants contain antioxidant properties (Habsah *et al*., 2000; Jitoe *et al*., 1992; Zaeoung *et al*., 2005), have tyrosinase inhibition properties (Lee *et al*., 1997)**,** and can produce skin lightening cosmeceutical products (Rozanida *et al*., 2006). Because all of these properties, it can be consumed by women during ailment, illness and confinement. Furthermore, it is also can be taken as carminatives for relieving flatulence and also been widely used as spices or condiments (Larsen *et al*., 1999). Leaves of ginger plants can be used for food flavoring and in traditional medicine. It contains antioxidant and tyrosinase inhibition properties. For example leaves of *Curcuma longa* can be used as ingredients of curries and also can be used to wrap fish before steaming or baking in order to give delicious flavoring to the fish (Larsen *et al*, 1999).

2.2 Genus of *Alpinia*

The genus *Alpinia* belongs to the family *Zingiberaceae*. *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). Moreover, genus of *Alpinia* also has 230 species occurring throughout tropical and subtropical Asia (Kress *et al*., 2005).

In Malaysia, *Alpinia* can be found rich in Peninsular Malaysia and Sarawak; some of the species found in Sarawak are *A. cylindrostachys*, *A. brachypoda*, *A. cenolophon*, *A. angustifolia*, and *A. flexistamen* (Ridley, 1906). In Peninsular Malaysia, the red-bracted form of *A. purpurata* and *A. mutica* can be found (Larsen *et al*., 1999).

According to Larsen *et al* (1999), *Alpinia* is a medium-sized to large forest plants and some of the species can reach height over of 3 m. *Alpinia* has a terminal inflorescence on the leafy shoot. The flowers are variety in color which is yellowish-green to creamy coloured or red.

Alpinia can be classified 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). *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).

Some of the important species in this family are *A. galanga* and *A. officinarum* (Raina *et al*., 2002; Kubota *et al*., 1999; Kubota *et al*., 1998)**.** *A. galanga*, generally termed as greater galangal and widely cultivated in Southeast Asia. In Malaysia, its rhizomes are called *lengkuas* and usually been used in the spicy meat dish called *rendang*. But *A. galanga* is not native to the Malay Peninsular, but it is widely cultivated (Larsen *et al*., 1999). Alternatively, *A. officinarum* or minor galangal distributed mainly in China is used.

2.3 Essential Oils and medicinal uses of *Alpinia*

Essential oil is the volatile oil which can be found in aromatic plants and isolated by steam or hydro-distillation (Parker, 1993). In early the aged, essential oils were end-point of metabolism, but now it is known that in times of stress the oils can be broken down to provide energy for the plant (John *et al*., 1987). The essential oils can be used as a food aroma and flavor in orange essence. Over 50% of the commercial of the essential oils and natural extracts are obtained from cultivated plants. For example are mint aromas, and flower essences such as rose, geranium, coriander, lavender and jasmine (Ian *et al*., 2000). There are various applications of essential oils in *Alpinia* such as in pharmaceutical industry, as spices and flavors in foods and as an essential source of traditional medicine.

In *A. Galanga*, the rhizomes usually been used for the treatment of skin disease, respiratory disease, for intestinal problems, mouth and stomach cancer and as an expectorant also, while the seeds are prescribed for diarrhoea, vomiting and herpes (Valkenburg and Bunyapraphatsara, 2001). Furthermore, *A. galanga* also act as an essential spice and food flavoring product (Raina *et al*., 2002; Mallavarapu *et al*., 2002; Kubota *et al*., 1999; Kubota *et al*., 1998) and also been consumed as fresh vegetables or *ulam* by village folk (Ibrahim, 1992).

According to Valkenburg and Bunyapraphatsara (2001), rhizome of *Alpinia malaccensis* can be used to cure wounds and sores. Furthermore, this plant can also be chewed together with betel nut to make the voice strong and clear.

A. officinarum also one types of *Alpinia* which the rhizomes are widely been used for the treatment of dyspepsia, flatulence, vomiting, gastralgia, colic disorder, diarrhea, fever and malaria. Moreover, the seeds can be used in the treatment of heartburn, cholera, toothache and colds (Valkenburg and Bunyapraphatsara, 2001).

A. zerumbet is traditionally used for the treatment of cardiovascular hypertension and act as an antispasmodic agent which can be obtained from its oil which can be extracted from its leaves (Bezerra *et al*., 2000). Furthermore, it is also can be used in folk medicine for its anti-inflammatory, bacteriostatic and fungistatic properties (Zoghbi *et al*., 1999). Furthermore, its leaves contain hypotensive, diuretic, anti-ulcerogenic properties and can be sold as an herbal tea, used to flavor noodles and to wrap rice cakes (Mpalantinos *et al.*, 1998).

2.3 Application of Essential Oils for Chemotaxonomy Purposes

Chemotaxonomy is the study to classify and identify organisms, originally plants, according to demonstrable differences and similarities in their biochemical compositions. Members of the *Zingiberaceae* are usually aromatic in all or most parts or at least one of the plant parts. Terpenoids are the richest compounds where can be found in the species of *Zingiberaceae* family. Other compounds such as alkaloids and phenolics are not well documented.

Phytochemical screenings of selected species have revealed that flavonoids and terpenoids are ubiquitos but alkaloids were detected in three species of *Alpinia*, *Hedychium* and two species *Zingiber* (Zakaria and Ibrahim, 1986). Since the *Zingiberaceae* family is a monocotyledons plant, the presence of alkaloids in gingers is an interesting finding because alkaloids are known to be more common in dicotyledons compared to monocotyledons. From the previous study, it is believed that saponins are the characteristic of *Costus spp.* from *Costaceae* family, but recently the studies conducted by Merh *et al.* (1986), the presence of saponins had been found in various genera of *Zingiberaceae*, such as in *Globba, Curcuma, Hedychium, Zingiber* and *Alpinia.*

Chemical studies have also revealed that gingers are rich in essential oils. There was an analysis of essential oils been conducted on three species which are *Zingiber spectabile, Z. officinale* (the red-rhizomed race) and *A. galanga* through steam distillation method and the results showed a total of 34 compounds with 18 compounds, 20 compounds and 31 compounds identified for the three species, respectively. The most common compounds which can be found present in the three species studied are α-pinene, β-pinene, limonene and β-elemene, etc. Moreover, the three species stated above also share many similar compounds, such as P-cymene, camphor, 1,8-cineole, citral-a, linalool, β-caryophyllene, α-humulene and β-bisabolene. The major compounds identified for each species were trans-d-bergamotene from *Z. spectabile*, α-terpineol from *A. galanga* and bornyl acetate from *Z. officinale* (Ibrahim and Zakaria, 1987).

In another study, two variants of *Elettariopsis triloba* were investigated for their volatile components (Mustafa *et al*., 1996). According to Mustafa *et al.* (1996), the results showed that the two variants showed differences in their volatile constituents and variations were also detected when different plant parts which are leaf, rhizome and root were compared from the same plant. Some examples of the major components in the leaves of one variant of *E. triloba* included α-citral, β-citral, 2,7-dimethyl-2 and geranyl isobutyrate, while its rhizome contained limonene and 2-carene. The percentage of volatile components in the roots was low. Similarly, examples of the major components of the leaves of a second variant of *E. triloba* included caryophyllene and eremophilene, while the rhizomes contained cineole and borneol. The roots were found to have camphene and caryophyllene. Overall, it appears that a larger number of similar components were detected between roots and rhizomes as compared to the leaves of two variants of this species.

2.5 Extraction methods

There are several method commonly been used in the essential oil extraction. Some of it is steam-distillation method, cold press extraction method and super critical fluid extraction (SFE). According to (Nakatsu *et al*., (2000), the proper and convenient methods should be selected for the essential oil extraction since the method could affect the chemicals constituents and composition of essential oil. The most convenient ways are to choose the method which can concentrate the targeted biologically active compound into the essential oil. Generally, most essential oils constituents are small, volatile and have lipophilic characteristic. The main objective of the method is to separate these compounds from aqueous plant materials to be analyzed.

2.5.1 Steam-distillation

In industrial scale extraction of essential oil, steam-distillation method commonly been used. The temperature for plant material in this method is set to 100˚C (Milner *et al.*, 1997) and usually the pH is not controlled. Thus, the composition of oil in the condensate is pH dependent (Milner et al., 1997). This technique becomes readily available to the community because of the simple apparatus used. Eventhough it is a very efficient process, saponification, isomerization and other reaction might occur due to the applied heat, water acidity/bacicity or trace metals in the sample. Thus the odor and flavor balance of the original essential oil might be affected (Nakatsu *et al.*, 2000).

2.5.2 Cold Press Extraction

Cold press extraction technique is the simplest extraction method compared to other extraction method. This method can be considered as 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. The purpose of this method is to isolate the oxygen containing species, which tend to rearrange or degrade when heat is applied (Nakatsu et al., 2000).

2.5.3 Super Fluid Extraction

Supercritical fluid is used in this extraction technique as the extraction solvent. Supercritical fluids have solvent strength that approach those of liquid solvent and will dissolve many substances. By capitalizing on the differences in component volatilities and differences in the specific interaction between the mixture components and the supercritical fluid solvents or the solubility, thus the fluids can make possible separation of multi component mixtures (Milner et al., 1997).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Plant Samples Collection

Fresh leaves, stems and rhizomes of samples were collected from various places in Sarawak, such as from Lanjak-Entimau of Ulu Ketibas, Matang, Bario, Lawas and from the forest within Universiti Malaysia Sarawak. The samples collected were cleaned and stored in the refrigerator in the laboratory in order to ensure the freshness of the samples.

3.2 Extraction of Essential Oils

The extraction of essential oils for the plant samples were performed by using procedure as described by Datta (1987). First and foremost, the essential oil was extracted by using hydrodistillation method in a Clevenger-type apparatus. About 100 grams of fresh sample 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 method was carried out for 6 hours. The flask was heated in order to maintain the distillation rates of two drops per second. The oil was separated and dried over anhydrous sodium sulphate. It was then stored in dark place at cool temperature (4˚C - 5˚C) before being analyzed. The extraction was repeated for two to three times and the average percentages were calculated.

3.3 Gas chromatography/flame ionization detector (GC/FID)

The oil obtained from the sample was then analysed by using Hewlett Packard (HP) 6890 gas chromatograph (Hewlett Packard, USA) equipped with FID detector. Fused silica capillary column DB-5 (25 m long x 0.3 mm internal diameter x 0.25 µm film thickness) was used. The initial temperature for the oven was programmed at 50˚C for 5 minutes. Then it was increased to 280˚C with the rate of 3.5˚C/min and was held for 5 minutes. Injector and detector temperature was set at 260˚C and 280˚C respectively. Nitrogen was used as the carrier gas with flow rates of 5.8mL/min. For sample injection, 1 μL of homologous sample was injected in the GC using splitless injection after been diluted with 199 μL hexane. Data obtained from the GC was analyzed by using Kovat Index.

3.4 Quantitative and Qualitative Analysis

3.4.1 Percentages of essential oils

The percentages of essential oils obtained were calculated. The yields were averaged over three experiments and calculated based on the dry weight of plant material.

$$\text{Percentage of yield, } \% = \frac{\text{weight of the essential oils}}{\text{weight of the dried sample used}} \times 100$$

3.4.2 Kovats index

GC data was analyzed in order to determine the chemical composition that present in the essential oils by using the Kovats Index equation (Robert, 1987):

$$KI = 100\ [(tR_x - tR_n\ /\ tR_{n+1} - tR_n\ ) + n]$$

Where:

n = Number of carbons

tR_n = Retention time of the saturated hydrocarbon containing n carbon.

tR_x = Retention time for component.

tR_{n+1} = Retention time of the saturated hydrocarbon containing n+1 carbon.

3.4.3 Semi Quantitative Analysis

GC data was analysed with semi-quantitative analysis by using normalization method in order to calculate the percentages of individual chemical components in the essential oils (Miller, 1988) based on the following equation:

$$X\% = A_x/A_T \times 100$$

Where:

A_x = Peak area of compound X

A_T = Peak area of all compounds in essential oil.

3.5 Statistical Analysis of Essential Oil Data

3.5.1 Cluster Analysis

This analysis was carried out by using SPSS version 17. According to Hibbert (1997), the purpose of this analysis is to determine whether the compounds are related to each other and this information can be used for the chemotaxonomy classification. In this analysis, the percentages of individual chemical components calculated from semi-quantitative analysis were grouped in the multi-dimensional space of the variables. The Euclidean, single linkage, and unweighted pair group average rules were then compared. In the absence of information on the shape of the groupings or their statistical distribution, the Euclidean distance were combined with unweighted pair group average linking; which was the average distance between all pairs of objects in the two clusters.