CHARACTERIZATION AND BIOLOGICAL ACTIVITY OF ESSENTIAL OILS FROM ETLINGERA SPP.

Aisyaidil Binti Hanri

Bachelor of Science with Honours
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This report is submitted in partial fulfillment of the requirements for the degree of Bachelor of Science with Honors in Resource Chemistry

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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 other university or institution of higher learning.

Aisyaidil binti Hanri
Programme of Resource Chemistry
Faculty of Science and Technology
Universiti Malaysia Sarawak
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ABSTRACT

Essential oils from six species of *Etlingera* were studied for their chemical composition and their biological activity on brine shrimp, *A. salina*. Essential oils were extracted by hydrodistillation and subsequently analyzed using gas chromatography/flame ionization detector (GC/FID). The percentage of essential oil obtained from the six species of *Etlingera* ranged from 0% to 2.15%, with the highest percentage was obtained from the leaves of *Etlingera* Species 2. Ethyl-(E,Z)-decanoate (79.53%), α-gurjunene (17.25%), geosmin (17.25%) and tetradecyl aldehyde (8.57%) were the major constituent of *E. elatior*. α-pinene and β-pinene were detected at high abundance in *E. littoralis* with 20.67% of α-pinene in leaf and 20.24% of β-pinene in rhizome. *E. punicea* was rich in octenol (73.27%), while *E. latilabris* contain high abundance of octadienone (66.55%), methyllethylpyrazine (29.30%) and α-pinene (25.91%). Octanol (31.02%) and β-pinene (31.02%) were the major constituents found in *Etlingera* species 1. *Etlingera* species 2 has α-pinene (25.39%), methyl laurate (16.29%) and phenol (15.69%) as the major components. Camphene and (E)-α-bergamotene were found in leaves of all *Etlingera* spp. analyzed. α-thujene and 2-ethylpyridine were found present in rhizome of all *Etlingera* spp. analyzed. Leaves of *E. elatior* and *Etlingera* species 1 as well as leaves of *E. littoralis* and *E. latilabris* shows close relationship. There was also close relationship between rhizomes of *Etlingera* species 2 and *E. latilabris* as well as close relationship between *E. littoralis* and *Etlingera* species 1. Toxicity tests on brine shrimp, *A. salina* shows only leaf oil from *Etlingera* species 1 (13.59 µg/mL), flower oils from *E. punicea* (13.59 µg/mL), leaf oil from *E. elatior* (56.67 µg/mL) and flower oil from *E. elatior* (100 µg/mL) have toxicity to brine shrimp.

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

Komposisi minyak pati daripada enam spesies *Etlingera* telah dikaji komposisi kimia dan aktiviti biologinya terhadap anak udang, *Artemia salina*. Minyak pati telah diekstrak dengan menggunakan kaedah penyulingan hidro dan seterusnya dianalisis menggunakan kromatografi gas/pengesan ion nyalaan. Peratusan minyak pati yang diperoleh daripada enam spesies *Etlingera* adalah dalam julat 0% hingga 2.15%, dengan peratusan tertinggi diperoleh daripada daun *Etlingera* spesies 2. Etil-(E,Z)-dekanoat (79.53%), α-gurjunen (17.25%), geosmin (17.25%) dan tetradesil aldehid (8.57%) merupakan komponen utama dalam *E. elatior*. α-pinena and β-pinena hadir dalam kelimpahan yang tinggi dalam *E. littoralis* dengan 20.67% α-pinena telah dijumpai pada daun dan 20.24% β-pinena telah dijumpai pada rizom. *E. punicea* kaya dengan octenol (73.27%), manakala *E. latilabris* mempunyai kelimpahan oktadienon (66.55%), metiletpirazina (29.30%) dan α-pinena (25.91%). Oktanol (31.02%) dan β-pinena (31.02 %) merupakan komponen utama yang dijumpai dalam *Etlingera* spesies 1. *Etlingera* spesies 2 mempunyai α-pinena (25.39%), metil laurat (16.29%) and fenol (15.69%) sebagai komponen utama. Kamfena and (E)-α-bergamoten dijumpai hadir di dalam daun dalam setiap *Etlingera* spp. yang dianalisis. α-thujen dan 2-etilpiridina hadir di dalam rizom dalam setiap *Etlingera* spp. yang dianalisis. Daun *E. elatior* dan *Etlingera* species 1 dan juga daun *E. littoralis* dan *E. latilabris* menunjukkan hubungan yang rapat antara satu sama lain. Selain itu, terdapat juga hubungan yang rapat antara rizom *Etlingera* spesies 2 dan *E. latilabris* disamping rizom *E. littoralis* and *Etlingera* species 1. Ujian ketoksikan terhadap anak udang *A. salina* menunjukkan hanya minyak pati daripada daun *Etlingera* species 1 (13.59 μg/mL), bunga *E. punicea* (13.59 μg/mL), daun *E. elatior* (56.67 μg/mL) dan bunga *E. elatior* (100 μg/mL) menunjukkan ketoksikan terhadap anak udang.

Kata kunci: *Etlingera*, kromatografi gas/pengesan ion nyalaan, minyak pati, penyulingan hidro, anak udang.
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1.1 INTRODUCTION

According to Anand and Kumar (1990), essential oil is an extremely heterogeneous mixture of volatile, lipophilic plant products with characteristic odors. The International Standard Organization (ISO) has defined the essential oil in the strict sense as the steam distillates of plants or oils obtained by pressing out the rinds of a few citrus fruits. Generally, essential oils are understood to be volatile compounds that are freely soluble in alcohol, ether and vegetable and mineral oils and are usually assumed to be the result of distillation or a steam-stripping process (Hernandez, 2000).

The use of essential oils in ancient times consisted of preparing ointments by mixing oils from flowers with fatty oils. This process was done by placing flowers and roots with the oil in glass bottles, which were then allowed to sit for periods of time. Sometimes the flowers or roots were macerated with wine before the fatty oil was added, and the product obtained by digestion filtered and boiled down to a thicker consistency (Hernandez, 2000). The production and use of essential oils did not become widespread until the second half of the 16th century when Hieronymus Brunschwig’s book on distillation, Liber De Arte Distillandi describe the distillation techniques for four essential oils published in 1507. The four essential oils were
According to Parker (1993), essential oils are composed of many kinds or classes of molecules such as terpenoids, phenolics, aromatics, cyclic and acyclic compounds, sulfur and nitrogen containing compounds, and acetonides compounds depending on the plant and extraction method. The main components are monoterpenes (C_{10}) and sesquiterpenes (C_{15}). The boiling point for monoterpenes is 140-180°C while for sesquiterpenes the boiling point is more than 180°C. This terpene can exist as an open chain, monocyclic or bicyclic and is usually composed of one or more double bond and hydroxyl group. Hernandez (2000) has grouped the essential oils into six classes based on their chemical properties as shown in Table 1.1.

Table 1.1: Classification of chemical components in Essential Oils

<table>
<thead>
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<th>Component</th>
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<td>Hydrocarbons</td>
<td>Limonene in lemon oil</td>
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<td>Alcohols</td>
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<td>Ketones</td>
<td>Menthone in oil of peppermint</td>
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<td>Lactones</td>
<td>Coumarin from Tonka beans</td>
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</table>

The Zingiberaceae is a moderately sized family of relatively advanced monocotyledonous plants of order Zingiberales. Zingiberaceous plants are rhizomatous, perennial and aromatic herbs often of large size, bearing flowers either
terminally on aerial leaf shoots or from ground level (Chen and Chen, 1998). Species of Zingiberaceae are the ground plants of the tropical forests. They mostly grow in damp and humid shady places. They also found infrequently in secondary forest. Some species can fully exposed to the sun, and grow on high elevation (Sirirugsra, 1997). These are plants from tropical and subtropical regions distributed mainly in Asia. The Zingiberaceae comprises about 1200 species of which about 1000 occur in tropical Asia. By far the richest area is the Malesian region, a floristically distinct region that includes Malaysia, Indonesia, Brunei, Singapore, the Philippines and Papua New Guinea, with 24 genera and about 600 species. According to Larsen et al. (1999), it is known that large areas such as Sumatra and Borneo are still very insufficiently known and largely under explored for the ginger flora. Therefore many species will undoubtedly be found in the years to come.

The current records indicate that the tribes of Alpinae and Zingibereae are quite well represented in Borneo including 2 genera (Burbidgea and Geanthus) unknown in Peninsular Malaysia. There are 20 species described for Boesenbergia, the tribe Hedychease, which is poorly represented in Borneo, with only 6 genera, recorded (Smith, 1987). The tribe Globeae is similarly poorly represented with only 8 species described so far (Smith, 1988). There are about 18 genera with more than 160 species of Zingiberaceae recorded in Peninsular Malaysia. Many of these are rare, very local in distribution, and consequently highly vulnerable to endangerment. The Zingiberaceae are mainly forest plants found from the lowlands to the highest elevations in Peninsular Malaysia (Larsen et al., 1999).
Etlingera, which is from the tribe Alpin eae and family, Zingiberaceae (Sirirugsa, 1997) is of interest in this project. The genus Etlingera is distributed from India to the Pacific Island with centers of species richness assumed in Borneo and New Guinea. At least 70 species are recorded from the Malesian region, but recent study shows that 29 are known species with 10 still undescribed. Species of Etlingera can be more than 5 m tall and become dominant in gaps (E. littoralis, E. brevilabrum, E. coccinea, and E. fimbriobracteata) and thus indicate disturbance; whilst other species are found reproductive in shady conditions as well, e.g. E. corrugate (Poulsen, 2003).

1.2 OBJECTIVES

The objectives of this study are:

i. To isolate the essential oils from several Etlingera spp. using hydro distillation method.

ii. To identify and characterize the chemical components of the essential oils using GC-FID.

iii. To conduct a biological activity study of the essential oil on brine shrimp, *Artemia salina*.

iv. To determine the chemical profile of Etlingera spp. for chemotaxonomy classification by cluster analysis.
CHAPTER 2
LITERATURE REVIEW

2.1 Essential Oils

In the early aged, essential oils were end-point of metabolism, but it is known that in times of stress the oils can be broken down to provide energy for the plant (John et al., 1987). Current specific uses of essential oils are to add flavor to foodstuffs and beverages and to scent perfumes, lotions, soaps, detergents and households' cleaners. For example, limonene from citrus peel is a very strong solvent and it is used in a wide variety of cleaning products. Essential oils are a major part of carbonated beverage flavorings; the most common flavors include lemon, lime, orange, cassia, cinnamon and nutmeg. Essential oils are also used to flavor many foods such as sweets and candies, cookies, snacks and chewing gum (Hernandez, 2000).

The use of essential oils as functional ingredients in foods, drinks, toiletries and cosmetics is gaining momentum, both for the growing interest of consumers in ingredients from natural sources and also because of increasing concern about potentially harmful synthetic additives (Reische et al., 1998). However, essential oils and their components are gaining increasing interest because of their relatively safe status, their wide acceptance by consumers and their exploitation potential multi-purpose functional use (Ormansey et al., 2001; Sawamura, 2000).
2.2 Extraction of Essential Oils

An essential oil is internationally defined as the product obtained by steam distillation, hydrodistillation or expression (for citrus) of a plant or a part of it. This definition is now less strictly applied and the fractions resulting from several other techniques that sample the volatile fraction of a plant are now erroneously classified as essential oils. In general it would be more correct to call them volatile fractions of a vegetable matrix, and to use the term essential oil more specifically for samples obtained by distillation or expression (Bicchi, 2000). The essential oils are natural product derived from aromatic plants and have a wide range of uses in flavoring and fragrances in the food and perfume industries, medicine and crop protection (Isman, 2000; Daferera et al., 2003) that can be isolated by steam distillation or hydrodistillation (Parker 1993).

There are three general methods to isolate the essential oil. They are boiling water, steam distillation, or the combination of both methods. The conditions for temperature, pressure or vacuum and processing time will depend on the characteristics of the essential oil, particularly as regards susceptibility to oxidation and heat decomposition (Hernandez, 2000). Other methods are hydrodistillation, solvent extraction, cold press extraction, steam distillation supercritical fluid extraction (SFE), microwave oven extraction, solid phase extraction (SPE), fluorocarbon extraction and various other techniques (Atta, 1999).
A simple distillation apparatus consists of a retort or still, a condenser and a receiver. This system is commonly used in fields for processing lavender. Some aroma systems involve the use of only steam in order to obtain a more concentrated essential oil in the condenser. This system is applicable where there is no agglomeration or agglutination of raw materials. Extraction methods by steam distillation, solvent extraction and supercritical CO₂ usually produce different results with regards to yield and composition profiles of the extracted materials. Steam distillation usually gives the lowest yield of recovered aroma but produces a concentrate that is a true essential oil. Solvent extraction with hexane or alcohols produces the highest yield but there is always the possibility that unwanted non-volatile materials might end up in the final product. Also, solvent extraction uses high temperature. Supercritical CO₂ extraction produces a lower yield than conventional solvent extraction but higher than steam distillation and it also has the advantage of using little or no heat and no steam or moisture in the process. However, this method is expensive since it involves high pressure and sophisticated equipment and controls (Hernandez, 2000).
2.3 *Zingiberaceae*

*Zingiberaceae* is one of the largest families of the plants kingdom. It is important natural sources that provide many useful products for food, spices, medicines, dyes, perfumes and aesthetics to man (Sririrugsa, 1997).

One of the common uses of the *Etlingera* is as food flavoring, vegetables and beverages. For example, *E. elatior* is known as *bunga kantan* used locally for flavoring food (Larsen et al., 1999). The part usually used is the young flower buds, which is used as an important ingredient for the spicy dish, *Laksah* (Sririrugsa, 1997) and a favorite noodle dish in Peninsular Malaysia (Larsen et al., 1999). *Kantan* is also used in the local dishes of *nasi kerabu* or *nasi ulam* (at the East Coast of the Malaysia Peninsula) and *laksa asam* (Larsen et al., 1999). In southern Thailand, flowers are eaten as vegetable. Whereas the flowers of *E. maingayi* can be eaten as vegetables. Furthermore, the fruit of *Etlingera* littoralis is edible. While the young stem of this species, after removing the outer parts, yields an aromatic, tender core, which is suitable for eating raw or cooked (Sririrugsa, 1997). In Sabah, the fruits of *E. fimbriobraceata* have been used as a kind of conserve, and are also eaten by *Ihans* (Poulsen, 2003). Furthermore, the fruits of some *Etlingera* spp. and *Hornstedtia* spp. are also edible. While in Sabah, *E. coccinea*, locally known as *tuhau* (by Dusuns and Muruts), is used as a condiment. It is also used by Kelabits (tubu nanung) and Iban (tepus) in Sarawak, a name also used in Java (Poulsen, 2003).
Species with very fibrous leaves can be processed for the production of fibers and paper. *E. clatior* was known to be successfully processed for papermaking albeit not high quality. The leaves of many species are quite fibrous and hence having good potential for their exploitation into economic products (Halijah, 1998).

The Zingiberaceae are mostly plants with showy inflorescences and often brightly colored bracts and floral parts. In this they are second to none, not even to the orchids. For ornamental purposes, however, most of the species have one drawback: the flowers of most species are very short-lived, often lasting only a few hours (Larsen *et al.*, 1999). In the western countries, especially the United States of America, there is increase in the popularity of Zingiberaceae as decorative plants in indoor gardens, landscapes and floral arrangements (Halijah, 1998). Farm in Australia and Costa Rica for instance are selling the inflorescences of *E. elatior* (the torch ginger), in shades of pink, deep red and purplish black, as cut flowers (Larsen *et al.*, 1999).

Rhizome decoction from *E. littoralis* and *E. peciosa* can be drink as antipyretic. While the juice of *E. solaris* is dropped into the ears to treat certain ear-ache. Decoction of the rhizome is used as an antiseptic for external wounds and the flowers are sometimes included in the herbal bath for a mother after childbirth (ARCBC, 2004).

Volatile for the flowers of *E. elatior* have been previously analyzed by Wong *et al.* (1993), Lechat-Vabirua *et al.* (1996) and Zoghbi *et al.* (2001). The study showed that flowers of *E. elatior* are rich in alcohols and aldehydes. Predominance
components identified in the oils of inflorescence and inflorescence axis of *E. elatior* were dodecanol, dodecanal and tetradecanol. α-pinene is the major component of monoterpenes group in the flower oil of *E. elatior* with 22.2% present in inflorescence and 6.3% present in inflorescence axis. According to The European Agency for the Evaluation of Medicinal Products in its Final Position Paper on the Use of Herbal Medicinal Products Containing Methyleugenol (2004), *E. cevuga* has found to have 47.7% of methyleugenol in the rhizome oil.
CHAPTER 3
MATERIALS AND METHOD

3.1 Plant Materials

Samples of *Etlingera* spp. were collected from several locations in Sarawak. The area of sampling including Sematan, Padawan and Kuching of Kuching Division, forest area of Unimas campus of Kota Samarahan and Lawas of Limbang Division. The sampling locations of *Etlingera* spp. are summarized in Table 3.1. Leaves, stems and rhizomes of the plant sample were separated during the sample collection. Upon arriving at the laboratory, the samples were cleaned and then cut into small pieces before grinding. Fresh samples were used for extraction.

**Table 3.1: Etlingera spp. and location collected**

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<td><em>Etlingera latilabris</em></td>
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<td><em>Etlingera</em> species 2</td>
<td>Unimas, Kota Samarahan</td>
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</table>
3.2 Extraction of Essential Oil

The extraction of essential oils from plant samples was carried out using method established by Lee and Ogg as described by Datta (1987). Briefly, essential oils were extracted and isolated using hydro distiller equipped with Clevenger-type apparatus. Approximately 100 g of fresh-grounded samples were weighed and transferred to 2 L-capacity flat bottom flask and mixed with 1.5 L distilled water. Several anti-bumping granules were added to the flask to avoid the solvent bumping. The flask were then assembled to the Clevenger trap and connected to the condenser. Hydrodistillation was carried out for 6-8 hours. The flask was heated to maintain the distillation rates of two drops per second. After 6-8 hours, the oil trapped in the Clevenger was then cooled to room temperature. The oily layers obtained were separated and dried over anhydrous sodium sulphate and stored at 4-5°C. The hydrodistillation process was done in triplicates and average percentages of the oils were calculated.

3.3 Instrumental Analysis

3.3.1 Gas Chromatography-Flame Ion Detector (GC/FID)

The analysis of the essential oils was performed on Shimadzu GC-17A gas chromatograph equipped with a flame ionization detector (FID) detector using a fused silica capillary column DB-5 (25 m X 0.3 mm). Prior to GC/FID analysis, 1 μL of essential oil were diluted with 200 μL n-hexane. Helium was used as a carrier gas
with velocity of 2 mL/min. The initial temperature was programmed at 50°C and held for 2 minutes. The temperature was then increased to 300°C at a rate of 6.5°C/min. The final temperature was held for ten minutes. The temperature for the injector and detector were set at 280°C and 320°C, respectively.

3.4 Data Analysis

3.4.1 Percentage of Essential Oils

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

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% \text{ Essential Oils (v/w)} = \frac{\text{volume of oil}}{\text{dry weight of samples}} \times 100\%
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