ESSENTIAL OIL PROFILE AND BIOLOGICAL ACTIVITY OF LITSEA SPP.

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ESSENTIAL OIL PROFILE AND BIOLOGICAL ACTIVITY
OF LITSEA SPP.
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DECLARATION

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

______________________________
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Essential oil profile and biological activities of *Litsea* spp.

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Resource Chemistry

ABSTRACT

The percentage, composition, and the toxicity of essential oils from *Litsea paludosa*, *Litsea sessilis*, *Litsea gracilipes* and *Litsea resinosa* were studied. The essential oils compositions for all samples were analysed using gas chromatography/flame ionization detector and gas chromatography/mass spectrometry. The percentage of essential oils from the four *Litsea* species studied varied from 0.08% to 4.79%. The highest percentage was obtained from the leaves of *Litsea sessilis* while the lowest percentage of oil obtained was from the roots of *Litsea paludosa*. The major chemical compositions in *Litsea paludosa* were α-guaiene (14.88%), β-selinene (14.82%), cis-linalool pyran oxide (36.57%) and methylene bis(methyl sulfide) (15.83%). The major chemical composition in the oils of *Litsea sessilis* were geranial (37.23%), ethyl undecanoate (33.55%) and elemicin (42.17%). For *Litsea gracilipes*, the major chemical compositions were elemicin (29.08%) and geranyl acetone (12.20%). *Litsea resinosa* contains heneicosane (4.51%) and acetovanillone (3.89%) as its major chemical composition. Some of the similar compounds presents in the oils were β-selinene from the leaves, barks and roots of *Litsea paludosa*, elemicin in both the oils from the barks of *Litsea sessilis* and the leaves of *Litsea gracilipes* while the oils from the leaves of *Litsea paludosa* and *Litsea resinosa* both contain similar chemical compositions such as isogeraniol, β-sesquiphellandrene and methyl eugenol. The toxicity test showed that only oils extracted from the leaves of *Litsea paludosa*, *Litsea gracilipes* and the barks of *Litsea sessilis* were toxic with the LC$_{50}$ values of 42.0μg/mL, 30.0μg/mL, 26.0μg/mL, and 10.0μg/mL respectively.

Key words: *Litsea* species, essential oil, hydrodistillation, kovats index, toxicity.
ABSTRAK
Peratusan, komposisi, dan ketoksikan minyak pati dari empat spesies Litsea iaitu Litsea paludosa, Litsea sessilis, Litsea gracilipes dan Litsea resinoso telah dikaji. Komponen bagi setiap sampel minyak pati yang diekstrak telah dianalisis menggunakan kromatografi gas/pengesan ion nyalaan dan kromatografi gas/spektrometri jisim. Peratus minyak pati yang diekstrak dari keempat-empat spesies berada dalam julat 0.08% sehingga 4.79%. Peratus minyak pati tertinggi diperolehi daripada bahagian daun Litsea sessilis manakala peratus hasil minyak terendah adalah daripada bahagian akar Litsea paludosa. Peratus komposisi sebatian kimia utama dalam minyak pati Litsea paludosa adalah a-guaiena (14.88%), β-selinena (14.82%), cis-linalool piranoksida (36.57%) dan metilena bis(metil sulfida) (15.83%). Bagi Litsea sessilis, komposisi sebatian kimia utama adalah terdiri daripada geranial (37.23%), etil undekanoat (33.55%) dan elemisin (42.17%). Litsea gracilipes memberikan elemisin (29.08%) dan geranil aseton (12.20%) sebagai komponen utama manakala Litsea resinoso pula mengandung heneikosan (4.15%) dan asetovanilon (3.89%). Sebahagian daripada sebatian kimia minyak pati yang serupa dalam spesies yang dikaji adalah β-selinena daripada bahagian daun, kulit batang dan akar Litsea paludosa dan elemisin daripada kulit batang Litsea sessilis dan daun Litsea gracilipes. Mnyak pati dari bahagian daun Litsea paludosa dan Litsea resinoso pula mengandungi sebatian kimia utama yang terdiri daripada isogeraniol, β-seskifelandrena and metil eugenol. Ujian ketoksikan menunjukkan minyak pati daripada bahagian daun Litsea paludosa, Litsea gracilipes dan Litsea resinoso serta minyak pati daripada bahagian kulit batang Litsea sessilis adalah toksik dengan nilai LC50 42.0 µg/mL, 30.0 µg/mL, 26.0 µg/mL, dan 10.0 µg/mL masing-masingnya.

Kata kunci : Litsea spp., minyak pati, penyulingan hidro, indeks kovats, ketoksikan.
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CHAPTER 1

Introduction

An essential oil is the volatile oil containing odiferous elements of the plant, produced by steam or hydro-distillation of aromatic vegetable plant matter (Parker, 1993). In previous years, it was considered that essential oils were an 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). Essential oils occur in many different parts of plants, like roots of grass (vetiver), bark (cinnamon), heartwood (sandalwood), leaves (bay and eucalyptus), herb (peppermint), seeds (nutmeg and caraway), flowers (cananga and jasmine), petals (rose), citrus rind (lemon), rhizomes (valerian), bulbs (garlic), the aerial or top parts of the plant (marjoram) or resin (frankincense), and sometimes in more than one part of the plant (John et al., 1987). Lavender, for instance, yields oil from both the flowers and the leaves, while the orange tree produces three different smelling essences with varying medicinal properties; the heady bitter-sweet neroli (flowers), the similar, less refined scent of petitgrain (leaves) and the cheery orange (rind of the fruit) (John et al., 1987).

Essential oils are products of the secondary metabolism of plants, and generally consist of complex mixtures of monoterpenes (C\(_{10}\)) and sesquiterpene (C\(_{15}\)) hydrocarbons, and oxygenated materials biogenically derived from them. The boiling point for this monoterpenes is 140-180°C while sesquiterpenes is more than 180°C. This terpene can exist as an open chain, monocyclic or bicyclic and it is usually composed of one or more double bond and hydroxyl group (Parker, 1993). Other common constituents include phenyl propanoids from the Shikimic acid pathway, and their bio-transformation
products, and other compounds from the metabolism of fatty acids and amino acids. As well as these major groups of compounds, a large number of other types of chemical components are also found, including nitrogen and sulphur (Ian et al., 2000). Essential oils are ideally isolated with minimum chemical changes from human intervention (Leifer, 1951). They should be produced by purely physical means, and be 100% pure and wholly derived from the named botanical source (Leifer, 1951). Advances in organic chemistry have allowed for the establishment of techniques to define the component profiles of many aromas and fragrances which permit the establishment of composition standards for trade regulations and for the synthesis of aromas using less costly starting materials. Chemically, essential oils are extremely complex mixtures containing compounds of every major functional-group class. Essential oils are commonly grouped into six classes according to their chemical nature as depicted in the following table (Watson et al., 1992).

Table 1: Common chemical groups found in essential oils.

<table>
<thead>
<tr>
<th>Components</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrocarbons</td>
<td>Limonene in lemon oil</td>
</tr>
<tr>
<td>Alcohols</td>
<td>Borneol in rosemary oil</td>
</tr>
<tr>
<td>Esters</td>
<td>Methyl salicylate in oil of wintergreen</td>
</tr>
<tr>
<td>Aldehydes</td>
<td>Benzaldehyde in almond nut oil</td>
</tr>
<tr>
<td>Ketones</td>
<td>Menthone in oil of peppermint</td>
</tr>
<tr>
<td>Lactones</td>
<td>Coumarin from tonka beans.</td>
</tr>
</tbody>
</table>

The oils are isolated by steam distillation, extraction, or mechanical expression of the plant material usually only from certain parts, such as roots, buds, leaves or flower petals (Parker, 1993). Essential oils have been produced and used for flavoring, incense,
and medicinal purposes for many centuries. There are indications that crude preparations were known in ancient Egypt and Persia. Distillation of the essence of plants was described about the year 1500 (Parker, 1993). In the beginning of modern organic chemistry, essential oils provided fertile source of compounds for studying the structures and reactions of organic molecules. Chemical investigations of essential oils also led directly to the development of terpene chemistry (Parker, 1993).

Natural essential oils fight infection, contain hormone balancing compounds and can rejuvenate and regenerate cells (Watson et al., 2000). Organic essential oils utilize all of the plant's chemical components synergistically, blending with the human body's natural chemical makeup to provide balance and healing from within while maintaining the body's homeostatic environment. Today, attractive aromas from essential oils which leave a pleasant memory association are used as marketing devices to sell edible cosmetic products, including unlikely materials as detergents. Current specific uses of essential oils are to add flavour to foodstuff and beverages, and to scent perfumes, lotions, soaps, detergents, and household cleaners (Ian et al., 2000). 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; some of the most common flavours include lemon, lime, orange, cassia, cinnamon and nutmeg. Essential oils are also used to flavour many foods such as sweets, candies, cookies, snacks and chewing gum (Miller, 1941).

The most common essential oils used as a food aroma and flavour is orange essence. Over 50% of the commercial essential oils and natural extracts are obtained from cultivated plants. Examples of these include mint aroma and flower essences such as
rose, geranium, mints, coriander, lavender and jasmine (Watson et al., 1992). The field of aromatherapy constitutes a small part of the essential oils industry but it is a fast-growing area and requires a wide variety of essences. Essential oils are also used as insect and animal repellents, in pharmaceutical preparations, and as anti-microbial agents.

Other properties of essential oils with commercial potential include antimicrobial effects. The inhibition of 25 different bacteria using an essential oil of marjoram has been reported (Ian et al., 2000). Similar effects are noted for other volatiles and essences derived from plant materials. It was found that the short chain volatiles such as 5-8 carbon aldehydes and ketones resulting from the distillation of vegetable oils had antimicrobial properties against bacteria such as Staphylococcus aureus and Escherichia coli (Ian et al., 2000).

1.2 Objective

The objective of this research is to study the chemical composition and the biological activities of essential oil from Litsea spp.
2.1 *Litsea* spp.

*Litsea* is one of the many genera in the family Lauraceae (Laurel family). The laurel family has about 2000 to 2500 species in 32 genera, found in tropical and subtropical regions. About 400 species of *Litsea* are native to warm regions of Asia, Australia and America. However, only 54 of the species can be found in Malaysia (Corner, 1988). Some other species in the Lauraceae family are the *Cinnamomum* spp. (e.g. *Cinnamomum zeylanicum*) which is commonly known as the cinnamon and *Persea* spp. (e.g. *Persea americana*) which is known as avocado. Other genera in the family Lauraceae includes *Actinodaphne, Alseodaphne, Beilschmiedia, Cassytha, Cryptocarya, Dehaasia, Endiandra, Lindera, Nothaphoebe, Phoebe* and *Potoxylon* (Lawless, 1996).

Essential oils may be present in many different types of plant materials such as wood, bark, leaves, stems, flowers, stigmas, and reproductive parts at concentrations ranging from thousandths of a percent to one or several percent. Oil is often contained in specialised secretory structures which include secretory cells, ducts, cavities, glandular trichomes and other structures. The yield of essential oils from seeds can often be high, mostly in the several tens of percentage. However, for the majority of other materials, the main range is 0.1% to 1% (Altschul, 1973).

The *Litsea* spp. can be found in various habitats, namely the peat swamp, mangrove swamps and other watery areas. They are also found in tropical and subtropical forests. The *Litsea* plant has a smooth bark in reddish brown colour. The leaves of the
plant are alternately arranged, individually, and have a white or grayish color on the back surface. Few of the species has been analysed for their essential oils chemical composition. Some of the important chemicals found in the plant are geraniol, nerol, geranial, citral, and citronellal (Lyth & Charles, 1998).

Analysis performed on *Litsea cubeba* has shown that a large amount of citral can be found in the fruits while geranial and nerol are abundant in the leaves and twigs (Lawless, 1996). Of Chinese origin, *cubeba* oil is the only source of internationally traded material. It is commonly known as May Chang. It is a tree which grows up to 10 metres, with bright green, lance shaped leaves on slender branches which bear fluffy, white flowers and small, round, green fruit about the size of a peppercorn. The oil of *Litsea cubeba* which is pale yellow or yellow in colour, has a strong, sharp, and complex lemony odour. Some of the major constituents of the plant is citral, limonene, myrcene, methyl heptone, linalool and linlyl acetate (Lawless, 1995).

![Figure 1: Major constituents in the essential oils of *Litsea cubeba*.](image)

The major use of oil of May Chang both in China and in international markets, is as a raw material source for the isolation of citral. This is used in its own right for flavour and fragrance purposes or converted by the chemical industry to a number of important
derivatives. May Chang is an excellent room deodoriser and insect repellent. A balsam or oil from the plant *Litsea cubeba* was implicated in contact sensitivity (Rudzki and Grzywa, 1976). To date, *Litsea cubeba* is the most studied species of all its other counterparts.

*Litsea citrata* is often mistaken for *Litsea cubeba* (May Chang), but in truth, *Litsea citrata* is a synonymous related species with a lower citral content and a wonderful citrus aroma which uplifts, cleanses and tones. *Litsea polyantha* is said to have a very irritant bark (Burkill, 1935). *Litsea garciae*, commonly known as Engkala has small fruits, only 1-1.5" across, with pink to purple skin and have an excellent delicate, avocado-like flavor. The fruit can be eaten fresh or used to prepare foods and can also be used in the same way as the avocado (Burkill, 1935). Its seeds contain fats that are used in the production of soaps and candles.

### 2.2 Chemical compounds and uses of essential oils

Essential oils from these plants are usually captured by steam distillation (Parker, 1993). Unlike ordinary vegetable oils, such as corn and olive, plant essences are highly volatile and will evaporate if left in the open air. The chemistry of essential oils is complex. Most consist of hundreds of components, such as terpenes, alcohols, aldehydes, and esters. For this reason, a single oil can help a wide variety of disorders. Lavender, for instance, is endowed with antiseptic, antibacterial, antibiotic, antidepressant, analgesic, decongestant and sedative properties. Moreover, due to their tiny molecular structure, essential oils applied to the skin can be absorbed into the bloodstream. They also reach the blood as a result of the aromatic molecules being inhaled. In the lungs, they pass
through the tiny air sacs to the surrounding blood capillaries by the process of diffusion. Once in the bloodstream the aromatic molecules interact with the body's chemistry.

*Litsea* is also widely used in the perfume and soap industries, providing a lemon-scented base note, often used to anchor the scents of the true citrus top notes that are far too fleeting, commonly extracted by steam-distillation or hydro-distillation. The strength of the initial aroma of *Litsea spp.* is strong with possible uses to treat acne and indigestion (Lawless, 1995). Although sometimes denigrated as 'waste products' of plant metabolism, studies have shown that plants utilize essential oils for such purposes as attracting pollinating insects, repelling predators and protecting themselves from disease which is quite a significant survival mechanism. *Litsea* has also been used in traditional Chinese medicine to treat indigestion, lower back pain, chills, headaches and travel sickness and is also useful in treating excessive perspiration and flatulence. Recent research indicates that it may have a use in treating cardiac arrhythmia. It is also an antispasmodic, specifically of the bronchia, and thus can be helpful in treating an asthma attack (John et al., 1987). According to most aromatherapists, *Litsea* is used for calming and as an inflammatory. It is also stated that it is anti-viral and regenerative. Practitioners of essential oil for medicinal purposes recommended that *Litsea* should not be used neat, as it is both an irritant and a potential sensitizer.

2.3 Extraction of essential oils

Extraction of essential oils achieved by hydro distillation is the most ancient method of distillation and the most versatile. Hydro-distillation of essential oils involves the vapours being condensed to yield a water condensate and an essential oil that can be
separated off, usually by gravity. Once tapped off, it is usually necessary to dry the oil over an inert material, such as anhydrous sodium sulphate. Hydrodistillation can be achieved by one of the two methods which are Clevenger distillation and steam distillation. In Clevenger distillation the material to be extracted is immersed in water, which is then boiled. In steam distillation, the steam passes through a bed of the material to be extracted. In both methods, the vapours of the volatile components are carried by the steam to a condenser. On condensation, oil-rich and water-rich layers are formed. These are separated by decantation. During both forms of hydrodistillation the sample is exposed to temperatures close to 100°C, which can lead to changes in 'thermolabile' components.

The essential oil of a plant usually consists of many compounds which generally boil between 150°C to 300°C. If attempts are made to remove these compounds by dry distillation many will decompose and the oil will be ruined. However, the compounds are steam volatile and can be distilled out of the vegetal materials at around 100°C. The distillation period depends on the plant material being processed. It can take from 15 to 30 minutes or longer and usually up to a few hours. Hydrodistillation seems to work best for powders like spice powders, ground wood and very tough materials like roots, wood, or nuts. Some material must be distilled immediately after harvesting, whereas others can be, and are best, stored for a day or two before distilling and finally there are materials which can be stored indefinitely before distillation. In general, flowers should be distilled immediately, whereas herbaceous material often benefits from wilting for one or two days before distillation. Woody materials may need to be ground and/or soaked before distillation. Most essential oils can be stored for long periods under suitable conditions.
They should be dry, not in contact with the air or direct sunlight and kept cool. Glass containers are often used for smaller amounts of oil but larger quantities are invariably stored in metal drums. Plastic containers like polythene should not be used because the oil may be absorbed by the plastic and contamination may occur.

2.4 Analyses of the essential oils

Analyses of essential oils are usually done by gas chromatography. One of the best techniques to identify the constituents of an essential oil is by using GC-MS. When properly used it can easily detect and identify major components of essential oils, and give some indications of the quality and authenticity of the oil. The technique does have limitations however. Many minor components of essential oils (<0.01%) do not register on GC detector systems, yet can be powerfully perceived by the nose, as indicated by aromagrams. Aromagrams are usually constructed by dividing the output of the GC column between the detector and an odour port, where components can be individually smelled and identified or described by perfumers/odour analysts, as they progressively elute from the column. In certain cases these undetected materials can contribute profoundly to the odour profile, and may also be responsible for psycho-physiological effects of the oils.
CHAPTER 3

Materials and Methods

3.1 Sampling

Litsea spp. used in this study were collected from the secondary forest of UNIMAS and in Simunjan. Fresh samples were used for extraction. The parts of the plants used in this study were the leaves, barks, and root.

3.2 Extraction

Extractions of essential oil from Litsea spp. were performed by hydro-distillation using a Clevenger-type apparatus (Datta, 1987). Approximately 100 g of grinded sample was weighed and transferred to a 2 L flat bottom flask and mixed up with 1.5 L of distilled water. The flask was assembled to the Clevenger trap and connected to the condenser. The hydro-distillation process was carried out for 8 hours continuously with the distillation rate of 1-2 drops per second. After 8 hours, oil trapped in the Clevenger was left to cool to room temperature. The oil was separated and dried up with anhydrous sodium sulphate and stored at 4-5°C. The process of hydro-distillation was replicated three times. The essential oil obtained was then stored in a cool and dark place before being analyzed using gas chromatography-flame ionization detector (GC-FID) and gas chromatography-mass spectrometry (GC-MS).
3.3 Instrumental analysis (GC-FID/GC-MS)

3.3.1 Gas Chromatography-Flame Ionization Detector (GC-FID)

The oils were analyzed on Shimadzu GC17A chromatograph equipped with a FID detector by using fused silica capillary column (25 cm x 0.3 mm). Helium was used as a carrier gas with the velocity of 2 mL/min. The initial temperature was programmed at 50°C and held for 2 minutes. The temperature was increased to 250°C at a rate of 6.5°C/min and held for 10 minutes. The temperature for the injector and detector were set at 280°C and 320°C respectively.

3.3.2 Gas Chromatography-Mass Spectrometry (GC-MS)

The oils were also analyzed on Shimadzu QP-5000 and medium polarity capillary column DB-5 (internal diameter of 30 mm x 0.25 mm). Film thickness of 0.25 μm was used to capture the presence of an organic compound in the essential oils with helium as the carrier gas. Exactly 1 μL of the sample was injected by using an indivisible mode. The initial temperature was held at 50°C and increased up to 250°C with the rate of 6.5°C/min and maintained for 10 minutes at the final temperature. The temperature for the injector and detector was set to 280°C and 320°C respectively.
3.4 Qualitative and Quantitative Analysis

3.4.1 Percentages of essential oils

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

3.4.2 Kovats Index

Kovats index was calculated to identify the chemical components in the essential oils. Calculation of Kovats index was based on chromatogram data for standard sample of C_{10} to C_{26}. Kovats index was calculated using the following formula:

\[ K_{lx} = 100 \left( \frac{\log T_{Rx} - \log T_{Rn}}{\log T_{Rn+1} - \log T_{Rn}} \right) + 100n \]

Where,

- \( T_{Rx} \) = retention time for component \( x \)
- \( T_{Rn} \) = retention time of aliphatic alkanes
- \( T_{Rn+1} \) = retention times of aliphatic alkenes
- \( K_{lx} \) = Kovats Index for component \( x \)