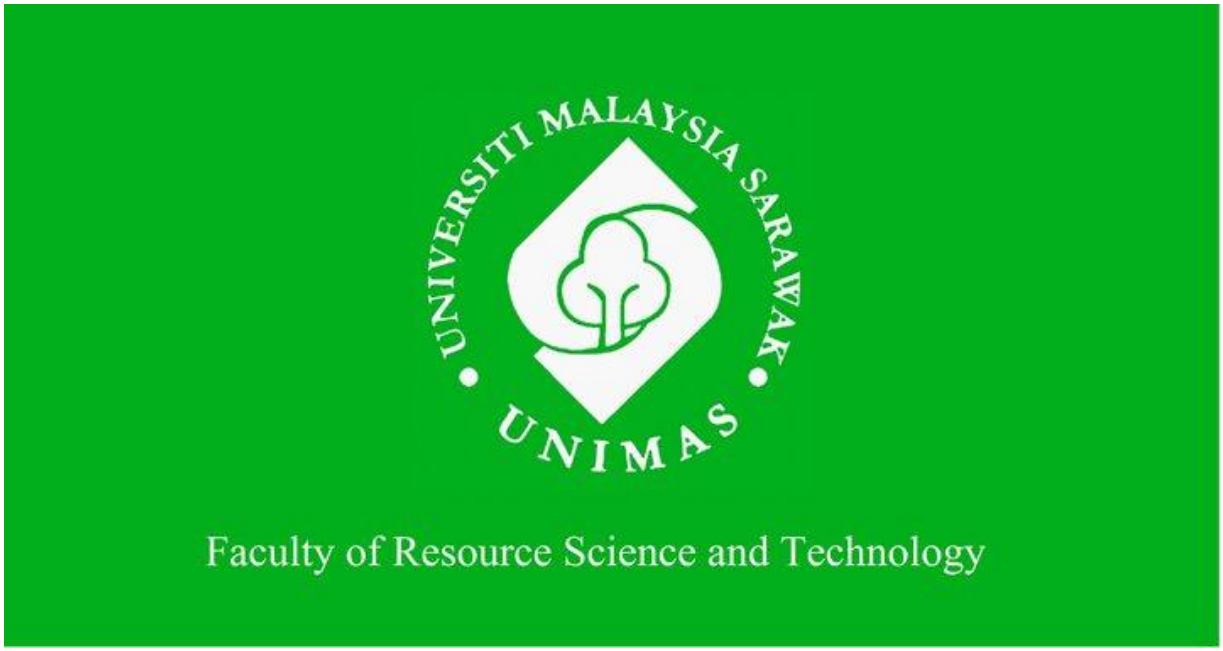

CHEMICAL COMPOSITION AND BIOLOGICAL ACTIVITY OF ESSENTIAL OIL FROM *CINNAMOMUM* SPP. AND *LITSEA* SPP.

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Bachelor of Science with Honours

(Resource Chemistry)

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Chemical Composition and Biological Activity of Essential Oil from *Cinnamomum* spp. and *Litsea* spp.

Noor Fasihah Binti Hasan

A final report submitted in partial fulfillment of the Final Year Project II STF 3015 Course

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Faculty of Resource Science and Technology

University Malaysia Sarawak

2011

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DECLARATION

No portion of the work referred 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.

NOOR FASIHAH BINTI HASAN

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Table of Contents

List of Figures

List of Tables

Chemical Composition and Biological Activities of Essential Oils of *Cinnamomum* spp. and *Litsea* spp.

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ABSTRACT

The essential oils from the leaves, barks, stems and roots of *Cinnamomum zeylanicum*, *Cinnamomum burmannii*, *Cinnamomum iners*, *Litsea gracilipes* and *Litsea resinosa* was extracted using hydrodistillation method. The essential oil obtained was analyzed by Gas Chromatography-Mass Spectroscopy (GC/MS). The percentage yields of essential oil from *Cinnamomum* and *Litsea* species ranged from 0.01-3.72 %. The highest percentage was obtained from the leaves of *C. burmannii* while the lowest percentage of oil obtained from the stem of *C. burmannii*. The major chemical compositions in *C. zeylanicum* were eugenol, camphor, tetradecanal and cinnamyl acetate. The chemical compositions in the oils of *C. burmannii* were rich in benzyl benzoate, (+)-2-bormanone, tetradecanal and caryophyllene. *C. iners* was contained (+)-2-bormanone and hexadecanoic acid as the major compound. The chemical compositions in the oil of *L. gracilipes* were rich in caryophyllene, α-bisabolene and spathulenol while the oil of *L. resinosa* contained o-cymene, caryophyllene, epizonarene and 3-methylacetophenone as the major constituents. The toxicity and antitermite activities of the essential oils were determined. All the essential oil showed inhibitory activity against the larvae of *Artemia salina* with the LC_{50} value in the ranged of 3.02-56.23 µg/mL. The leaf oil of *C. burmannii* showed stronger biological activity against the larvae of *A.salina* and termites of *Coptotermes* sp. with LC_{50} values of 3.02 µg/mL and 100 % mortality after 3 days treatment at 1.0 % concentration respectively. Other essential oil from *Cinnamomum* spp. was exhibited moderately inhibitory activity towards termites *Coptotermes* sp. with 50-61 % mortality at 10 % concentration after 3 days of contact.

Key words: *Cinnamomum* spp., *Litsea* spp., essential oil, toxicity, termiticidal activity.

ABSTRAK

Minyak pati daripada daun, kulit, batang dan akar bagi Cinnamomum zeylanicum, Cinnamomum burmannii, Cinnamomum iner, Litsea gracilipes dan Litsea resinosa telah diekstrak dengan menggunakan kaedah penyulingan hidro. Minyak pati yang telah diekstrak dianalisis menggunakan Gas Kromatografi-Spektroskopi Jisim (GC/MS). Peratusan hasil minyak pati yang diperolehi daripada spesies Cinnamomum dan Litsea adalah dalam julat 0.01-3.27 %. Minyak pati dari daun C. burmanii memberikan peratusan yang tertinggi manakala minyak pati dari batang C .burmannii memberikan peratusan yang terendah. Komponen utama minyak pati bagi C. zeylanicum ialah eugenol, kamphor, tetradecanal dan asetat sinamil. Komponen utama bagi C. burmannii ialah benzil benzoate, (+)-2-bornanone, tetradecanal dan karyophylin. C. iners, mengandungi (+)-2-bornanone dan asid hexadecanoik sebagai komponen utama. Komponen utama minyak pati bagi L. gracilipes ialah karyophylin, α-bisabolin dan spatulenol manakala minyak pati bagi L. resinosa mengandungi o-cymene, karyophylin, epizonarin dan 3-metilacetophenone sebagai komponen utama. Kesan ketosikkan dan aktiviti anti-serangga setiap minyak pati telah dikenalpasti. Kesemua minyak pati adalah toksik terhadap anak udang, Artemia salina dengan nilai LC_{50} dalam julat 3.02-56.23 µg/mL. Minyak pati daripada daun C. burmannii menunjukan aktiviti biologi yang sangat tinggi terhadap anak udang, A.salina dan anai-anai, Coptotermes sp. dengan nilai LC_{50} 3.02 µg/mL dan 100 % kematian selepas 3 hari berturut-turut diuji dalam 1.0 % kepekatan minyak pati. Minyak pati lain daripada Cinnamomum spp. Menunjukan aktiviti biologi yang sederhana terhadap anai-anai, Coptotermes sp. dengan 50-61 % kematian pada 10 % kepekatan selepas 3 hari diuji.

Kata kunci: Cinnamomum spp., Litsea spp., minyak pati, ketoksikan, anti-serangga aktiviti.

CHAPTER 1

INTRODUCTION

The genus of *Cinnamomum* and *Litsea* are belonging to the Lauraceae family. The family of Lauraceae is a large family. The Lauraceae family contains about 50 genera and approximately 2500-3500 species of trees, shrubs or climbers mostly distributed in tropical and subtropical low land forest of Africa, South America, Southeast Asia and Australia.

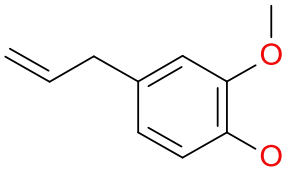
Cinnamomum species contain essential oils that can be obtained from their stem-bark, leaves, root and root-bark. Cinnamon, the most important commercial product obtained from the bark of four *Cinnamomum* species, *viz. C. zeylanicum, C. loureirii, C. burmannii* and C. *cassia,* depends on cinnamaldehyde for its characteristic taste (Lawrence 1967). The bark oil of C. *zeylanicum* is used as flavour in foods and drinks, as a component of perfumes and in many pharmaceutical preparations (Reynolds 1989). The leaf oil from the same species is an important natural source of eugenol **(1)** (Guenther 1975).

Some of the chemical constituents which are presence in the essential oils of *Cinnamomum* species are very important due to their biological activity. The chemical constituents of the essential oils of *Cinnamomum* species gave a valuable effect especially in biological activities such as antifungal (Wang *et al.*, 2004; Cheng *et al.*, 2005), antibacterial (Chang *et al.*, 2001) and others. For example, the antimicrobial properties of cinnamon are thought to be due to eugenol **(1)** and a derivative of cinnamaldehyde **(2)**.

Litsea species also contain essential oil that can be obtained from the leaf, stem, bark, root and fruit. According to Kirtikar and Basu (1993), the root of *L. chinensis* is bitter and sweetish and it is useful in aphrodisiac, tonic, throat troubles, burning sensations, bronchitis,

fever, consumption and inflammations. Its bark is slightly balsamic and most popular as native drugs. It is used as mild astringent in diarrhea and dysentery. Besides that, it is also used either dry or mixed with water and milk, where it is applied on bruises and wounds. The oil extracted from its berry can be used to treat rheumatism.

The efficient method for the extraction of the essential oils of *Cinnamomum* and *Litsea* species is required due to their uses for consumer product. So that, the study on the chemical compositions and the biological activities of the essential oils of *Cinnamomum* and *Litsea* species are found essential to be studied.



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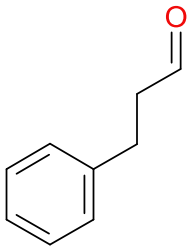

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The objectives of this research are:

1. To characterize and identify the chemical composition of the essential oil from several *Cinnamomum* spp. and *Litsea* spp.
2. To evaluate the biological activity of essential oils towards brine shrimp larvae, *Artemia salina* for toxicity test and antitermite activity against *Coptotermes sp.*

CHAPTER 2

LITERATURE REVIEW

2.1 *Cinnamomum* spp.

The genus *Cinnamomum* belongs to the Lauraceae family which consists of 250 species (Fang *et al*., 2005). The genus *Cinnamomum* is probably native from East and Southeast Asia to Australia (Wee and Hsuan, 1990). *Cinnamomum* species is an evergreen tree or shrubs which are found in tropical rain forest. The flowers of genus *Cinnamomum* are small, in axillary and subterminal panicles, where the females are usually the largest flowers (Kirtikar and Basu, 1993). The leaves are opposite or alternate which is usually 3-nerved. Some of the species from the genus of *Cinnamomum* are *C. camphora*, *C. cassia*, *C. burmannii*, *C. iners*, *C. javanicum*, *C. sintoc*, *C. tamala* and *C. zeylanicum*.

Cinnamomum zeylanicum is also known as cinnamon which is native to India, Sri Lanka and Penisular Malaysia. Cinnamon trees grow best under conditions of constant warmth and moisture where they thrive in tropical forest at an altitude of about 1500 feet. The bark of *C. zeylanicum* is aromatic and stimulant which contains from 0.5 to 1 percent essential oil (Foster and Rebecca, 2006). The essential oil derived from the bark of cinnamon is used to stimulate digestion, respiration and blood circulation (Wee and Hsuan, 1990). Besides that, the oil from bark of *C. zeylanicum* contains cinnamaldehydes **(2)** which has antispasmodic, antifungal, antibacterial and carminative properties (Foster and Rebecca, 2006). In pharmaceutical, the essential oil is used for dental preparation and as oral hygiene products (IMR, 2000). *C. zeylanicum* leaf oil is also used in perfumes, spices and in the synthesis of vanillin.

Cinnamomum iners is moderately sized tree and can be found in Western India, Myanmar, Java, Malaysia and Sumatra (Kirtikar and Basu, 1993; IMR, 2000). In traditional medicine, the seeds of *C. iners* are bruised and mixed with honey or sugar to treat children with dysentery and coughs (Kirtikar and Basu, 1993). The roots and leaves are laxative and have been used to treat poisoning, wounds and fever. *C. iners* can be made into tonic and is applied as poultice to relieve rheumatism. The bark of *C. iners* is used to treat abdominal colic and acts as laxative (IMR, 2000).

Cinnamomum camphora is an evergreen tree which is native of China, Japan and Formosa and introduced into and cultivated in many countries including India. The tree attains a height of about 100 feet with a girth of 6 to 20 feet. The leaves are leathery, shining and aromatic (Kapoor, 2001). The fruits are dark green, ovoid, rather dry and globose. When ripe they turn black. The odor of bruised leaves of the camphor-yielding plant resembles that of camphor. Camphor is formed in the oil cells distributed in all parts of plant (Kapoor, 2001). Camphor is used externally to relieve pain, muscle aches and pains and chest congestion, resulting from colds and bronchitis (Wee and Hsuan, 1990).

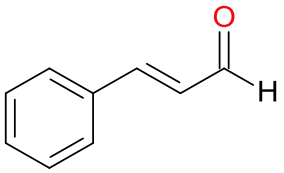
Besides that, the essentail oil of *C. camphora* also contains safrole which is used in the preparation of expensive perfumes (Duke, 1985). Camphor is esteemed as an analeptic in various cardiac depressions and has been used in the treatment of myocarditis. It is very useful in hysteria and nervousness and is used in the treatment of serious diarrhea. It is also employed in external application as a counterirritant in the treatment of muscular strains, reumatic conditions and inflammations (Kapoor, 2001).

Cinnamomum tamala is a moderately sized evergreen tree which is native to Philippines. The tree is up to 1.4 m girth and 7.5 m high. The leaves are glabrous and usually 10 to 13 cm long. The leaves also leathery rarely alternate which is 3-nerved, rarely elliptical and obtuse (Kapoor, 2001). The leaf of *C. tamala* is bitter taste and is used in folk medicine to treat heart disease, ozoena, diuretic, inflammation, sore eyes and good for liver and spleen. The oil of *C. tamala* is powerful stimulant and the bark of *C. tamala* is used to treat gonorrhoea (Kirtikar and Basu, 1993).

2.2 Chemical composition of essential oil from *Cinnamomum* spp.

According to the Wang *et al*. (2009), eugenol **(1)** (79.75%) is the major volatile compound component in the essential oil of *Cinnamomum zeylanicum* instead of trans-cinnamaldehyde **(3)** (16.25%) and the other compounds including aldehydes, alcohols, alkanes, alkenes, ketones, ethers and sulfides. The chemical composition in the bark and leaf oil of C. *zeylanicum* consists of high levels of eugenol **(1)** (90.2%) and cinnamaldehyde **(2)** (44.2%).

The chemical constituents of C. *zeylanicum* bark essential oil are composed of three major and six minor constituents (Yang *et al.,* 2005). The three major constituents are cinnamaldehyde **(2)** (58.1%), benzaldehyde **(4)** (12.2%) and eugenol **(1)** (5.1%) while the six minor constituents are α-phellandrene **(5)** (1.1%), linalool **(6)** (1.1%), benzoic acid **(7)** (0.8%), β-caryophyllene **(8)** (0.7%), linalyl acetate **(9)** (0.6%) and benzyl cinnamate **(10)** (0.6%). The main constituents identified in the leaf oil of *Cinnamomum zeylanicum* grown in Little Andaman, India are eugenol **(1)** (76.60%), linalool **(6)** (8.5%) and piperitone **(11)** (3.31%) (Raina *et al.,* 2001).



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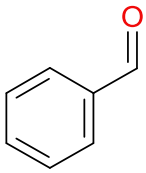

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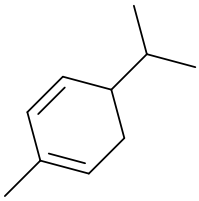

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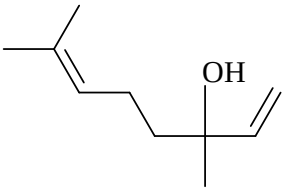

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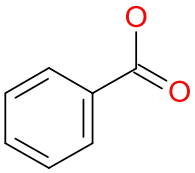

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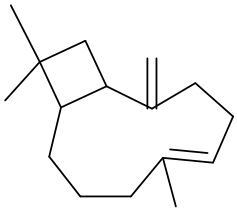

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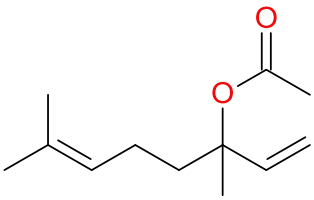

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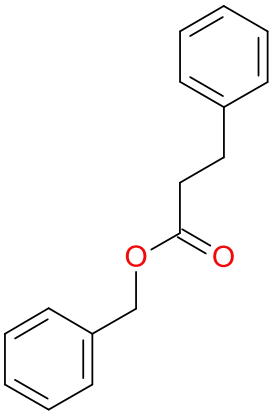

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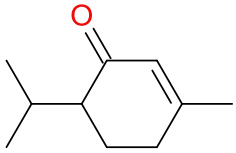

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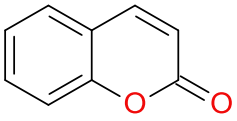
The chemical constituents in the leaf oil of *Cinnamomum burmanii* are trans-cinnamaldehyde **(3)** (60.17%), eugenol **(1)** (17.62%) and coumarin **(12)** (13.39%). Other constituents identified in the oils are alcohols, aldehydes and ketones (Wang *et al.*, 2009). The

major component in the stem bark oil of *Cinnamomum iners* are 1,8-cineole **(13)** (40.76%), α-terpineol **(14)** (15.06%) and terpinen-4-ol **(15)** (13.85%). The other components identified are β-pinene **(16)** (4.75%), γ-terpinolene **(17)** (1.61%) and caryophyllene oxide **(18)** (4.37%) (Baruah *et al.*, 2001).



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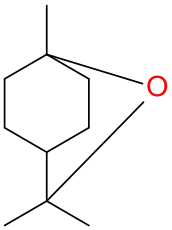

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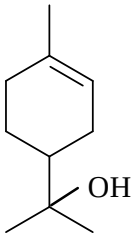

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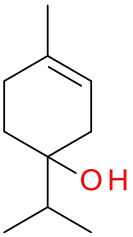

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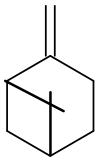

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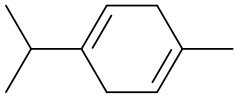

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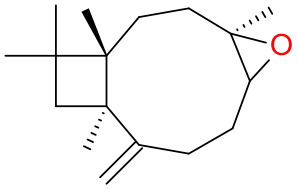

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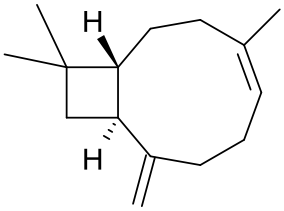
The major constituents in the leaf oil of *Cinnamomum camphora* is 1,8-cineole **(13)** (Senanayake and Wijesekera, 1989). Other chemical constituents identified in the oils include caryophyllene **(19)**, dipentene **(20)**, pinene **(21)** and terpineol **(14)** (Duke, 1985). In *C. camphora* seed oil, the major chemical constituents present are linalool **(6)**, camphor **(22)** and limonene **(23)** (Liu *et al.*, 2005).



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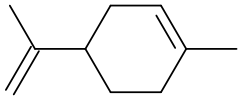

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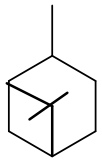

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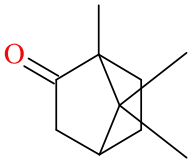

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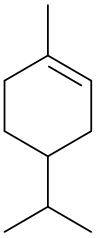

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2.3 Biological activity of *Cinnamomum* spp.

A recent study on *Psoroptes cuniculi*, showed that the essential oil of *Cinnamomum zeylanicum* which consist of several compounds such as linalool, cinnamyl acetate and eugenol showed insecticidal activity (Fichi *et al.*, 2005). According to Yang *et al.*, (2005), the pediculicidal constituents of *C. zeylanicum* bark essential oil were identified as benzaldehyde, linalool and cinnamaldehyde by GC-MS analysis. Additionally, benzaldehyde, benzyl cinnamate, cinnamaldehyde and salicylaldehyde were also highly effective ovicides against *Pediculus humanus capitis* (human head louse).

According to Seenivasan *et al.* (2006), the essential oil of cinnamon is the most effective antibacterial agent. The antibacterial activity has been attributed to the presence of some active constituents in the oils. Cinnamaldehyde is the major constituent of cinnamon oil. Cinnamaldehyde is a natural antioxidant and the animal studies suggest that an extract of cinnamon bark may help prevent stomach ulcer. Cinnamon oil is locally applied with much benefit in neuralgia and headache. As an antiseptic, it is used is an injection gonorrhea and also used as germicide that used internally in typhoid fever. Besides that, the cinnamon oil is also used in the treatment of cancer and other microbial diseases. It can be incorporated into creams, lotions, drops and other which are externally on the body to treat diseases caused by *Aspergillus niger*.

The major chemical constituents of *Cinnamomum camphora* essential oil are linalool, limonene and camphor were reported to be toxic, repellent or fumigant and some of them acted synergistically (Tripathi *et al.*, 2000, 2003; Hummelbrunner and Isman, 2001). Studies performed by Liu *et al.* (2005) showed that the high concentration of linalool in the oils of *C.*

camphora was responsible for the phytotoxic effect on seed germination of wheat and broad bean. A recent study has been performed to investigate the inhibitory effects of *C. camphora* extracts to explore its potential anti-inflammatory mechanisms under non-cytotoxic (less than 100 μg/mL) conditions (Lee *et al.*, 2005).

2.4 *Litsea* spp.

Litsea is a genus of evergreen or deciduous trees or shrubs belonging to the Lauraceae family. There are about 622 species distributed mainly in tropical and subtropical Australia, New Zealand, North America, South America and Asia (Agrawal *et al.,* 2011). More than 10 species of this genus found in China which mostly growing in warm regions of the South and Southwest.

Litsea spp. can be found at the peat swamp, mangrove swamps and other watery areas. The leaf of *Litsea* spp. is alternate, penninerved, with naked or scaly buds. The flower of the genus *Litsea* is small, dioeciously, with four to six flowered umbels, sessile or shortly pedunculate, axillary or in the scars of fallen leaf (Kirtikar and Basu, 1993). The *Litsea* plant also has a smooth bark in reddish brown color.

Litsea umbellata is known as *medang ayer* in IndoMalaya. *L. umbellata* is a lowland forest tree that can be found in India and Southeast Asia. The stem of *L. umbellata* is petioles and the leaves are simple, alternate and exstipulate. The leaves of *L. umbellata* are used as a poultice to heal boils (Wiart, 2006).

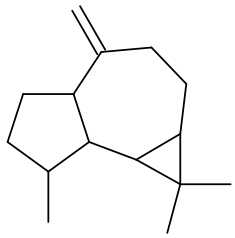
Litsea glutinosa is a medium-sized evergreen tree up to 75 ft in height and it can be found throughout India ascending up to an altitude of 1400 m in the Himalayas (Kapoor, 2001; Sukh Dev, 2006). The leaves of *L. glutinosa* are 7-15 cm long, aromatic and pubescent. The

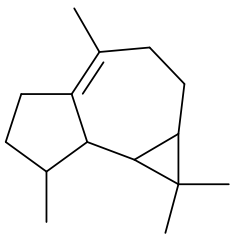
bark of *L. glutinosa* is thick and has brownish grey color on the outside and pale with a reddish tinge color on the inside. The flowers are small, yellowish and borne in umbellate heads. The fruits are fleshy berries and the seeds are exalbuminous (Kapoor, 2001; Dev, 2006).

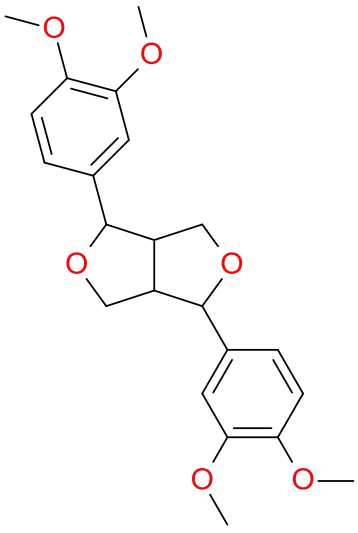
Besides that, the bark of *L. glutinosa* is medicinally useful which is used in diarrhea and dysentery. The freshly ground bark is used as an emollient application to bruises, sprains, rheumatic and gouty joints. The wood of *L. glutinosa* is also stated to be durable and resistant to insect attacks (Kapoor, 2001).

2.5 Chemical composition of essential oil from *Litsea* spp.

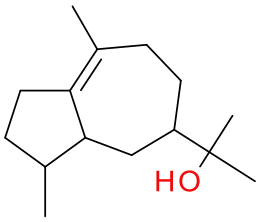
The chemical composition of essential oil from several *Litsea* spp. had been reported by Nisha *et al.* (2011), Sura (2008) and Ahmad *et al.* (2005). According to Agrawal *et al.* (2011), aromadendrene **(24)**, ledene **(25)** and (+)-Eudesmin **(26)** were detected in *L. gracilipes*; also bulnesol **(27)**, β-caryophyllene **(8)** and β-elemene **(28)** in *L. resinosa*.

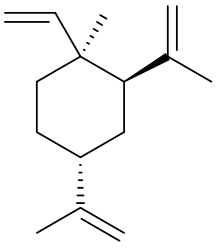






24 **25** **26**

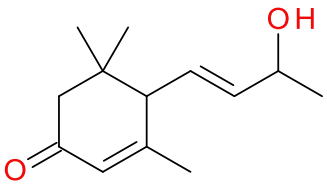




27 **28**

The study conducted by Ahmad *et al*. (2005) has shown that the chemical composition in the leaf oil of *L. resinosa* contain high percentage of bulnesol **(27)** (14.9%), β-caryophyllene **(8)** (10.2%), β-elemene **(28)** (10.2%) and other sesquiterpenoids. Ledene **(25)** (9.0%) and aromadendrene **(24)** (8.3%) were the major components in the leaf oil of *L. gracilipes.*

Previous study by Sura (2008) has shown that the major chemical composition in essential oil of *L. resinosa* were 3-oxo-α-ionol **(29)** (50.59%), tricosane **(30)** (43.14%) and acetovanillone **(31)** (23.49%).



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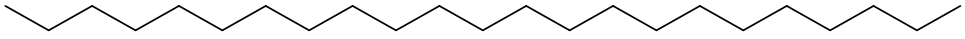

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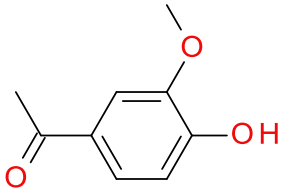
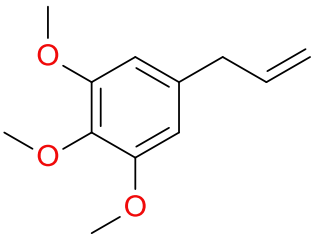


31

According to Aimy (2005), the major components in the essential oil from leaves of *L. gracilipes* were elemicin **(32)** (29.08%), geranyl acetone **(33)** (12.20%), hexadecane **(34)** (8.00%), calamenene **(35)** (7.23%), T-muurolol **(36)** (6.23%). Chemical composition in the essential oil from leaves of *L. resinosa* were heneicosane **(37)** (4.51%), acetovanillone **(31)** (3.89%), methyl eugenol **(38)** (3.23%), (E)-isoelemicin **(32)** (3.13%) (Aimy, 2005).



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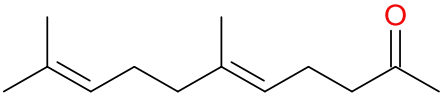

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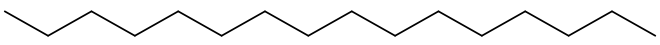

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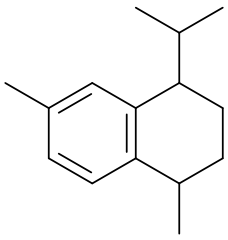

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