ESSENTIAL OILS PROFILE AND BIOLOGICAL ACTIVITIES OF 
LITSEA SPP. AND CINNAMOMUM SPP. (LAURACEAE)

Wan Nur Aishah Bt Wan Abdullah

Bachelor of Science with Honours 
(Resource Chemistry) 
2013
Essential Oils Profile and Biological Activities of *Litsea* spp. and *Cinnamomum* spp. (Lauraceae)

Wan Nur Aishah bt Wan Abdullah (28613)

A final report submitted in partial fulfillment of the
Final Year Project 2 STF 3015

Supervisor: Prof. Dr. Fasihuddin Badaruddin Ahmad
Co-supervisor: Prof. Dr. Zaini Assim

Resource Chemistry
Department of Chemistry
Faculty Resource Science and Technology
Universiti Malaysia Sarawak
2013
DECLARATION

This thesis entitled “Essential Oils Profile and Biological Activities of Litsea spp. and Cinnamomum spp. (Lauraceae)” is a presentation of my own research work and has not been submitted to any other university for and degree. Whenever contribution of others are involved, every effort is made to indicate this clearly with due reference to the literature.

Wan Nur Aishah bt Wan Abdullah

Resource Chemistry

Faculty of Resource Science and Technology

Universiti Malaysia Sarawak
ACKNOWLEDGEMENT

Alhamdulillah my most gratitude to Allah S.W.T to His blessing for the strength and courage that gives me as I can finally finished my final year project. This project could not been done without the guidance and fill support of all the people around me, who I greatly indebted and cherish most.

My important source of knowledge that always support me when I lost, my most gratitude and appriciation is extended to my supervisor Prof. Dr. Fasihuddin Badruddin Ahmad for always helping me in this project. Thanks also to my co-supervisor Prof. Dr. Zaini Assim for his guidance throughout compliting this thesis report. Besides, I also want to express my gratitude to all lecturers from Chemistry Department for guided me in the progress of carriying out this project. Special thank also to my family and my other half for always supporting me in my studies with unlimited advice and courage for me completing this project.

Last but not least, I would like to thank to the lab assistant and all my beloved friends who always being beside me help and support thoughout completion of this project.
# Table of Content

Acknowledgement ........................................................................................................ ii  
Table of Content ........................................................................................................... ii  
List of Figures ................................................................................................................ vi  
List of Tables .................................................................................................................. vi  
Abstract .......................................................................................................................... 1  
1.0 Introduction .............................................................................................................. 2-3  
2.0 Literature Review  
  2.1 Introduction ........................................................................................................... 4  
  2.2 Extraction of Essential Oil ....................................................................................... 5  
  2.3 Phytochemical and Biological activities studied on *Litsea* spp. ...................... 6-7  
  2.4 Phytochemical and Biological activities studied on *Cinnamomum* spp. ........ 8-9  
3.0 Material and Methods  
  3.1 Plant Sample ........................................................................................................ 10  
  3.2 Extraction of Essential Oil ...................................................................................... 10  
  3.3 Instrumental Analysis ............................................................................................ 11  
  3.4 Qualitative and Quantitative Analysis ................................................................... 12  
  3.5 Bioassay ................................................................................................................. 13
4.0 Results and Discussion

4.1 Percentage of Essential Oil

4.1.1 *Litsea* spp................................................................. 14

4.1.2 *Cinnamomum* spp.......................................................... 15

4.2 Chemical Composition of Essential Oil

4.2.1 Chemical Composition of Essential Oil of *L. resinosa*......... 16

4.2.2 Chemical Composition of Essential Oil of *L. nidularis*......... 19

4.2.3 Chemical Composition of Essential Oil of *C. javanicum*....... 22

4.2.4 Chemical Composition of Essential Oil of *C. microphyllum*.... 26

4.3 Brine shrimp *Artemia salina* toxicity test.......................... 29

5.0 Conclusion.............................................................................. 34

References.................................................................................. 36
List of Figures

Figures | Pages
---|---
**Figure 4.1**: Gas chromatogram of root oil of *L. resinosa*. | 18
**Figure 4.2**: Gas chromatogram of leaves oil of *L. resinosa*. | 18
**Figure 4.3**: Gas chromatogram of bark oil of *L. nidularis*. | 21
**Figure 4.4**: Gas chromatogram of leave oil of *L. nidularis*. | 21
**Figure 4.5**: Gas chromatogram of leave oil of *C. javanicum*. | 24
**Figure 4.6**: Gas chromatogram of bark oil of *C. javanicum*. | 24
**Figure 4.7**: Gas chromatogram of root oil of *C. javanicum*. | 25
**Figure 4.8**: Gas chromatogram of leave oil of *C. microphyllum*. | 28
**Figure 4.9**: Gas chromatogram of bark oil of *C. microphyllum*. | 28
**Figure 4.10**: Percentage average death of *Artemia salina* against concentration of root oil *L. resinosa*. | 30
**Figure 4.11**: Percentage Average death of *Artemia salina* against concentration of leave oil *L. resinosa*. | 30
**Figure 4.12**: Percentage average death of *Artemia salina* against concentration of bark oil *L. nidularis*. | 31
**Figure 4.13**: Percentage average death of *Artemia salina* against concentration of root oil *L. nidularis*. | 31
**Figure 4.14**: Percentage average death of *Artemia salina* against concentration of leave oil *L. nidularis*. | 31
**Figure 4.15**: Percentage average death of *Artemia salina* against concentration of bark oil *C. javanicum*. | 32
**Figure 4.16**: Percentage average death of *Artemia salina* against concentration of root oil *C. javanicum*. | 32
Figure 4.17: Percentage average death of *Artemia salina* against concentration of 32 leave oil *C. javanicum*.

Figure 4.18: Percentage average death of *Artemia salina* against concentration of 33 bark oil *C. microphyllum*.

Figure 4.19: Percentage average death of *Artemia salina* against concentration of 33 root oil *C. microphyllum*.

Figure 4.20: Percentage average death of *Artemia salina* against concentration of 33 leave oil *C. microphyllum*. 
List of Table

Tables                                      Pages

Table 4.1 : Percentage yield and color of the essential oils from *Litsea* spp.   14
Table 4.2 : Percentage yield and color of the essential oils from *Cinnamomum* spp.  15
Table 4.3: Chemical Composition of essential oil extracted from *L. resinosa*        17
Table 4.4: Chemical Composition of essential oil extracted from *L. nidularis*      20
Table 4.5 : Chemical Composition of essential oil extracted from *C. javanicum*      23
Table 4.6 : Chemical Composition of essential oil extracted from *C. microphyllum*   27
Table 4.7 : Toxicity of essential oil from *Litsea* and *Cinnamomum* spp. against  29
  *Artemia salina* at different concentration and their LC$_{50}$ values.
Essential Oils Profile and Biological Activities of Litsea spp. and Cinnamomum spp. (Lauraceae)

Wan Nur Aishah bt Wan Abdullah

Resource Chemistry Programme
Faculty of Resource Science and Technology
Universiti Malaysia Sarawak

ABSTRACT

The essential oils from various parts of Litsea nidularis, Litsea resinosa, Cinnamomum javanicum and Cinnamomum microphyllum (Lauraceae) was extracted using hydrodistillation method. The essential oils obtained were analyzed using Gas Chromatography Mass Spectrometry (GC-MS). The percentage of essential oil extracted from two Litsea spp ranged from 0.5% to 1.5% (v/w). The percentage of essential oils from two Cinnamomum spp. ranged from 1.20% to 8.12%. The highest yield of oils was obtained from the roots of C. javanicum (8.12%) and lowest oils obtained from the barks of C. microphyllum (1.20%). The root oil and the leaves oil of L. resinosa was rich in (Z, E)- α-farnesene (12.40%) and cadina-3, 9-diene (27.11%). Elixene was the highest composition compound in the bark oil of L. nidularis with 9.17% compared to the leaves oil which was rich in selina-6-en-4-ol (20.62%). The bark, leaves and root oil of of C. javanicum was rich in 1-Isopropyl-4,8-dimethylspiro [4.5] dec-8-en-7-one (18.97%), 3,5-Diisopropylphenol (12.79%) and eugenol methyl ether (23.67%) respectively. The leaves oil of C. microphyllum was rich in longipinocarveol (18.48%) while the bark oil of C. microphyllum was rich in 6-Isopropenyl-4,8a-dimethyldecahydro-1-naphthalenol (22.29%). Toxicity test against brine shrimp Artemia salina showed that some of the essential oil from Litsea spp. and Cinnamomum spp. were toxic. The leaves oil of L. nidularis and bark oil of C. javanicum showed the highest toxicity with LC_{50} less than 10μg/mL which showed 100% mortality at concentration of 10μg/mL.

Key words: Lauraceae, Litsea spp., Cinnamomum spp., hydrodistillation, essential oil, toxicity test

ABSTRAK

Minyak pati dari pelbagai bahagian Litsea nidularis, Litsea resinosa, Cinnamomum javanicum dan Cinnamomum microphyllum (Lauraceae) telah diekstrak menggunakan kaedah penyulingan hidro. Minyak pati yang diperoleh telah dianalisis menggunakan Kromato Gas Spektrum Jisim (GC-MS). Peratusan minyak pati yang diekstrak daripada dua Litsea spp adalah pelbagai dari 0.5% kepada 1.5% (v/w). Peratusan minyak pati daripada dua Cinnamomum spp. antara 1.20% hingga 8.12% di mana hasil tertinggi minyak diperoleh daripada akar C. javanicum (8.12%) dan minyak paling rendah diperoleh daripada kulit C. microphyllum (1.20%). Minyak akar dan daun L. resinosa adalah kaya dengan (Z, E) - α-farnesene (12.40%) dan cadina-3, 9-diene (27.11%). Elixene merupakan komposisi tertinggi dalam minyak batang L. nidularis dengan 9.17% berbanding dengan minyak daun yang kaya dengan Selina-6-en-4-ol (20.62%). Minyak batang, daun dan akar daripada C. javanicum adalah kaya dengan 1-isopropyl-4,8-dimethylspiro[4.5]dec-8-en-7-one (18.97%), 3,5- Diisopropylphenol (12.79%) dan eugenol metil etar (23.67%) masing-masingnya. Minyak daun C. microphyllum kaya dengan longipinocarveol (18.48%) manakala minyak batang daripada C. microphyllum kaya dengan 6-isopropenyl-4,8 a-dimethyldecahydro-1-naphthalenol (22.29%). Ujian ketoksikan terhadap anak udang Artemia salina menunjukkan bahawa sesetengah minyak pati dari Litsea spp. dan Cinnamomum spp. adalah toksik. Minyak daun L. nidularis dan minyak batang C. javanicum menunjukkan tahap ketoksikan yang tertinggi dengan LC_{50} kurang daripada 10 μg / mL yang menunjukkan kadar kematian 100% pada kepekatan 10 μg / mL.

Kata kunci : Lauraceae, Litsea spp., Cinnamomum spp., penyulingan hidro, minyak pati, ujian ketoksikan
CHAPTER 1
INTRODUCTION

1.1 General Introduction

Essential oils are widely used for various applications. Essential oils are known as aromatic substances and produced by specific plant species. Most of these oils have been used as raw materials of the fragrance and flavoring agents since ancient years. Oil is "essential" in the sense that it carries a distinctive scent, or essence, of the plant. These oils are usually extracted by distillation. Essential oil can be extracted from various part of plant including flower, fruit, stem, leaves and roots (Nakatsu et al., 2000).

Essential oil from plant can be extracted by using several methods. The most commonly used is steam or hydrodistillation and solvent extraction (Milner et al., 1997). Essential oils are most studied chemical compound in plants as regard their composition and physical and chemicals properties. Advance in organic chemistry has allowed the establishment of the techniques to identify the component profiles of many aromas and fragrances that permit the establishment of composition standard for trade regulation and for the synthesis using less costly starting raw materials (Hernandez, 2005).

Lauraceae family consist about 55 genera and over 2,000 species distributed in the tropical and subtropical regions of the world, especially in the warm or tropical regions of Southeast Asia and Brazil (Van Der Werff, 1996). Some of the important genera in the Lauraceae family include Litsea and Cinnamomum.
The essential oil obtained by steam distillation of the floral calyces of *Ocotea bofo Kunth* (Lauraceae), consisted of 25 compounds, including the major components estragole (48.7%), α-phellandrene (19.6%) and sabinene (10.4%) have demonstrated radical scavenging and chain breaking antioxidant properties comparable to or better than those provided by synthetic controls (Guerrini et al., 2006). The evaluation of putative bioactivities and potential medicinal application, including antifungal tests, antioxidant evaluation, anti inflammation activity and cytotoxicity against human umbilical vein endothelial cell line (HUVEC) on the leaves and branches of 27 Lauraceae tree species in Taiwan suggested that these Lauraceae have a great potential for further development as cancer chemoprevention agents or food supplements for promoting human health (Lin et al., 2007).

1.2 Objectives of Study

The objective of this study are to extract essential oil from selected genera in Lauraceae family using hydrodistillation method, to characterize and identify the chemical composition of the essential oil and to evaluate the toxicity of essential oil against brine shrimp larvae, *Artemia salina.*
CHAPTER 2
LITERATURE REVIEW

2.1 Introduction

The fragrant mixture of liquids, obtained through distillation of aromatic plant materials, is known as an essential oil (Burt, 2004). Essential oil is a naturally occurring substance in a form of concentrated liquid (Christine, 2007). It can be present in the plant body of aromatic plants as isolated cells (organelles or idioblasts) or more at the surface of the plant material as glandular hairs, or in cavities, ducts or canals (Malingre, 1981; Kubeczka and Bohn, 1986). Volatile compounds of many plant extracts and essential oils consist of alkanes, alcohols aldehydes and terpenoids, particularly monoterpenoids (Coats et al., 1991). Essential oil has been used as antimicrobial agent in food spoilage against a specific pathogen like *Listeria* and *Salmonella* (Bhusita et al., 2009). Other uses of essential oils are to add flavor to the foodstuff and beverages and to scent perfumes, lotions, soaps, detergents, and household cleaner (Hernandez, 2005). Essential oils are isolated from various parts of the plant, such as leaves (basil), fruits (mandarin), bark (cinnamon), root (ginger), grass (citronella), gum (balsam oils), berries (pimenta), seed (caraway), flowers (rose), twigs (clove stem) and wood (amyris) (Cava et al., 2007; Hussain et al., 2008).
2.2 Extraction of Essential Oil

This essential oil can be extract through various methods. Some of the distillation methods that are widely used to extract essential oil include, hydrodistillation, steam distillation, simultaneous micro steam distillation/solvent extraction (SD/SE), hydrodiffusion, turbo distillation, rectification and fractional distillation (Nakatsu et al., 2000; Wang et al., 2005; Cheng et al., 2006). The hydrodistillation method is featured by the fact that the plant material is heated with water and the stem is generated within the still (Boelens et al., 1989).

Hydrodistillation or steam distillation is the most widely utilized physical method for isolating essential oils from the botanical material (Masango, 2004). Most studies which focus on the essential oil of herbs have made use of hydrodistillation in Clevenger-type apparatus (Hussain et al., 2008). In hydrodistillation procedure, the material is immersed in water, which is heated to boiling point using an external heat source. In both hydro-, and steam-distillation techniques, the vapors are allowed to condense and the oil is then separated from the aqueous phase (Houghton and Raman, 1998). In hydrodistillation the plant material and water are combined in the still and the whole things is then brought to a boil. The hot water draw out the oils, just as steam does, and it is carried to the condenser and cooled into hydrosol and essential oil. This method produces a finer, more complete product, as hot water is collar than steam distillation and shocks the plant material less (Ackerman, 2001). Sefidkon et al. (2007) isolated the essential oils from the aerial parts of Satureja rechingeri by steam, hydro- and water-steam-distillation. The highest oil yield was obtained with hydrodistillation and the lowest with steam distillation (Sefidkon et al., 2007).
2.3 Phytochemical and Biological Activities Studies on Litsea spp

Litsea is one of the largest genera in Lauraceae family which consist about 400 species in tropical and subtropical region of both hemispheres. This species are widely distributed between Southeast Asia, including Australasia (more than 350 spp.) and America (8 spp.) (Rohwer, 1993). This genus is commonly found in forest or dense bushes which is cloud-covered for much of the year. Large numbers of this genus are found in Asia and other in Australia, Pacific Islands and America. The local uses this plant is as pest repellent. Bifenthrin (1) isolated from L. elliptica can be used in controlling dengue vectors (Sulaiman et al., 2008). Sulaiman et al. (2006) also reported that the L. elliptica extract, such as bifenthrin (1), could be potentially used in the control of the Aedes aegypti adults. In addition, the essential oils of Litsea elliptica have been reported to have adulticidal effects on Aedes mosquito (Hidayatulfathi et al., 2004).

The anti-diarrheal activity of methanol extract of dried bark was aerial parts of Litsea polyantha has been evaluated in mice using different models (Poonia et al., 2007). The antioxidant activity of phenolic fractions of bark extract was evaluated by various chemical and enzymatic methods (Arfan et al., 2008). Phytoconstituents of essential oil can be studied by using gas chromatography–mass spectrometry (GC–MS) technique. This technique is suitable for the detection of the biological volatile organic compounds and corresponding volatile profile characteristics (Zhang et al., 2009). Devi et al. (2010) investigated antioxidant and anti-inflammatory and wound healing effects of Litsea glutinosa in rats. The aqueous extract of Litsea glutinosa (250 and 500 mg/kg body weight) was studied for anti-inflammatory in animal models. The activity was studied in some acute models Viz carragenan, histamine and dextrin induced rats paw edema against indomethacin as standard, and it showed significant anti-inflammatory activity in all the three models.
The terpenoids commonly found in all the *Litsea* species studied are linalool (2), α-pinene (3), β-pinene (4) and α-terpineol (5), as major constituent (Agusta et al., 1999). However, their relative abundances are different, giving rise to a characteristic chromatographic profile for each of the surveyed species. These compounds are also common to other Lauraceae species, including *Cinnamomum*, *Laurus*, *Lindera*, *Ocotea*, and *Persea* genera (Setzer et al., 2007).
2.4 Phytochemical and Biological Activities Studies on Cinnamomum spp.

*Cinnamomum* is one of the genera in Lauraceae family which is well-known and widely used as medicinal plant (Wiart, 2006). This genus consists over 250 species and distributed in tropical and subtropical region of China, India, East Africa and South Asia (Ibrahim et al., 1995). *Cinnamomum* spp. is used to treat blood clotting, cough, fever and scar treatment and also to control blood sugar (Wiart, 2002). The leaves, bark and strip are also used to reduce toxin and painful in their body (Mat Salleh et al., 2002; Wiart, 2002). Research by Agarwal et al. (2012), showed that the essential oils of *Cinnamomum* species have effective antimicrobial and antioxidant activities.

Previous studies on the leaves oils of Lauraceae species revealed antioxidant, antibacterial, fungicidal, cytotoxic, and cruzain inhibitory activities (Wright et al., 2007). Most of the chemical components of essential oils are terpenoid, including monoterpenes, sesquiterpenes, and their oxygenated derivatives. These low-molecular weight compounds can easily diffuse across cell membranes to induce biological reactions (Chao et al., 2005). The composition of the oil, and therefore, its value and the use to which it can be put, depends very much on the species that are distilled as well as the parts of the plant that are utilized (Chericoni et al., 2005).

Various interesting biological activities have been reported as the essential oil of *cinnamomum*. Studies by Lui 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.
Previous study on biological activity of *Cinnamomum cassia* on *Eschericia coli* revealed that the bacterial cells which were treated with cinnamic aldehyde from the extract oil suffered from nerve damages in their surface structure (Kim et al., 2003). High concentration of cinnamaldehyde in the essential oil also exhibited strong antifungal activity against *Candida albicans* (Giordani et al., 2005). Most essential oil of the *Cinnamomum* species are shown possess antimicrobial properties and may inhibit the growth of *Bacillus cereus* (Araar, 2009). Moreover, one of the secondary compounds which are proanthocyanidin catechins from *Cinnamomum* bark possesses strong antibacterial properties (Shang et al., 2007). The antimicrobial activity of *Cinnamomum zeylanium* essential oil against 21 bacteria and 4 *Candida* species using disc diffusion and minimum inhibitory concentration (MIC) methods was reported (Unlu et al., 2010). Cinnamaldehyde and eugenol has been proposed to inhibit the important enzyme production by bacteria and cause damage to the bacteria cell wall. Therefore, the high antimicrobial activity of this *Cinnamomum* oil may be due the presence of high amount of cinnamaldehyde (Unlu, 2010).

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 (58.1%) (6), benzaldehyde (12.2%) (7) and eugenol (5.1%) (8).
CHAPTER 3

MATERIALS AND METHODS

3.1 Plant Samples

*Litsea nidularis* and *Litsea resinosa* were collected from secondary forest of Universiti Malaysia Sarawak, while *Cinnamomum javanicum* and *Cinnamomum microphyllum* found around Samatan, Sarawak. The samples were separated into leaves, bark and roots. The fresh samples were used for extraction.

3.2 Extraction of the essential oil

The essential oil was extracted using hydrodistillation method in a Clevenger apparatus according to the method described by Makgwane (2006). The ground sample was weighed and approximately 100g sample was placed into 2L flat bottom flask. About 1.5L distilled water was added into the flask. The flat bottom flask was then assembled to the Clevenger trap connected to the condenser. The distillation process was carried out for six hours. After six hours, oil trapped in the Clevenger was left to cool to room temperature. The essential oil from Clevenger was separated and dried with sodium sulphate anhydrous. The oil was stored at 4-5°C. The hydrodistillation process was repeated for three times. The percentage yield of essential oil was calculated based on the dry weight of sample according to the following formula:

\[
\text{Percentage of essential oils (\%)} = \frac{\text{Volume of essential oil (ml)}}{\text{Dry weight of sample (g)}} \times 100\%
\]
3.3 Instrumental Analysis

Essential oil was diluted with n-hexane and the oil constituent was analyzed by using Gas Chromatography-Mass Spectrometry (GC-MS)

3.3.1 Gas Chromatography-Mass Spectrometry

Gas Chromatography-Mass Spectrometry (GC-MS) model Shimadzu QP2010 system series was used to analyze the essential oils by determining molecular mass of the compound. The analysis was performed using non-polar DB-5 cross linked (30 m long × 0.25 mm ID × 0.25 μm film thickness composed of 5% of phenyl methyl polysiloxane). The initial temperature was programmed at 50°C for 2 minutes. Then it was increased to 300°C with the rate of 6.5°C/min for 10 minutes. The injector and detector temperature was set to 280°C and 300°C respectively. Helium was used as carrier gas. Exactly 1 μL of sample was diluted in 100 μL of n-hexane and 1 μL diluted sample injected into GC-MS (Houghton and Raman, 1998; Pavia et al., 2002).
3.4 Qualitative and Quantitative Analysis

3.4.1 Kovats Index

Kovats index was calculated to identify the chemicals component in the essential oils based on method described by Lucero et al. (2009). Kovats index was calculated based on chromatogram data for standard sample of C10-C26. Kovats index was calculated using following formula:

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

\( t_{Rx} = \) retention time for component

\( t_{R(n+1)} = \) retention time of aliphatic alkane with \( n+1 \)

\( t_{Rn} = \) retention time for aliphatic alkane

\( K_{Ix} = \) Kovats index of component \( x \)

3.4.2 Semi-quantitative Analysis

Gas chromatography was used to analyze with semi-quantitative analysis to calculate the percentage of individual chemicals components in the essential oils using normalization methods.

\[ \% X = \left( \frac{A_x}{\sum A_i} \right) \times 100 \]

\( A_x = \) area of \( X \)

\( \sum A_i = \) sums of all the area
3.5 Brine Shrimp, *Artemia salina* Toxicity Test

Procedure developed by McLaughin (1991), were used and tested against brine shrimp, *Artemia salina* in order to determine the toxicity of the essential oil. Initially, 20g of *A. salina* eggs (biomarine) was added into small clear container and filled with seawater for hatching process. Aerator was placed inside the container to produce air continuously. The brine shrimp was used after 48 hours for bioassay. Exactly 2 mg of essential oil was dissolved in 2 mL methanol. Exactly 600 μL, 200 μL and 20 μL of the samples were transferred into different vials in triplicate. Solvent was then removed completely using evaporator and 2.0 mL of seawater was added to each vial to give the final concentration of 300 ppm, 100 ppm and 10 ppm. Ten *A. salina* larvae were then added to each vial. The number of survivors was observed every 1, 4, 6, 12, and 24 hours of contact. After 24 hours of contact, the number of survivors countered and the percentage of death against concentration was plotted and \( LC_{50} \) values from the plot was determined. Seawater was used as negative control and 10 ppm of thymol was used as positive control.
CHAPTER 4
RESULT AND DISCUSSION

4.1 Percentage of Essential Oil

4.1.1 Litsea spp.

The percentage of essential oils extracted from the two Litsea spp ranged from 0.50% to 1.49% (v/w) (Table 4.1). The highest yield of oils was obtained from the leaves of L. resinosa (1.50%) and the lowest oil was obtained from barks of L. nidularis.

Table 4.1: Percentage yield and color of the essential oils of Litsea spp.

<table>
<thead>
<tr>
<th>Species</th>
<th>Plant part Used</th>
<th>Oil percentage (%)</th>
<th>Color</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Bark</td>
<td>0.50</td>
<td>Clear</td>
</tr>
<tr>
<td>L. nidularis</td>
<td>Leaves</td>
<td>1.30</td>
<td>Clear</td>
</tr>
<tr>
<td>L. resinosa</td>
<td>Root</td>
<td>0.51</td>
<td>Light yellow</td>
</tr>
<tr>
<td></td>
<td>Leaves</td>
<td>1.49</td>
<td>Light yellow</td>
</tr>
</tbody>
</table>
4.1.2 *Cinnamomum* spp.

The percentage of essential oils from the two *Cinnamomum* spp. studied ranged from 1.20% to 8.12% (Table 4.2). The highest yield of oils was obtained from the roots of *C. javanicum* (8.12%) and lowest oils obtained from the barks of *C. microphyllum* (1.20%).

All of these essential oils existed as liquid form at room temperature.

Table 4.2 : Percentage yield and color of the essential oils from *Cinnamomum* spp.

<table>
<thead>
<tr>
<th>Species</th>
<th>Plant part Used</th>
<th>Oil percentage (%)</th>
<th>Color</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Barks</td>
<td>6.91</td>
<td>Yellow</td>
</tr>
<tr>
<td><em>C. javanicum</em></td>
<td>Roots</td>
<td>8.12</td>
<td>Yellow</td>
</tr>
<tr>
<td></td>
<td>Leaves</td>
<td>5.50</td>
<td>Yellow</td>
</tr>
<tr>
<td><em>C. microphyllum</em></td>
<td>Barks</td>
<td>1.20</td>
<td>Clear</td>
</tr>
<tr>
<td></td>
<td>Roots</td>
<td>2.97</td>
<td>Clear</td>
</tr>
<tr>
<td></td>
<td>Leaves</td>
<td>3.47</td>
<td>Clear</td>
</tr>
</tbody>
</table>