CHEMICAL STUDIES ON THE ESSENTIAL OILS OF SEVERAL CINNAMOMUM SPECIES AND THEIR BIOLOGICAL ACTIVITIES

Christine Anak Jinang

Bachelor of Science with Honours (Resource Chemistry) 2007
CHEMICAL STUDIES ON THE ESSENTIAL OILS OF SEVERAL CINNAMOMUM SPECIES AND THEIR BIOLOGICAL ACTIVITIES

CHRISTINE ANAK JINANG

This project is submitted in partial fulfillment of the requirements for the degree of Bachelor of Science with Honours (Resource Chemistry)

Faculty of Resource Science and Technology
UNIVERSITI MALAYSIA SARAWAK
2007
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.

Christine anak Jinang  
Program of Resource Chemistry  
Faculty of Resource Science and Technology  
Universiti Malaysia Sarawak
ACKNOWLEDGEMENT

I would like to express my gratitude to my supervisor, Assoc. Prof. Dr. Fasihuddin Badruddin Ahmad for the precious guidance and assistance throughout my final year project. My appreciation also goes to my family, friends and anyone who has in one way or another contributed towards the improvement and completeness of my final year project.
TABLE OF CONTENTS

DECLARATION ii
ACKNOWLEDGEMENTS iii
TABLE OF CONTENTS iv-vi
LIST OF FIGURES vii-viii
LIST OF TABLES ix-x
ABSTRACT xi
ABSTRAK xii
CHAPTER 1
INTRODUCTION 1

1.1 Introduction 1-4
1.2 Objectives 4

CHAPTER 2
LITERATURE REVIEW 5

2.1 Cinnamomum spp. 5-9
2.2 Extraction and Isolation 9-13
of Essential Oils
2.3 Characterization and Identification 13-16
of Chemical Constituents from
Cinnamomum spp. Essential Oils
CHAPTER 3

MATERIALS AND METHODS

3.1 Plant Materials

3.2 Preparation of Extracts

3.3 Instrumental Analysis of Essential Oil
   3.3.1 Thin Layer Chromatography (TLC)
   3.3.2 Preparative Thin Layer Chromatography (PTLC)
   3.3.3 Gas Chromatography-Mass Spectrometry (GC-MS)

3.4 Quantitative Analysis
   3.4.1 Essential Oils Yield
   3.4.2 Semi-Quantitative Analysis

3.5 Qualitative Analysis
   3.5.1 Kovats Index

3.6 Biological Activity-Brine Shrimp
   (Artemia salina) Lethality Assay
CHAPTER 4

RESULTS AND DISCUSSION

4.1 Essential Oil Yield

4.2 Chemical Component of Essential Oils

4.2.1 Kovats Index

4.2.2 *Cinnamomum* sp. 1

4.2.3 *Cinnamomum tahijanum*

4.2.4 *Cinnamomum griffithii*

4.2.5 *Cinnamomum javanicum*

4.3 Purification of Essential Oil

4.3.1 Thin Layer Chromatography

(TLC)

4.3.2 Preparative Thin Layer Chromatography (PTLC)

4.4 Brine Shrimp Lethality Test

CHAPTER 5

CONCLUSIONS

REFERENCES

APPENDICES
**LIST OF FIGURES**

<table>
<thead>
<tr>
<th>Figure 4.1: GC spectra of n-alkane standard</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Figure 4.2: GC spectra of <em>Cinnamomum</em> sp. 1 leaves essential oil</td>
<td>34</td>
</tr>
<tr>
<td>Figure 4.3: GC spectra of <em>Cinnamomum</em> sp. 1 root essential oil</td>
<td>35</td>
</tr>
<tr>
<td>Figure 4.4: GC spectra of <em>Cinnamomum</em> sp. 1 root-bark essential oil</td>
<td>35</td>
</tr>
<tr>
<td>Figure 4.5: GC spectra of <em>Cinnamomum</em> sp. 1 stem-bark essential oil</td>
<td>36</td>
</tr>
<tr>
<td>Figure 4.6: GC spectra of <em>C. tajijanum</em> leaves essential oil</td>
<td>41</td>
</tr>
<tr>
<td>Figure 4.7: GC spectra of <em>C. tajijanum</em> root essential oil</td>
<td>41</td>
</tr>
<tr>
<td>Figure 4.8: GC spectra of <em>C. tajijanum</em> root-bark essential oil</td>
<td>42</td>
</tr>
<tr>
<td>Figure 4.9: GC spectra of <em>C. tajijanum</em> stem-bark essential oil</td>
<td>42</td>
</tr>
<tr>
<td>Figure 4.10: GC spectra of <em>C. griffithii</em> leaves essential oil</td>
<td>48</td>
</tr>
<tr>
<td>Figure 4.11: GC spectra of <em>C. griffithii</em> root essential oil</td>
<td>49</td>
</tr>
<tr>
<td>Figure 4.12: GC spectra of <em>C. griffithii</em> root-bark essential oil</td>
<td>49</td>
</tr>
<tr>
<td>Figure 4.13: GC spectra of <em>C. griffithii</em> stem-bark essential oil</td>
<td>50</td>
</tr>
<tr>
<td>Figure 4.14: GC spectra of <em>C. javanicum</em> young leaves essential oil</td>
<td>54</td>
</tr>
<tr>
<td>Figure 4.15: GC spectra of <em>C. javanicum</em> matured leaves essential oil</td>
<td>54</td>
</tr>
<tr>
<td>Figure 4.16: GC spectra of <em>C. javanicum</em> root essential oil</td>
<td>55</td>
</tr>
<tr>
<td>Figure 4.17: GC spectra of <em>C. javanicum</em> root-bark essential oil</td>
<td>55</td>
</tr>
<tr>
<td>Figure 4.18: GC spectra of <em>C. javanicum</em> stem-bark essential oil</td>
<td>56</td>
</tr>
<tr>
<td>Figure 4.19: Mass spectra of Band 1-2 <em>C. javanicum</em> root essential oil</td>
<td>64</td>
</tr>
<tr>
<td>Figure 4.20: Mass spectra of Band 2-2 <em>C. javanicum</em> root essential oil</td>
<td>65</td>
</tr>
</tbody>
</table>
Figure 4.21: Average death of *Artemia salina* (%) as a function of different concentration of *Cinnamomum* sp. 1 essential oils

Figure 4.22: Average death of *Artemia salina* (%) as a function of different concentration of *C. tahijanum* essential oils

Figure 4.23: Average death of *Artemia salina* (%) as a function of different concentration of *C. griffithii* essential oils

Figure 4.24: Average death of *Artemia salina* (%) as a function of different concentration of *C. javanicum* essential oils
**LIST OF TABLES**

<table>
<thead>
<tr>
<th>Table 4.1: Average percentage of yield and colour of the essential oils</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>27</td>
<td></td>
</tr>
<tr>
<td>Table 4.2: Retention time for n-alkane standard analyzed by GC-MS</td>
<td>29</td>
</tr>
<tr>
<td>Table 4.3: Chemical composition of <em>Cinnamomum</em> sp. I essential oils</td>
<td>31-34</td>
</tr>
<tr>
<td>Table 4.4: Chemical composition of <em>C. taitianum</em> essential oils</td>
<td>37-40</td>
</tr>
<tr>
<td>Table 4.5: Chemical composition of <em>C. griffithii</em> essential oils</td>
<td>44-48</td>
</tr>
<tr>
<td>Table 4.6: Chemical composition of <em>C. javanicum</em> essential oils</td>
<td>51-53</td>
</tr>
<tr>
<td>Table 4.7: Rf value of chemical constituents of <em>Cinnamomum</em> sp. I essential oils developed in hexane-ethyl acetate (9:1, v/v) and visualize under UV lamp and vanillin</td>
<td>58</td>
</tr>
<tr>
<td>Table 4.8: Rf value of chemical constituents of <em>C. taitianum</em> essential oils developed in hexane-ethyl acetate (9:1, v/v) and visualize under UV lamp and vanillin</td>
<td>59-60</td>
</tr>
<tr>
<td>Table 4.9: Rf value of chemical constituents of <em>C. griffithii</em> essential oils developed in hexane-ethyl acetate (9:1, v/v) and visualize under UV lamp and vanillin</td>
<td>60-61</td>
</tr>
<tr>
<td>Table 4.10: Rf value of chemical constituents of <em>C. javanicum</em> essential oils developed in hexane-ethyl acetate (9:1, v/v) and visualize under UV lamp and vanillin</td>
<td>61-62</td>
</tr>
<tr>
<td>Table 4.11: Rf value of chemical constituents of <em>C. javanicum</em> root essential oil</td>
<td>63</td>
</tr>
<tr>
<td>Table 4.12: Rf value of chemical constituents of <em>C. javanicum</em> root essential oil</td>
<td>64</td>
</tr>
</tbody>
</table>
Table 4.13: Identified major compounds for Band 1-2 based on the comparison between the mass spectra data with Wiley Incorporated database

Table 4.14: Identified major compounds for Band 2-2 based on the comparison between the mass spectra data with Wiley Incorporated database

Table 4.15: Average death of *Artemia salina* at different concentration and the LC$_{50}$ values
Chemical studies on essential oils of several Cinnamomum species and their biological activities

Christine anak Jinang

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

ABSTRACT

The essential oils isolated from leaves, root, root-bark and stem-bark of four Cinnamomum spp. (Cinnamomum sp. 1, C. tahijanum, C. griffithii and C. javanicum) using hydrodistillation method were analyzed by gas chromatography-mass spectrometry (GC-MS) and their chemical constituents were compared. Kovat indices for individual component in the essential oils were determined and these indices were used for identification. The percentage yield of the essential oils ranged between 0.26-9.56% and stem-bark of Cinnamomum sp. 1 gave the highest yield percentage of essential oil. The major components in the essential oils of Cinnamomum sp. 1 were δ-cadinol (11.78%), diethyl malonate (7.89%), 2-octenal (7.16%), isoborneol (6.94%), hydrocinnamic acid (42.70%), (E)-α-bergamotene (44.27%), isocoryophyllene (8.74%), cinnamyl alcohol (8.70%), (E)-isoelemicin (7.47%), (−)-γ-elemene (48.11%) and β-bisabolol (10.86%). Meanwhile, the major components of the C. tahijanum essential oils were β-phellandrene (9.23%), α-cadinol (6.52%), (−)-γ-elemene (24.53%), β-selinene (16.71%), wine lactone (19.60%), β-farnesene (12.71%), (E)-α-bergamotene (11.12%), geranyl isovalerate (14.73%), γ-dodecalactone (14.07%), cedrol (18.95%) and β-eudesmol (6.28%). C. griffithii essential oils contain nitro-phenylethane (7.16%), 1,3-p-menthadiene-7-ol (6.57%), α-copaene (7.31%), (−)-γ-elemene (8.82%), eudesmol (7.75%), (Z)-3-hexenyl-2-methylbutanoate (7.58%), nonanoic acid (24.69%), β-bourbonene (17.94%) and ethyl benzoate (14.72%) as the major components. The major components in the essential oils of C. javanicum were perilla aldehyde (47.23%), (E)-β-damascone (25.18%), decadienal (26.57%), (−)-γ-elemene (24.76%), hydrocinnamic acid (16.24%), (E)-α-bergamotene (12.14%), dill apiole (11.97%), methyl cinnamate (11.81%), styrene glycol (8.23%) and 10-epi-γ-eudesmol (7.45%). C. javanicum young and matured leaves essential oils contain similar major compounds with similar amount which were perilla aldehyde (47.23% and 36.61%), methyl cinnamate (11.81%), styrene glycol (8.23%) and 10-epi-γ-eudesmol (7.45%). The toxicity of each essential oil from the four Cinnamomum spp. was investigated using brine shrimp, Artemia salina. Results from the brine shrimp lethality test showed that the essential oils of Cinnamomum sp. 1 stem-bark, C. tahijanum root, C. griffithii root-bark and C. javanicum root-bark possessed the strongest lethality activities against Artemia salina with the lethal concentration (LC₅₀) value of 50 μg/mL.

Keywords: Cinnamomum spp., essential oils, chemical compositions, GC-MS, brine shrimp lethality test
ABSTRAK

Minyak pati yang diekstrak dari bagian daun, akar, kulit akar dan kulit batang empat spesies Cinnamomum (Cinnamomum sp. I, C. tahitianum, C. griffithii dan C. Javanicum) dengan menggunakan kaedah penyulingan hidro dan tanah dialalis menggunakan kromatografi gas-spektroskopi jisim (GC-MS) serta komposisi kimia setiap spesis dibandingkan. Indeks Kovat bagi setiap komponen minyak pati telah ditentukan dan indeks ini digunakan untuk tujuan pencirian. Peratusan hasil minyak pati yang diperoleh adalah dalam julat 0.26-9.56% dan minyak pati dari kulit batang Cinnamomum sp. I memberikan peratusan yang tertinggi. Komponen utama minyak pati Cinnamomum sp. I adalah δ-cadinol (11.78%), dictil malonat (7.89%), 2-oktenal (7.16%), isobornenol (6.94%), asid hidrosinamik (42.70%), (E)-α-bergamotene (44.27%), isocaryophyllene (8.74%), alkohol sinamil (8.70%), (E)-isoelemicin (7.47%), (–)-γ-elemene (64.81%) dan β-bisabolol (10.86%). Komponen utama bagi minyak pati C. tahitianum ialah β-phellandrene (9.23%), α-cadinol (6.52%), (–)-γ-elemene (24.53%), β-selinene (16.71%), wine lactone (19.60%), β-farnesene (12.71%), (E)-α-bergamotene (11.12%), geranil isovaleriat (14.73%), γ-dodecalactone (14.07%), cedrenol (18.95%) dan β-eudesmol (6.28%). Komponen utama minyak pati dari C. griffithii ialah nitro-phenylethane (7.16%), 1,3-b-methyliden-7-ol (6.57%), α-copaene (7.31%), (–)-γ-elemene (8.82%), eudesmol (7.75%), (Z)-3-hexenyl-2-methylbutanoate (7.58%), nonanoic acid (24.69%), β-bourbonene (17.94%) dan etil benzoate (14.72%). Komponen minyak pati C. javanicum yang dikenalpasti ialah perilla aldehyde (47.23%), (E)-β-damascone (25.18%), decadienal (26.57%), (–)-γ-elemene (24.78%), hydrocinclamic acid (16.24%), (E)-α-bergamotene (12.14%), diall apiol (11.97%), metil cinnamate (11.81%), styrene glycol (8.23%) dan 10-e-γ-eudesmol (7.45%). Minyak pati daun muda dan daun tua C. javanicum didapati mengandungi amuah komponen utama yang banyi sama iaitu perilla aldehyde (47.23% dan 36.61%) dan (E)-β-damascone (27.18% dan 24.62%). Kesal ketoksikan setiap minyak pati dari Cinnamomum spp. tersebut dikaji dengan menggunakan larva anak udang, Artemia salina. Keputusan mengunjukkan minyak pati yang diperoleh dari kulit batang Cinnamomum sp. I, akar C. tahitianum, kulit akar C. griffithii dan C. javanicum menunjukkan ketoksikan pada tinggi dengan nilai LC₅₀ 50 μg/mL.

Kata kunci: Cinnamomum spp., minyak pati, komposisi kimia, GC-MS, ujian ketoksikan pada anak udang.
CHAPTER 1

INTRODUCTION

The genus *Cinnamomum* belongs to the Lauraceae family which consists of 250 species (Fang et al., 2005). The genus *Cinnamomum* is a group of aromatic trees and shrubs, native to the region stretching from East and Southeast Asia to Australia (Wee and Hsuan, 1990), and some of the Pacific Islands (Senanayake and Wijesekera, 1989). The leaves of *Cinnamomum* spp. are evergreen and in opposite pairs, where each has three prominent main veins and is leathery in texture (Wee and Hsuan, 1990). *Cinnamomum* spp. which are of commercial values include *C. zeylanicum*, *C. cassia*, *C. camphora*, *C. loureirii*, *C. burmannii*, *C. obtusifolium*, *C. tamala* and *C. sintoc* (Senanayake and Wijesekera, 1989).

*Cinnamomum* species contain essential oils that can be obtained from their stem-bark, leaves, root and root-bark. Essential oils from *Cinnamomum* species consist of complex mixtures of volatile, aromatic compounds which are used as flavouring and fragrances. These essential oils are a concentrated, hydrophobic liquid and mainly composed of mono- and sesquiterpenes and phenolic compounds (Peter and Amala, 1998). Several *Cinnamomum* spp. are important sources of essential oils which have been traded since antiquity. Many of the *Cinnamomum* spp. have been used in folk medicine for their interesting biological activities such as anti-diabetic, anti-inflammatory, intestinal infections, astringent and diuretic (Huang, 2003). The essential oils from *Cinnamomum* spp. such as *C. camphora* are used externally to relieve pain, muscle aches and
chest congestion, resulting from colds and bronchitis (Wee and Hsuan, 1990). The bark of *C. zeylanicum* is used as spice or condiment, for flavouring cakes and sweets, in curry powder and in incenses and perfumes (Wee and Hsuan, 1990). The oil obtained from unripe fruits of *C. cassia* is used in chocolate and allied industries (Wee and Hsuan, 1990).

The extraction of the essential oils from *Cinnamomum* spp. can be performed using several methods such as steam distillation (Sritharan *et al.*, 1994; Nakatsu *et al.*, 2000) or hydrodistillation (Wang *et al.*, 2004; Cheng *et al.*, 2005), simultaneous micro steam distillation/solvent extraction, gas-phase sparging, headspace analysis (Jayatilaka *et al.*, 1994; Nakatsu *et al.*, 2000), cold press extraction, solvent extraction and supercritical fluid extraction (Nakatsu *et al.*, 2000).

The volatiles constituents in the essential oils of some *Cinnamomum* species have been studied. There are several methods that can be used to identify the constituents of essential oils from stem-bark, leaves and root-bark of plant species such as thin layer chromatography (TLC) (Sritharan *et al.*, 1994), gas chromatography (Jayatilaka *et al.*, 1994; Wang *et al.*, 2004), gas chromatography-mass spectrometry (GC-MS) (Jayatilaka *et al.*, 1994; Ali and Jantan, 1995; Cheng *et al.*, 2005) and high performance liquid chromatography (HPLC) (Shen *et al.*, 2004).

Each of the *Cinnamomum* species contains the common and unique volatile constituents. For example in *C. cassia*, the major component of its bark oil is cinnamaldehyde with smaller amounts of terpenes, aromatic compounds and esters (Jayatilaka *et al.*, 1994). The major
component of its leaf oil is eugenol and its root bark oil is camphor (Jayatilaka et al., 1994). The bark oil of C. zeylanicum contains eugenol and only traces of coumarin (Jayatilaka et al., 1994). There were some variations among the Cinnamomum species in the main components of leaf and stem bark oils. The major constituents in the oil extracted from the leaf were either eugenol (C. capparucoronde, C. rivulorum, C. sinharajanse and C. verum), linalool (C. litseaefoliunm), α-terpineol (C. dubium and C. ovalifolium) or citronellal (C. ciriroidorum) (Sritharan et al., 1994). In the stem-bark oil, the major constituents were either cinnamaldehyde (C. citriodorwn and C. verum), linalool (C. dubium and C. ovalifolium), α-terpineol (C. litseaefoliunm) or eugenol (C. capparucoronde and C. rivulorum) (Sritharan et al., 1994).

Some of the chemical constituents, which are active ingredients presence in the essential oils of Cinnamomum species, are very important due to their broad spectrum of 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.

Some of the Malaysian Cinnamomum spp. has also been investigated for their essential oil chemical constituents (Jantan and Goh, 1992). Most of the Cinnamomum spp. oils studied contain linalool, safrole, camphor, eugenol, 1,8-cineole and benzyl benzoate (Ali and Jantan, 1995). Based on the study of the essential oil constituents, the basis of chemotaxonomy analysis of Cinnamomum spp. were reported where the study of the secondary metabolites such as
terpenes, alkaloids, phenolics and other compounds can be of considerable taxonomic value (Bandoni et al., 1975; Stahl and Kubeczka, 1979).

Studies of *Cinnamomum* spp. in Malaysia, especially in Sarawak are very rare. Therefore, this study is performed to determine the chemical constituents of *Cinnamomum* spp. essential oils and its biological activity.

The objectives of this study are to isolate the essential oils of several *Cinnamomum* spp. using the hydrodistillation method, and to determine the percentage yields. The essential oils will be characterize and the major chemical constituents of the essential oils in *Cinnamomum* spp. will be identified by using various chromatographic procedures include thin layer chromatography (TLC), preparative thin layer chromatography (PTLC) and gas chromatography-mass spectrometry (GC-MS). The biological activity of the essential oil isolated from the *Cinnamomum* spp. will be evaluated using brine shrimp (*Artemia salina*) lethality test.
CHAPTER 2
LITERATURE REVIEW

2.1 *Cinnamomum* spp.

Lauraceae is a very large family of 35 genera, 2500 species of trees and shrubs (Francis, 1992) and can be found throughout the tropics and subtropics. Some of the genus from the Lauraceae family includes *Cinnamomum*, *Litsea*, *Laurus*, *Phoebe*, *Cassytha* and *Eusideroxylon* (Francis, 1992). The Lauraceae trees or shrubs usually are of aromatic values. Its leaves are simple, rarely lobed, usually alternate, pinninerved or pininerved. Flowers are generally small, regular, hermaphrodite or dioecious, sometimes polygamous-dioecious and generally fragrant (Pulle, 1966). The Lauraceae family fruit a one-seeded berry, with fleshy or leathery wall, often with a basal persistent calyx. Although consisting of many genera, the Lauraceae family is remarkably uniform in the structure of the fruit and seed, and also in the seedling morphology (Francis, 1992).

The genus *Cinnamomum* which is found in tropical rain forests at a range of altitudes and are rare in areas that have seasonal climate (Kirtikar and Basu, 1993) is an evergreen tree or shrubs. It has leaves that are opposite or alternate which is usually 3-nerved. The flowers of the genus *Cinnamomum* are small, hermaphrodite or by abortion polygamous, in axillary and subterminal panicles, where the females usually have the largest flowers and sometimes with fewer parts (Kirtikar and Basu, 1993). The genus *Cinnamomum* also fruit a berry which is resting on the
spreading more or less enlarged perianth, the segments of which are wholly or partly deciduous and less often persistent. The seeds of this genus are covered with thin testa (Kirtikar and Basu, 1993). Some of the species from the genus of *Cinnamomum* are *C. camphora, C. cassia, C. loureirii, C. iners, C. javanicum, C. sintoc, C. tamala* and *C. zeylanicum* (Duke, 1985; Kirtikar and Basu, 1993).

There are several *Cinnamomum* spp. that are of important values in medicinal field and other application. *Cinnamomum zeylanicum* which is also known as cinnamon is a native to India, Sri Lanka, and Peninsular Malaysia (Wee and Hsuan, 1990), but it is now widely cultivated in Sri Lanka, the Seychelles and Madagascar (Jayatilaka *et al.*, 1994). *C. zeylanicum* is a moderate-sized evergreen tree and grow well in tropical regions (Jayatilaka *et al.*, 1994). *C. zeylanicum* tree can reach up to 20 m high. The bark of *C. zeylanicum* is aromatic and stimulant. The fragrant cordial of *C. zeylanicum* is used especially to treat digestive problems, nausea and vomiting (Kirtikar and Basu, 1993). The cinnamon oil which is distilled from the dried green leaves is used externally in Chinese medicine, as an astringent, carminative and antiseptic (Wee and Hsuan, 1990). The oil derived from the bark of *C. zeylanicum* is used to stimulate digestion, respiration and blood circulation (Wee and Hsuan, 1990), and to treat diarrhea (Kirtikar and Basu, 1993). Beside that, the essential oil from the bark and pure cinnamic aldehyde are quite infective as anthelmintic (Kirtikar and Basu, 1993). In pharmaceutical, the essential oil is used for dental preparation and as oral hygiene products (IMR, 2000). The essential oil of *C. zeylanicum* acts as a rubefacient to treat acute and chronic rheumatism. It is also used to treat stomach cramps, toothache, cancer, tuberculosis, leucorrhoea, hypertension, arthritis and as
external remedy for skin disorders and ulcer (IMR, 2000). *C. zeylanicum* leaf oil is also a powerful germicide and used in perfumes, spices and in the synthesis of vanillin. The essential oils are antiseptic (Duke, 1985).

*Cinnamomum camphora* is an evergreen tree which is native to China, Taiwan and Japan. This species is known as camphor tree. The camphor tree grows in localities with temperatures above 13°C and altitudes below 800 m (Senanayake and Wijesekera, 1989). Its leaves are alternate, chartaceous or coriaceous and long (Kirtikar and Basu, 1993). The leaves, twigs and wood are distilled to produce camphor. Each part of the *C. camphora* tree is credited with sedative, anodyne, antispasmodic, diaphoretic and anthelmintic properties (Kirtikar and Basu, 1993). Camphor may also be obtained from wounds made on the tree (Wee and Hsuan, 1990). Camphor and camphorated oil are used externally to relieve pain, muscle aches and pains and chest congestion, resulting from colds and bronchitis (Wee and Hsuan, 1990). Beside that, the oil that contains camphor and safrole is used in the preparation of expensive perfumes (Duke, 1985).

Camphor is used extensively as a plasticizer to make celluloid and in the preparation of explosives, disinfectants and other chemicals (Duke, 1985). Camphor is also used in external substances as a counter-irritant in the treatment of muscular strains, gout, rheumatic conditions and inflammations, in relieving itching skin and as a weak antiseptic. Beside that, it is employed to treat asthma, bronchitis, emphysema, lung congestion and rhinitis. It is used as an analeptic in cardiac depressions, myocarditis and paralysis, has a calmative influence in cholera, convulsions, epilepsy, hysteria and nervousness, and it is also used in the treatment of serious diarrhea, farus
and toothache. Safrole which is produced from the residual oil after camphor extraction is used to manufacture soap (Duke, 1985).

*Cinnamomum cassia* which is also known as Chinese cinnamon or cassia is native to Myanmar, but now it is cultivated in southern China and Indonesia, and also in Laos and Vietnam. *C. cassia* is an evergreen tree, up to 10 m high. The odour is delicate, fragrant and aromatic, and the taste is warm, sweet and pungent (Wee and Hsuan, 1990; Kirtikar and Basu, 1993). The bark was known in Europe in the 14th century as cinnamon and is used to produce incense, drugs and oils, as well as to mull wine, but it is not used as a table spice. Cassia oil which is obtained from the distillation process of the unripe fruits is used in chocolate and allied industries. The dried unripe fruits which are commonly known as cassia buds are used as a spice. The barks and twigs of this species are used to increase the flow of urine, induce lumbago in old people, stomachache, indigestion, common cold, to treat fever and headache, promote blood circulation and treat pain in joints and abdomen (Wee and Hsuan, 1990). The bark is tonic, stomachic and carminative (Kirtikar and Basu, 1993). Beside that, *C. cassia* is used to treat clammy extremities, cough and wheezing, pains in the lower part of the body and knees, dysmenorrhoea, amenorrhoea, low blood pressure and frost-bite (Wee and Hsuan, 1990; Kirtikar and Basu, 1993). *C. cassia* has been used traditionally for treating dyspepsia, gastritis, blood circulation disturbances and inflammatory disease in both Eastern and Western countries (Ahn, 1998). The leaves and bark are used as spices and condiments (Joy et al., 1998).
*Cinnamomum tamala* which is native to Philippines is a small evergreen tree up to 1.4 m girth and 7.5 m high. The leaf of *C. tamala* is bitter in taste and is used in folk medicine to treat heart problems, ozoena, diuretic, inflammation, sore eyes and good for liver and spleen. In Punjab, the leaves are used to treat rheumatism, colic and diarrhea and being considered as stimulant. The bark of *C. tamala* is used to treat gonorrhoea (Kirtikar and Basu, 1993).

*Cinnamomum iners* is a 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).

### 2.2 Extraction and Isolation of Essential Oils

Essential oil is a naturally occurring substance in a form of concentrated liquid. It contains the true essence of the plant it was derived from. Essential oils 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). It can be produced by distillation, expression, or solvent extraction from the leaves, stems, flowers, bark, roots, or
other elements of a plant. The type of extraction methods is one of the factors that affect the yield composition and chemical constituents of the essential oils.

The distillation methods are the most common method to extract and isolate the essential oils in laboratory. Some of the distillation methods that are widely used are hydrodistillation, steam distillation, simultaneous micro steam distillation/solvent extraction (SD/SE), hydrodiffusion, turbo distillation, rectification and fractional distillation (Jayatilaka et al., 1994; Sritharan et al., 1994; Nakatsu et al., 2000; Wang et al., 2004; Cheng et al., 2005). The hydrodistillation method is featured by the fact that the plant material is heated with water and the steam is generated within the still (Boelens et al., 1989). The hydrodistillation of essential oils from plant material is a complicated method because the oil has to diffuse from the inside of the material to the surface, and due to the composition of the plant material which also contains non-volatiles compound (e.g. fatty oils) that will retain the volatiles compound (Boelens et al., 1989). Moreover, during the isolation process of the volatiles with steam from plant material, there are several considerations need to be taken with the following physical parameters: partition coefficients, diffusion rates, water solubility, partial vapour pressures, times and velocities of heat transfer (Boelens et al., 1989). For hydrodistillation method, the order of the transfer of the volatile chemical constituents is determined more by the isolation sequence of their water solubility (Koedam et al., 1979, 1981). Steam distillation is a common macroscale sample preparation method but is less commonly employed for routine analysis where a microscale approach is sufficient to yield enough sample for analysis. Steam distillation is performed with indirect (dry) steam (Boelens et al., 1989). One primary problem in miniaturizing steam distillation to achieve
microscale sample processing conditions is the poor efficiency of condensation as a sample collection procedure (Jayatilaka et al., 1994). Therefore, to overcome this problem, the steam distillation method is combined with the solvent extraction method. This method is called the simultaneous micro steam distillation with solvent extraction (SD/SE) which provides an effective method of isolating semivolatile compounds (Jayatilaka et al., 1994).

Hydrodiffusion method is primarily used for extracting essential oils from seeds. In hydrodiffusion, the plant material is directly extracted with steam that enter from the top of the vessel containing the plant material and being condensed below the vessel (Näf, 1989) instead of from the bottom as in normal steam distillation. For turbo distillation extraction, it is a method used for hard to extract plants, like bark or roots or sometimes seeds. The plant materials are soaked in water, and steam is run through the materials. The same water and steam is used to extract the oils, gives a much quicker way to extract the oils from hard substances (Robutz, 2005). Fractional distillation method is a normal distillation process, but the difference is the essential oil yield is collected in batches (fractions) instead of being collected continuously. The plant material that commonly used this extraction method is Ylang-Ylang and other flowers (Extraction, 2006). Some of the essential oils contain impurities. Therefore, to overcome this problem the essential oil can be purified by re-distillation, either in steam or vacuum. This purification by re-distillation is called rectification. Example of the essential oil produce by this method is eucalyptus oil (Extraction, 2006).