Faculty of Resource Science and Technology

PHYTOCHEMICAL STUDIES AND BIOLOGICAL ACTIVITY OF *CINNAMOMUM MICROPHYLLUM*

Nur Syaida binti Yusof (21962)

Bachelor of Science with Honours
(Resource Chemistry)
2012

PHYTOCHEMICALSTUDIES AND BIOLOGICAL ACTIVITY OF *CINNAMOMUM MICROPHYLLUM*

NUR SYAIDA BINTI YUSOF

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

Bachelor of Science with Honours (Resource Chemistry)

Faculty of Resource Science and Technology

UNIVERSITI MALAYSIA SARAWAK

2012

DECLARATION

This thesis entitled "Phytochemical and Biological Studies of *Cinnamomum microphyllum*" is a presentation of my own research work and has not been submitted to any other University for any degree. Wherever contribution of others are involved, every effort is made to indicate this clearly with due reference to the literature.

_____

Nur Syaida Binti Yusof

Resource Chemisstry

Faculty of Resource Science and Technology

Universiti Malaysia Sarawak

ACKNOWLEDGEMENT

Alhamdulillah, all praises to Allah for the strength and His blessing in completing this thesis. I would extremely indebted to my supervisor Prof Fasihuddin Badruddin Ahmad for his supervision and constant support. During my thesis-writing period, he provided encouragement, sound advice, good teaching, and lots of good ideas. His invaluable help of constructive comment and suggestion throughout experimental and thesis work has contributed to the success of this research.

Special appreciation goes to Madam Wong, Miss Syazaliza, and Miss Nabihah for their immense support, knowledge, and encouragement. I successfully overcame many difficulties and learned a lot from them. Not forgotten my appreciation to Encik Benedict, Puan Norhayati, and Encik Wahab for their guiding and helping me to use some kind of instruments in order to complete my project.

Sincerely thanks to all my friends especially my lab-mates: Farahen, Aidelia, Alin, Cecilia, Syafiqah, Kamilah, and Atul for their kindness and moral support during completing this project. Thanks for the friendship and memories.

Last but not least, my deepest gratitude goes to my family members for their love, prayers and encouragement throughout my studies in UNIMAS. The incessant support from them influenced me to complete this thesis with strong effort and enthusiasm.

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

Potassium Bromide	KBr
Thin Layer Chromatography	TLC
Gas Chromatography-Flame Ionization Detector	GC-FID
Column Chromatography	CC
Preparative Thin Layer Chromatography	PTLC
Dichloromethane	DCM
Methanol	MeOH
Ethyl Acetate	EtOAc
Sulfuric Acid	H_2SO_4
Retention Factor	R_f
Fourier Transform Infra Red spectrometer	FTIR
Gas Chromatograph-Mass Spectroscopy	GC-MS
Ultra Violet light	UV
Nuclear Magnetic Resonance	NMR
Carbon NMR	13C'NMR
Proton NMR	1H'NMR
Helium gas	He
Micro Litre	µL
Mili Gram	mg
Mili Litre	mL

Celcius	ºC
Lethal Concentration 50	LC_{50}
Part per million	ppm

LIST OF TABLES

LIST OF FIGURE

Phytochemical Studies and Biological Activity of *Cinnamomum microphyllum*

Nur Syaida binti Yusof

Resource Chemistry

Faculty of Resource Science and Technology

Universiti Malaysia Sarawak

ABSTRACT

The stem bark, root, and bark, and leaves of *Cinnamomum microphyllum* was extracted using MeOH. Their filtrates were evaporated to dryness to gave 7.20 %, 12.86 %, 6.21%, and 10.22% of crude extracts respectively. Stem bark and root were chosen to perform solvent partition using solvent with increasing polarities and resulted in four partitions for each part where the stem bark yield 0.35% of hexane partition, 0.28% dichloromethane, 0.68% ethyl acetate, and 0.48% of methanol partition. The root part gave in 0.68% of hexane partition, 0.47% dichloromethane partition, 1.14% ethyl acetate, and 2.23% methanol partition. Separation of dichloromethane crude partition of stem bark by column chromatography resulted in the isolation of one pure compound which identified and characterized as methyl cinnamate. The pure compound was isolated as white crystal needle in hexane- dichloromethane (1:1) fraction with R_f value of 0.54 and gave a molecular mass of 162 g/mol corresponded to molecular formula of $C_{10}H_{10}O_2$. Various spectroscopic data especially mass spectrum, infrared, nuclear magnetic resonance, and comparison with the published data were established in order to determine the structure of the pure compound. The semi-pure compound was found in one combined fractions from the root sample which was expected to be methyl eugenol with its molecular mass of 178 g/mol. None of crude partitions of *Cinnamomum microphyllum* showed significant toxicity against *Artemia salina*.

Keywords: *Cinnamomum microphyllum*, methyl cinnamate, methyl eugenol, toxicity

ABSTRAK

Bahagian kulit batang, akar, kulit, dan daun Cinnamomum microphyllum telah diekstrak dengan menggunakan pelarut MeOH. Eskstrak mentah telah dikeringkan bagi memberikan peratus hasil masing-masingnya 7.20 %, 12.86 %, 6.21%, dan 10.22%. Kulit batang dan akar dipilih untuk menjalani pempartisian pelarut dengan menggunakan pelarut dengan dalam urutan pertambahan kepolaran heksana, diklorometana, etil asetat, dan methanol. Hasil partisi yang diperolehi heksana sebanyak 0.35%, diklorometana 0.28%, etil asetat 0.68%, dan metanol 0.48%. Bahagian akar pula memberikan hasil partisi heksana sebanyak 68%, diklorometana 0.47%, etil asetat1.14%, dan metanol 2.23%. Pemisahan telah dilakukan ke atas partisi diklorometana dari bahagian kulit batang dengan menggunakan teknik kromatografi dan satu sebatian tulen telah dipencilkan dan dikenalpasti sebagai metal sinamat. Sebatian tulen ini dipisahkan di dalam sistem pelarut heksana-diklorometana (1:1) dan dalam bentuk kristal putih yang tajam dengan nilai R_f 0.54. bBerat molekul bagi sebatian ini adalah 162 g/mol dan berpadanan dengan jisim formula $C_{10}H_{10}O_2$. Teknik spektroskopi jisim, inframerah, resonansi magnetik nuklear, dan perbandingan dengan data yang telah diterbitkan bagi menentukanstruktur sebatian tulen yang telah dipisahkan. Sebatian separa tulen telah dipencilkan dalam gabungan fraksi dari bahagian akar adalah sebatian metil eugenol dengan jism molekul 178 g/mol. Ujian ketoksidan menunjukkan tiada kesan toksik terhadap Artemia salina oleh semua partisi kasar dari Cinnamomum microphyllum.

***Katakunci**: Cinnamomum microphyllum, metil sinamat, metil eugenol, ketoksidan*

CHAPTER 1.0

INTRODUCTION

1.1 Background of Studies

Lauraceae family is a green medium size tree found in the tropics especially in India, China, East Africa, South Asian Countries such Malaysia, Indonesia, Philippines and Australia. This family consists of 50 genus and 2000 species of trees and shrubs. The Lauraceae family is recognized in the field by their aroma, the smooth and thick bark, the bay-like leaves, and their fruits which are glossy and ovoid seated on a copular vestigial perianth (Wiart, 2006). Some of the genus in Lauraceae family includes *Cinnamomum, Litsea, Animba, Lindera, Cryptocarya* and *Nathapoeba* (Burkill, 1966). This family is very important economically and used widely in traditional medicine in the treatment of various ailments (Mat Salleh & Latiff, 2002; Burkill, 1966). They are important sources of medicine, timber, nutritious fruits such *Persia Americana,* spices like *Cinnamomum cassia and Cinnamomum subavenium,* and perfumes. Besides that, the fruits of *Actinodaphne, Cinnamomum, Cryptocarya, Lindera, Litsea* and *Syndiclis* contain abundant oil and fat which are used for making perfumes and medicines. For instance, *Cinnamomum camphora* is the common species which is widely used in making perfumes and medicinal properties due to presence of camphor (Mat Salleh & Latiff, 2002). This family is quite interesting due to numerous biologically active compounds isolated including alkaloids, terpenes, flavanoids, polyphenol, and others. These secondary metabolites show many biological activities such as antidiabetic, antiinflammatory, antitumor, antivirus, antifungal, antibacterial, antihelmentic and other biological activities. (Lee *et al*., 2005; Wang *et al*., 2005; Simic *et al*., 2004).

From the reachable information, there are many advantages of *Cinnamomum* plant species which mostly involve in medicinal uses. However, there are no detailed studies on chemical and biological activities of *Cinnamomum* spp. Even they are quite many species exist in Malaysia but some of them are very difficult to find. Many researchers perform their studies on common *Cinnamomum* spp. such as *Cinnamomum zeylanicum, Cinnamomum verum, Cinnamomum iners* and *Cinnamomum cassia*. Based on the previous studies, *Cinnamomum* spp are widely used to treat various diseases due to the secondary metabolites compound derived from them and shown various biological activities. Thus, it is very important to perform scientific study on various species belong to *Cinnamomum* especially in Sarawak, Malaysia.

1.2 Objectives

The key objectives of this study are to purify and characterize the biologically active compound from *Cinnamomum microphyllum*. The specific objectives are:

a. to extract the dried stem bark, root, and bark of *Cinnamomum microphyllum*.
b. to isolate and purify the secondary metabolite of *Cinnamomum microphyllum* using chromatography methods.
c. to elucidate the chemical structure of the pure compound isolated based on various spectroscopic techniques
d. to perform toxicity test against *Artemia salina* larvae of the crude extracts.

CHAPTER 2.0

LITERATURE REVIEW

Phytochemical study is generally referred to the study of chemical compounds that are produced by plants. There is some evidence that certain phytochemicals may help prevent formation of potential carcinogens, block the action of carcinogens on their target organs or tissue, or act on cells to suppress cancer development. From this, many experts suggest that people may diminish their risk of cancer significantly by eating more vegetables, fruit or herbs that contain phytochemicals. Based on previous research, there are many phytochemicals reported in *Cinnamomum* spp.

2.1 *Cinnamomum* spp.

Cinnamomum spp. is one of the genus in Lauraceae family that is most well-known and widely used as medicinal plant (Wiart, 2006). This genus is recognized as small or medium size trees, smooth and thick barks, waxy-like leaves and have a nice aroma. This genus contains over 250 species and distributed in tropical and subtropical region of China, India, East Africa and South Asia Countries (Ibrahim *et al*., 1995; Kochummen, 1989). Mawardi *et al. (*2000) and Burkill (1966) reported that about 30 species of *Cinnnamomum* can be found in Malaysia such as *Cinnamomum iners, Cinnamomum mollissimim, Cinnamomum sintok, Cinnamomum microphyllum, Cinnamomum zeylanicum Cinnamomum verum Cinnamomum camphora, Cinnamomum cassia, Cinnamomum burmannii, Cinnamomum mercadoi, Cinnamomum porrectum, Cinnamomum subavenium, Cinnamomum javanicum,* and *Cinnamomum loureirii.*

In Malaysia, *Cinnamomum* spp. is well known as “Kayu Manis” and “Tejur”. Many of this genus have been extensively used in various medicinal aspect. Generally, most people in Malaysia believed that *Cinnamomum* spp. can be used to treat blood clotting,

cough, fever, constipation, skin and scar treatment and control the blood sugar (Wiart, 2002). The bark of the *Cinnamomum mercadoi* is used to treat headache and intestinal problem like stomachache and diarrhea. Besides that, the roots of the *Cinnamomum iners* can be used to treat cough, asthma, fever and also act as a tonic for women after giving birth. This type of cinnamon is used by almost Bidayuh community. They also take the leaves, bark and strip to reduce toxin and painful in their body (Mat Salleh & Latiff, 2002; Wiart, 2002). Another previous study is *Cinnamomum zeylanicum* and *Cinnamomum verum* are used as a tonic, anti-toxin, reduce fungal infection, treat the scar on skin, and menstrual pain (Mat Salleh & Latiff, 2002; Burkill, 1966). *Cinnamomum porrectum* is another species where particularly the bark and seed being used to treat rheumatism, tonic for teenager's menstrual pain and also good for women after giving birth (Mat Salleh & Latiff, 2002; Burkill, 1966). Table 1.1 gives information on some of the *Cinnamomum* species in Malaysia along with their medicinal purposes.

Apart from medicinal uses, *Cinnamomum* spp. is also one of the famous medicinal plants which have always been used as a spice in cooking since ancient times. The main part that used as a spice is their bark. The barks of *C. zeylanicum*, *C. burmanni*, *C. loureirii*, and *C. cassia* are the most common species entering the trade market as cinnamon. Cinnamon has been known in Southern Europe for over a thousand years, but become very widely known in other parts of Europe during the Middle Ages when the Islamic World extended its influence to the Orient and East to the Siberia starting in the eighth century (Smith *et al*., 1992). In ancient Egypt, cinnamon was used as a flavoring agent for beverages and embalming where the body cavities will be filled with spiced preservatives.

Table 1.1: Uses of some common *Cinnamomum* sp. in Malaysia

Species	Medicinal purposes
C. camphora	Antiinflammatory and antioxidant
C. iners	Treat fever, cough, asthma, tonic for women after giving birth
C. javanicum	Reduce the spleen problem
C. mercadoi	Treat headache, diarrhea, stomachache
C. mollisimum	Treat fever, tonic (eaten with *Piper betle* and tobacco)
C. porrectum	Tonic for women pain, treat rheumatism,
C. sintoc	Tonic, cure stomachache
C.verum	Reduce the intestinal problem and constipation, antiseptic
C. zeylanicum	Tonic, anti-toxin, skin and scar treatment, constipation, reduce intestinal problem

Adapted from Mat Salleh & Latiff, 2002; Burkill, 1966

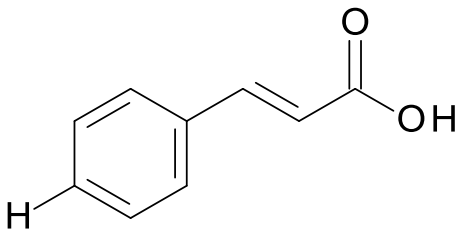
2.2 Secondary Metabolites of *Cinnamomum* species

Basically, plant are capable of synthesizing an overwhelming variety of small organic molecules called secondary metabolites. They are normally consists of very complex and unique skeleton structures (Sarker *et al*., 2005). Furthermore, they also have often attracted interest because of their biological activity. On the other hand, the primary metabolites exert their biological effect within the cell or organism that is responsible for their production (Hanson, 2003). The secondary metabolites are not important for the growth and plant development but they are required for the interaction of plants with the environment (Kuchan & Dixon, 2005). Most of secondary metabolites have a signaling function that may influence the activities of other cell, control their metabolic activity and coordinate the development of the whole plant. In general, there are few classes of the secondary metabolites namely polyketides and fatty acids, terpenoids and steroids, phenylpropanoids, alkaloids, specialized amino acid and peptides, and specialized carbohydrates.

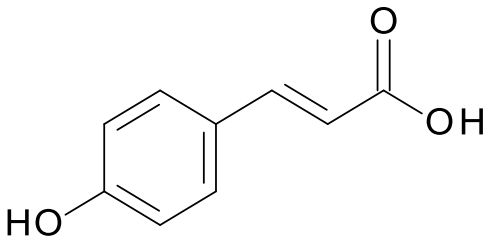
In the genus of *Cinnamomum*, there are various types of secondary metabolites produced by them. Based on previous studies, the most common secondary metabolites compound found in the *Cinnamomum* species are cinnamaldehydes, alkaloids, eugenol, proantocyanidin, flavonoid, and terpenoid (Wiart, 2002).

2.2.1 Phenylpropanoids

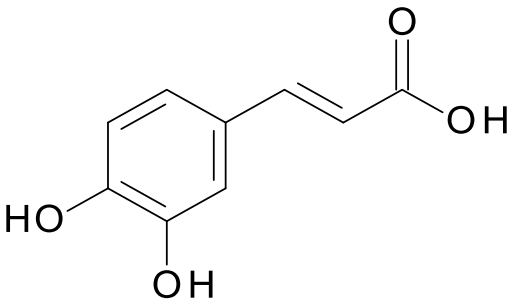
Phenylpropanoids are basically six membered aromatic compounds which possessed a three carbon chain attached to an aromatic ring. They are produced by biosynthetic pathway called shikimate pathway. In the late 19th century, the structures of a number of simple propanoids were established and widespread in plant product especially *Cinnamomum* sp. such as cinnamic acid (**1**), 4-hydroxycinnamic acid (coumaric acid, **2**), and 3, 4-dihydrocinnamic acid (caffeic acid, **3**) (Hanson, 2003).



1

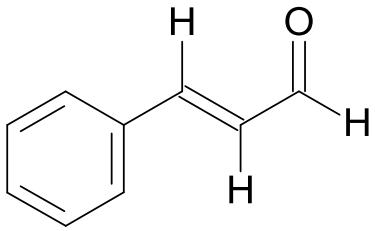


2



3

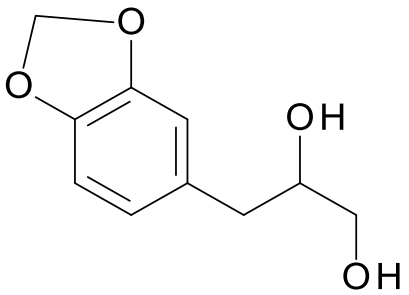
Cinnamaldehyde (**4**) is one type of phenylpropanoid compound most widely reported in *Cinnamomum* sp. and this compound usually present in the stem bark oil and root bark oil. For instance, *Cinnamomum zeylanicum* possess a very delicate aroma with a sweet and pungent taste due to the presence of 75% cinnamaldehyde (**4**) (Fazilah *et al*., 2006).



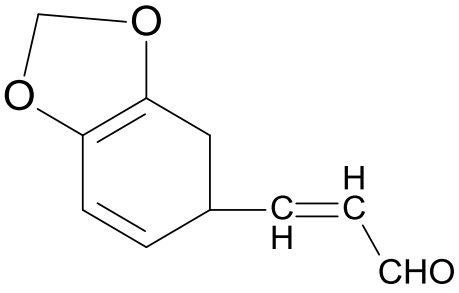
4

Cinnamaldehyde is an oily yellow liquid at room temperature with a boiling point of 246 °C. It is mainly used as a flavoring agent or as a scent of candles. It is also non toxic but may irritate the skin if in contact for too long. Moreover, there are various biological activities shown by cinnamaldehyde such as antioxidant, antiviral, antifungal and antibacterial. Cinnamaldehyde is a natural antioxidant and the animal studies suggest that the extracted cinnamon bark may help to prevent stomach ulcer (Joshi *et al*., 2009). In addition, it can completely inhibit both sensitive and resistant stain of *Helicobacter* (Joshi *et al*., 2009).

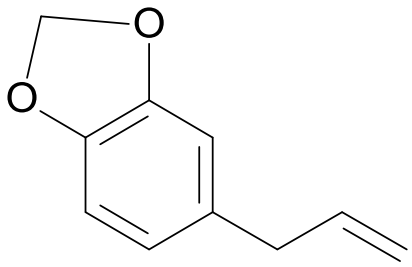
Thitima *et al* (2000) has been reported that the wood of *Cinnamomum parthenoxylon* contain phenylpropanoids compound such as (+)-3-(3, 4-methylenedioxyphenyl)-1, 2-propane-diol (**5**), 3, 4-methylenedioxycinnamaldehyde (**6**), safrole (**7**), and a mixture of six alkyl *trans*-ferulates bearing alkyl group of C22 and CN to C28 (**8**).



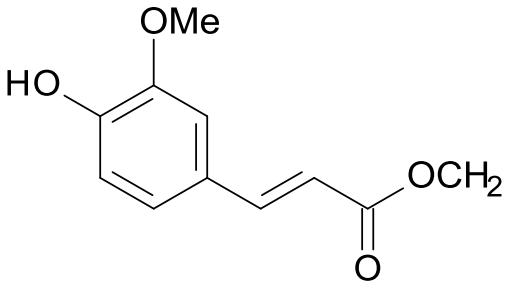
5



6



7



8