PHYTOCHEMICALS AND BIOACTIVITIES OF *Cinnamomum porrectum* (ROXB.) KOSTERM AND *Cinnamomum mollissimum* HOOK F.

FATIN FASIHAH BINTI MASNON

UNIVERSITI TEKNOLOGI MALAYSIA

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FATIN FASIHAH BINTI MASNON

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Dedicated to:

My father, Masnon bin Ab Rahim

My mother, Masitah binti Md Tab

My brothers and my sister

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PREFACE

This thesis is the result of my work carried out in the Department of Chemistry, Universiti Teknologi Malaysia between September 2011 and September 2013 under the supervision of Assoc Prof. Dr. Farediah Ahmad. Parts of my works described in this thesis have been reported in the following publications:

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2. Fatin Fasihah Masnon and Farediah Ahmad. (2012). Phytochemicals and Antimicrobial Activity of *Cinnamomum porrectum* (Roxb.) Kosterm. Fasihuddin Ahmad, Zaini Kassim, Laily Din and Ibrahim Jantan (Eds.), *Integrated Research in Natural Products Chemistry* (pp. 185-195). Faculty of Resource Science & Technology, Universiti Malaysia Sarawak.

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ABSTRACT

Cinnamomum porrectum (Roxb.) Kosterm and *Cinnamomum mollissimum* Hook f. which belong to the Lauraceae family are widely distributed in Peninsular Malaysia. They are locally known as "medang kemangi" and "medang lawang", respectively. The leaves and barks of *C. porrectum* and the leaves of *C. mollissimum* were extracted by cold extraction using methanol and the extracts were then partitioned using different solvents with increasing polarity to yield the petroleum ether, chloroform and ethyl acetate extracts. Acidification, basification and extraction of the methanol extract from the barks of *C. mollissimum* with chloroform produced the neutral and alkaloid crude extracts. The isolation and purification on the crude extracts were achieved using chromatographic techniques and have resulted in the isolation of prenylpropanoid, triterpenes, ester, carboxylic acid and aporphine alkaloids. Structure of the isolated compounds were elucidated using spectroscopic techniques including infrared, ultraviolet-visible, nuclear magnetic resonance spectroscopies, mass spectrometry and also by comparison of the spectral data with those previously reported in the literatures. Purification process of the leaves extracts of *C. porrectum* have yielded three compounds identified as methyl eugenol, β-sitosterol and stigmast-4-en-3-one. Benzyl benzoate and benzoic acid have been isolated from the leaves of *C. mollissimum*. Purification of the alkaloid extract from the barks of *C. mollissimum* produced five aporphines, namely isocorydine, *N*-methylhernagine, *N*-methylhernovine, hernagine and hernovine. Several bioactivities such as antibacterial, antioxidant and antityrosinase have been investigated for the crude extracts and selected compounds. The antibacterial assays were performed using disc diffusion method, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). The results showed that the alkaloid extract, methyl eugenol and benzyl benzoate exhibited strong antibacterial activity towards selective bacterial strains with the concentration ranged less than 500 μg/mL. The antioxidant activity by DPPH showed significance results on the alkaloid extract and hernovine with SC_{50} 50.1 μg/mL and 50 μg/mL, respectively. The crude extracts which were screened for antityrosinase activity using mushroom tyrosinase were found to be inactive with $IC_{50} > 1000$ μg/mL. As a conclusion, the alkaloid extract showed good activity towards all the tested bioassays except for the tyrosinase inhibition assay. The activity portrayed was due to the synergistic effect between the compounds presence in the extract.

ABSTRAK

Spesies *Cinnamomum porrectum* (Roxb.) Kosterm dan *Cinnamomum mollissimum* Hook f. tergolong dalam keluarga Lauraceae ditemui dengan meluas di Semenanjung Malaysia. Nama tempatan untuk masing-masing spesies ini adalah medang kemangi dan medang lawang. Daun dan batang kering *C. porrectum* dan *C. mollissimum* telah diekstrak menggunakan teknik rendaman dengan pelarut metanol dan kemudiannya ekstrak tersebut diperingkatkan menggunakan pelarut yang berbeza kekutuban untuk menghasilkan ekstrak petroleum eter, kloroform dan etil asetat. Pengasidan, pembesan dan pengekstrakkan ekstrak metanol daripada batang *C. mollissimum* dengan kloroform telah menghasilkan ekstrak neutral dan ekstrak alkaloid. Pengasingan dan penulenan setiap ekstrak mentah dijalankan dengan menggunakan teknik kromatografi dan telah berjaya menghasilkan sebatian fenilpropanoid, triterpena, ester, asid karboksilik dan alkaloid. Pengenalpastian struktur kimia sebatian tulen dilakukan dengan menggunakan kaedah spektroskopi inframerah, ultralembayung-nampak, resonans magnet nukleus, spektrometri jisim, dan juga perbandingan data spektrum dengan data yang telah diterbitkan dalam literatur. Proses penulenan terhadap ekstrak mentah daripada daun *C. porrectum* telah menghasilkan sebatian kimia yang dikenalpasti sebagai metil eugenol, β-sitosterol dan stigmast-4-en-3-on. Benzil benzoat dan asid benzoik telah berjaya dipisahkan daripada ekstrak daun *C. mollissimum*. Penulenan terhadap ekstrak alkaloid daripada batang *C. mollissimum* telah menghasilkan lima alkaloid dinamakan sebagai isokoridin, *N*-metilhernagin, *N*-metilhernovin, hernagin dan hernovin. Beberapa bioaktiviti seperti antibakteria, antioksidan dan antitirosinase telah dikaji ke atas setiap ekstrak mentah dan sebatian terpilih. Saringan antibakteria yang telah telah dilakukan menggunakan kaedah pembauran cakera, penentuan nilai kepekatan rencatan minimum (MIC) dan kepekatan bakterisida minimum (MBC). Keputusan telah menunjukkan bahawa ekstrak alkaloid, metil eugenol dan benzil benzoat mempunyai aktiviti antibakteria yang kuat terhadap beberapa jenis bakteria terpilih dalam julat kepekatan kurang daripada 500 μg/mL. Aktiviti antioksidan dengan menggunakan DPPH menunjukkan keputusan yang signifikan ke atas ekstrak alkaloid dan hernovin masing-masing dengan SC_{50} 50.1 μg/mL dan 50 μg/mL. Semua ekstrak mentah yang disaring untuk aktiviti tirosinase menggunakan tirosinase cendawan didapati tidak aktif dengan IC_{50} > 1000 μg/mL. Kesimpulannya, ekstrak alkaloid mempunyai aktiviti yang baik terhadap kesemua ujian bioaktiviti kecuali saringan perencatan tirosinase. Aktiviti yang dipamerkan disebabkan oleh kesan sinergi yang berlaku antara sebatian yang terdapat di dalam ekstrak tersebut.

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LIST OF ABBREVIATIONS

%	Percentage
δ	Chemical shift
α	Alpha
β	Beta
λ	Lambda
$BaCl_2$	Barium chloride
BHT	Butylated hydroxyl toluene
^{13}C	Carbon-13
cm	Centimeter
cm^{-1}	Per centimeter
°C	Degree Celsius
$CDCl_3$	Deuterated chloroform
$CHCl_3$	Chloroform
CC	Column Chromatography
COSY	Correlation Spectroscopy
d	Doublet
dd	Doublet of doublets
DEPT	Distortionless Enhancement of Polarization Transfer
DMSO	Dimethylsulfoxide
DPPH	2,2-diphenyl-1-picrylhydrazyl
EC_{50}	Effective concentration at 50%
EIMS	Electron Impact Mass Spectrometry
Et_2O	Diethyl ether
EtOAc	Ethyl acetate
EtOH	Ethanol
GC	Gas Chromatography
GC-MS	Gas Chromatography-Mass spectrometry

g	Gram
^{1}H	Proton
H_2SO_4	Sulfuric acid
HMBC	Heteronuclear multiple bond correlation
HMQC	Heteronuclear multiple quantum coherence
Hz	Hertz
IC_{50}	Inhibition concentration at 50%
IR	Infrared Spectroscopy
J	Coupling constant
lit.	Literature
mg	Milligram
mm	Millimeter
MBC	Minimum bactericidal concentration
MIC	Minimum inhibition concentration
MeOH	Methanol
MHz	Megahertz
m.p.	Melting point
m/z	Mass per Charge
mL	Milliliter
$MgSO_4$	Magnesium sulfate
M^+	Molecular ion
MS	Mass spectrum
m	Multiplet
Na_2HPO_4	Sodium hydrogen phosphate
NaH_2PO_4	Sodium dihydrogen phosphate
NA	Nutrient agar
NB	Nutrient broth
NMR	Nuclear Magnetic Resonance
NaCl	Sodium chloride
nm	nanometer
PE	Petroleum ether
ppm	parts per million
R_f	Retention factor
SD	Standard deviation

SC_{50}	Scavenging concentration at 50%
s	Singlet
SiO_2	Silicon dioxide
t	Triplet
TLC	Thin Layer Chromatography
UV	Ultraviolet
µg/mL	Microgram per milliliter
µL	Microliter
µM	Micromolar
VLC	Vacuum Liquid Chromatography

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CHAPTER 1

INTRODUCTION

1.1 General Introduction

Natural product chemistry becomes mankind's interest about color, odour, taste and treatment for animal, human and plant diseases. Natural product is related to materials originated from plants, microorganisms, invertebrates and vertebrates. All of them are involved in biochemical factories for the biosynthesis of both primary and secondary metabolites. The secondary metabolites play ecologically significant roles in how the organisms deal with their surroundings which are important for their survival. Natural products include alkaloids, flavonoids, terpenoids, steroids, amino acids, proteins, carbohydrates and others [1].

Natural product research continues to be one of the major studies of discovering biologically active compounds. The discovery of bioactive metabolites is the beginning step in the search for potentially useful compounds. The evidences for the existence of bioactive compounds can turn up from different sources. From the evolution of time and experiences, conventional medicine has assembled certain group of plants that have shown to be useful to human beings. Further evidences come from the inspections by the researchers who are skilled and expert in the interaction among organisms. The other sources come from the chances for discovery of new metabolites exhibiting pharmacological effects and from testing in a huge quantity of organisms for a specific effect [2]. On the other hand, Malaysia is also known for the research on natural products as Malaysia has plenty of natural resources. There are many types of plants in Malaysia which are not only useful in our daily life but also beneficial as medicines. This includes plants from Lauraceae

family such as *Cinnamomum zeylanicum* (kayu manis) and *Cinnamomum sintok* (kayu sintok).

1.2 Lauraceae Family

Family of Lauraceae is distributed in tropical and subtropical regions but mostly in tropical South East Asia and tropical America. They consist about 45 genera such as *Cinnamomum*, *Actinodaphne*, *Persea*, Dodecadenia, *Litsea* and *Lindera*.

The Lauraceae are much known for their economically benefits and uses. They are important as sources of medicine, nutritious fruits (e.g., *Persea americana*), perfumes and spices (e.g., *Cinnamomum cassia*, *Cinnamomum subavenium*). *Cinnamomum* trees such as the barks of *Cinnamomum sintok* are internally used for the treatment of diarrhea and externally used for wounds and numbness of the skin [3].

Actinodaphne lancifolia is an evergreen tree also belongs to Lauraceae family and very useful for treating arthritis, edema, overexertion and stomachache [4]. *Litsea tsinlingensis* is mostly cultivated in Sri Lanka also has its own uses. The oil extracted from the seeds is used to cure the rheumatism and the leaves and fruits are used for relieving soreness of bruises and sprains. Meanwhile, the bark is used as a mild astringent for diarrhea and food poisoning [5]. *Machilus thunbergii* is mostly distributed in the southern part of Korea and have been used medicinally as a folk medicine. The bark is used to treat leg edema, abdominal distension and pain [6].

Persea bombycina which is formerly known as *Machilus bombycina* is also belongs to this family. The local name of the tree is “Som” and the distributions are mostly in India. “Som” supplies the primary food for “Muga” silk worm which produces the golden silk called “Muga” silk and it is a unique silk and can be found nowhere except at the northeastern states of India [7].

1.3 Genus *Cinnamomum*

The genus *Cinnamomum* consists of 250 aromatic shrubs and evergreen trees and widely distributed in Australia and Asia. The common name of the genus is called cinnamon or cassia [8]. There are twenty one *Cinnamomum* species from Peninsular Malaysia such as *C. pubescens, C. javanicum, C. iners, C. impressicostatum, C. mollissimum, C. porrectum, C. camphora* and others [9].

1.4 Statement of Problems

The existence of several chemotypes within the species make the chemistry of the genus *Cinnamomum* become more interesting to be studied thoroughly [10]. Previous studies of the genus *Cinnamomum* and related species especially *Cinnamomum mollissimum* and *Cinnamomum porrectum* were only focusing on the volatile oils and their compositions. Therefore, it is essential to carry out the phytochemicals study of the dried parts of these species. It is also vital to study the bioactivities of the crude extracts and phytochemicals to determine the pharmaceutical and medicinal value of the plants.

1.5 Objectives of Study

The objectives of this study are as follows:

1. To isolate the phytochemicals from the leaves and barks of both *Cinnamomum mollissimum* and *Cinnamomum porrectum*.
2. To characterize the structures of the pure phytochemicals using spectroscopic methods.
3. To investigate the bioactivities such as antibacterial, antioxidant and anti-tyrosinase of the crude extracts and pure phytochemicals.

1.6 Scope of Study

In this study, the samples were the leaves and barks of *C. mollissimum* and *C. porrectum*. The leaves of *C. mollissimum* and the leaves and barks of *C. porrectum* will be extracted by cold extraction method using methanol. The methanol extract will be subjected to liquid-liquid extraction using petroleum ether, chloroform and ethyl acetate. Meanwhile, the sample of the barks from *C. mollissimum* will be extracted by acid-base extraction. Each extracts will be evaporated, fractionated by vacuum liquid chromatography (VLC) followed by purification using column chromatography (CC) to yield pure phytochemicals. Structural elucidations of the phytochemicals will be carried out by spectroscopic methods such as IR, MS, NMR (1D and 2D) and UV.

The crude extracts and characterized isolated phytochemicals will then be subjected to several bioactivity tests which include antibacterial, antioxidant and antityrosinase. The evaluation of antibacterial activity will be carried out using disc diffusion method, minimum inhibition concentration (MIC) and minimum bactericidal concentration (MBC) against bacterial strains of Gram-positive and Gram-negative. Meanwhile, 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay will be used for the antioxidant activity. The antityrosinase activity of the crude extracts will be examined through the inhibition activity of dopachrome formation at 475 nm.

1.7 Significance of the Study

The phytochemicals investigation of *C. mollissimum* and *C. porrectum* were expected to yield various classes of secondary metabolites which include alkaloids, prenylpropanoids and triterpenoids that may have several bioactivities. The results of this research will be valuable to the database of Malaysian *Cinnamomum* species. In addition, the plants which have the biologically active phytochemicals could be developed for cosmeceutical or pharmaceutical products in future.

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