



PHYTOCHEMISTRY AND ANTIOXIDANT ACTIVITY OF SELECTED SPECIES FROM LAURACEAE FAMILY



05-4506832



MUHAMMAD AMMAR BIN MOHD AZHAR



ptbupsi

UNIVERSITI PENDIDIKAN SULTAN IDRIS

2021





**PHYTOCHEMISTRY AND ANTIOXIDANT ACTIVITY OF
SELECTED SPECIES FROM LAURACEAE FAMILY**

MUHAMMAD AMMAR BIN MOHD AZHAR



**DISSERTATION PRESENTED TO QUALIFY FOR A
MASTERS IN SCIENCE
(RESEARCH MODE)**

**FACULTY OF SCIENCE AND MATHEMATICS
UNIVERSITI PENDIDIKAN SULTAN IDRIS**

2021





Please tick (✓)

Project Paper

Masters by Research

Master by Mixed Mode

PhD

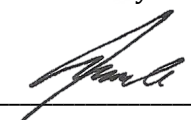
INSTITUTE OF GRADUATE STUDIES

DECLARATION OF ORIGINAL WORK

This declaration is made on the JULY day of 9 2021

i. Student's Declaration:

I, MUHAMMAD AMMAR BIN MOHD AZHAR, M20191000774 (PLEASE INDICATE STUDENT'S NAME, MATRIC NO. AND FACULTY) hereby declare that the work entitled PHYTOCHEMISTRY AND ANTIOXIDANT ACTIVITY OF SELECTED SPECIES FROM LAURACEAE FAMILY is my original work. I have not copied from any other students' work or from any other sources except where due reference or acknowledgement is made explicitly in the text, nor has any part been written for me by another person.


Signature of the student

ii. Supervisor's Declaration:

I DR. WAN MOHD NUZUL HAKIMI W SALLEH (SUPERVISOR'S NAME) hereby certifies that the work entitled PHYTOCHEMISTRY AND ANTIOXIDANT ACTIVITY OF SELECTED SPECIES FROM LAURACEAE FAMILY (TITLE) was prepared by the above named student, and was submitted to the Institute of Graduate Studies as a * partial/full fulfillment for the conferment of MASTER OF SCIENCE (NATURAL PRODUCTS) (PLEASE INDICATE THE DEGREE), and the aforementioned work, to the best of my knowledge, is the said student's work.

9 JULY 2021

Date



Signature of the Supervisor

DR. WAN MOHD NUZUL HAKIMI W SALLEH
Senior Lecturer
Department of Chemistry
Faculty of Science and Mathematics
Universiti Pendidikan Sultan Idris (UPSI)



**INSTITUT PENGAJIAN SISWAZAH /
INSTITUTE OF GRADUATE STUDIES**

**BORANG PENGESAHAN PENYERAHAN TESIS/DISERTASI/LAPORAN KERTAS PROJEK
DECLARATION OF THESIS/DISSERTATION/PROJECT PAPER FORM**

Tajuk / Title: PHYTOCHEMISTRY AND ANTIOXIDANT ACTIVITY OF SELECTED SPECIES
FROM LAURACEAE FAMILY

No. Matrik /Matric's No.: M20191000774

Saya / I: MUHAMMAD AMMAR BIN MOHD AZHAR

(Nama pelajar / Student's Name)

mengaku membenarkan Tesis/Disertasi/Laporan Kertas Projek (Kedoktoran/Sarjana)* ini disimpan di Universiti Pendidikan Sultan Idris (Perpustakaan Tuanku Bainun) dengan syarat-syarat kegunaan seperti berikut:-

acknowledged that Universiti Pendidikan Sultan Idris (Tuanku Bainun Library) reserves the right as follows:-

1. Tesis/Disertasi/Laporan Kertas Projek ini adalah hak milik UPSI.
The thesis is the property of Universiti Pendidikan Sultan Idris
2. Perpustakaan Tuanku Bainun dibenarkan membuat salinan untuk tujuan rujukan dan penyelidikan.
Tuanku Bainun Library has the right to make copies for the purpose of reference and research.
3. Perpustakaan dibenarkan membuat salinan Tesis/Disertasi ini sebagai bahan pertukaran antara Institusi Pengajian Tinggi.
The Library has the right to make copies of the thesis for academic exchange.
4. Sila tandakan (☒) bagi pilihan kategori di bawah / Please tick (☒) for category below:-

☐

SULIT/CONFIDENTIAL

Mengandungi maklumat yang berdarjah keselamatan atau kepentingan Malaysia seperti yang termaktub dalam Akta Rahsia Rasmi 1972. / Contains confidential information under the Official Secret Act 1972


☐

TERHAD/RESTRICTED

Mengandungi maklumat terhad yang telah ditentukan oleh organisasi/badan di mana penyelidikan ini dijalankan. / Contains restricted information as specified by the organization where research was done.

☒

TIDAK TERHAD / OPEN ACCESS


(Tandatangan Pelajar/ Signature)


(Tandatangan Penyelia / Signature of Supervisor)
& (Nama & Cop Rasmi / Name & Official Stamp)

DR. WAN MOHD NUZUL HAKIMI W SALLEH
Senior Lecturer
Department of Chemistry
Faculty of Science and Mathematics
Universiti Pendidikan Sultan Idris (UPSI)

Tarikh: 9 JULAI 2021

Catatan: Jika Tesis/Disertasi ini **SULIT @ TERHAD**, sila lampirkan surat daripada pihak berkuasa/organisasi berkenaan dengan menyatakan sekali sebab dan tempoh laporan ini perlu dikelaskan sebagai **SULIT** dan **TERHAD**.

Notes: If the thesis is CONFIDENTIAL or RESTRICTED, please attach with the letter from the organization with period and reasons for confidentiality or restriction.



05-4506832



pustaka.upsi.edu.my



Perpustakaan Tuanku Bainun
Kampus Sultan Abdul Jalil Shah



PustakaTBainun



ptbupsi

iii

*Special dedication of this grateful feeling to my family
for their love, support and best wishes...*



05-4506832



pustaka.upsi.edu.my



Perpustakaan Tuanku Bainun
Kampus Sultan Abdul Jalil Shah



PustakaTBainun



ptbupsi



05-4506832



pustaka.upsi.edu.my



Perpustakaan Tuanku Bainun
Kampus Sultan Abdul Jalil Shah



PustakaTBainun



ptbupsi

ACKNOWLEDGEMENT

First and foremost, Alhamdulillah, praises and thanks to Allah, the Almighty, for his countless blessings, throughout my research work to complete the research. I would like to express my deep and sincere gratitude to Dr. Wan Mohd Nuzul Hakimi W Salleh, my supervisor, for giving me the opportunity to do this research under his supervision and providing me with valuable guidance and support throughout this research. I was truly inspired by his hard work and passion. It was a great privilege and honor to work and study under his supervision. I would also like to thank Dr. Shazlyn Milleana Shaharudin for her help in multivariate statistical analysis. Sincere thanks to the Department of Chemistry, Faculty of Science and Mathematics, UPSI for the access to the laboratories and instruments. Last but not least, special thanks to my lab mates and friends for their moral support, advice, and guidance throughout my research.



ABSTRACT

The purposes of the study were to analyse the essential oils composition, phytochemicals, and antioxidant activity of selected species of the genus *Litsea* (*L. costalis*, *L. machilifolia* and *L. globularia*), *Beilschmiedia* (*B. kunstleri*, *B. insignis* and *B. pahangensis*) and *Cryptocarya* (*C. impressa*, *C. infectoria* and *C. rugulosa*) from Lauraceae family. The essential oils were obtained via hydrodistillation technique and their chemical compositions were determined by gas chromatography (GC-FID) and gas chromatography-mass spectrometry (GC-MS). The phytochemicals were obtained using chromatography techniques and their structures were confirmed by spectroscopic analysis and comparison with literature. The antioxidant activity of the essential oils and extracts was determined using DPPH free radical scavenging assay. The study showed that the major components of *L. costalis*, *L. machilifolia* and *L. globularia* essential oils were β -caryophyllene (12.6%), β -sesquiphellandrene (29.5%) and β -caryophyllene (25.2%), respectively. Besides, α -longipinene (11.0%), (*E*)-nerolidol (32.4%) and δ -cadinene were the major components from the essential oils of *B. kunstleri*, *B. insignis* and *B. pahangensis*, respectively. As for *C. impressa*, *C. infectoria* and *C. rugulosa*, the essential oils consisted of α -cadinol (40.7%), β -caryophyllene (25.4%) and bicyclogermacrene (15.6%), respectively. The dichloromethane and methanol extracts of *B. insignis* yielded seven phytochemicals identified as vanillic acid, vanillin, benzyl benzoate, benzoic acid, betulin, β -sitosterol and β -sitostenone. All essential oils displayed weak activity in DPPH radical scavenging assay, while the *L. costalis* methanolic extract showed the strongest activity (IC_{50} 21.9 ppm) comparable to ascorbic acid (IC_{50} 2.9 ppm). In conclusion, the major composition of essential oils was sesquiterpene hydrocarbons, meanwhile carboxylic acids and terpenes were successfully isolated from *B. insignis* extracts. The implication of the study showed the essential oils and extracts of the genus *Litsea*, *Beilschmiedia* and *Cryptocarya* have potential in pharmaceutical applications.





FITOKIMIA DAN AKTIVITI ANTIOKSIDAN DARIPADA SPESIES TERPILIH FAMILI LAURACEAE

ABSTRAK

Tujuan kajian untuk menganalisis komposisi minyak pati, fitokimia dan aktiviti antioksidan bagi spesies terpilih genus *Litsea* (*L. costalis*, *L. machilifolia* dan *L. globularia*), *Beilschmiedia* (*B. kunstleri*, *B. insignis* dan *B. pahangensis*) dan *Cryptocarya* (*C. impressa*, *C. infectoria* dan *C. rugulosa*) daripada famili Lauraceae. Minyak pati diperoleh melalui teknik penyulingan hidro dan kandungan kimianya ditentukan melalui kromatografi gas (GC-FID) dan kromatografi gas-spektrometri jisim (GC-MS). Sebatian fitokimia diperolehi menggunakan teknik kromatografi dan strukturnya disahkan melalui analisis spektroskopi dan perbandingan dengan literatur. Antioksidan aktiviti minyak pati dan ekstrak ditentukan menggunakan asai radikal bebas DPPH. Kajian menunjukkan komponen utama daripada minyak pati *L. costalis*, *L. machilifolia* dan *L. globularia* masing-masingnya adalah β -karyofailena (12.6%), β -seskuifelandrena (29.5%) dan β -karyofailena (25.2%). Selain tu, α -longifainena (11.0%), (*E*)-nerolidol (32.4%) dan δ -kadinena (21.9%) merupakan komponen utama minyak pati masing-masingnya daripada *B. kunstleri*, *B. insignis* dan *B. pahangensis*. Bagi *C. impressa*, *C. infectoria* dan *C. rugulosa*, minyak pati masing-masingnya terdiri daripada α -kadinol (40.7%), β -karyopfaillena (25.4%) dan bisiklogermakrena (15.6%). Ekstrak diklorometana dan metanol daripada *B. insignis* menghasilkan tujuh sebatian fitokimia yang dikenalpasti sebagai asid vanilik, vanilin, benzil benzoat, asid benzoik, betulin, β -sitosterol dan β -sitostenon. Kesemua minyak pati mempamerkan aktiviti yang lemah dalam asai radikal bebas DPPH, manakala ekstrak metanol *L. costalis* menunjukkan aktiviti terkuat (IC₅₀ 21.9 ppm) berbanding asid askorbik (IC₅₀ 2.9 ppm). Kesimpulannya, komposisi utama minyak pati adalah hidrokarbon seskuiterpena, manakala asid karboksilik dan terpena telah berjaya dipencilkan daripada ekstrak *B. insignis*. Implikasi kajian menunjukkan minyak pati dan ekstrak daripada genus *Litsea*, *Beilschmiedia* dan *Cryptocarya* berpotensi dalam aplikasi farmaseutikal.





CONTENTS

	Page
DECLARATION	ii
DEDICATION	iii
ACKNOWLEDGEMENT	iv
ABSTRACT	v
ABSTRAK	vi
CONTENTS	vii
LIST OF TABLES	x
LIST OF FIGURES	xii
LIST OF SCHEME	xv
LIST OF ABBREVIATIONS	xvi
CHAPTER 1 INTRODUCTION	1
1.1 General Introduction	1
1.2 Problem Statement	3
1.3 Objectives of Study	3
1.4 Scopes of Study	4
CHAPTER 2 LITERATURE REVIEW	5
2.1 Botany and Taxonomy of Lauraceae Family	5
2.1.1 The Genus <i>Litsea</i>	6
2.1.2 The Genus <i>Beilschmiedia</i>	9
2.1.3 The Genus <i>Cryptocarya</i>	11
2.2 Chemistry of Essential Oils	13





2.3	Chemical Composition of Essential Oils	16
2.3.1	The Genus <i>Litsea</i>	16
2.3.2	The Genus <i>Beilschmiedia</i>	35
2.3.3	The Genus <i>Cryptocarya</i>	40
2.4	Biological Activities of Essential Oils	44
2.5	Phytochemistry of Lauraceae Family	53
CHAPTER 3	EXPERIMENTAL	61
3.1	Plant Materials	61
3.2	Plant Extraction	62
3.3	Instrumentation	63
3.4	Isolation of Phytochemicals from <i>B. insignis</i>	65
3.5	Spectral Data of Isolated Phytochemicals	67
3.5.1	Vanillic acid (160)	67
3.5.2	Vanillin (161)	68
3.5.3	Benzyl benzoate (162)	69
3.5.4	Benzoic acid (163)	70
3.5.5	Betulin (164)	71
3.5.6	β -Sitosterol (165)	72
3.5.7	β -Sitostenone (166)	73
3.6	Antioxidant Activity	74
3.7	Statistical Analysis	74
CHAPTER 4	RESULTS AND DISCUSSION	75
4.1	Extraction of Essential Oils	75
4.2	Analysis of Essential Oils	76
4.2.1	<i>Litsea</i> Essential Oils	76



4.2.2	<i>Beilschmiedia</i> Essential Oils	84
4.2.3	<i>Cryptocarya</i> Essential Oils	92
4.3	Plant Extraction	101
4.4	Phytochemical Studies of <i>Beilschmiedia insignis</i>	102
4.4.1	Vanillic acid (160)	102
4.4.2	Vanillin (161)	107
4.4.3	Benzyl benzoate (162)	111
4.4.4	Benzoic acid (163)	115
4.4.5	Betulin (164)	118
4.4.6	β -Sitosterol (165)	125
4.4.7	β -Sitostenone (166)	131
4.5	Antioxidant Activity	138

CHAPTER 5 CONCLUSION AND RECOMMENDATIONS 142

5.1	Conclusions	142
5.2	Recommendations	143

REFERENCES 145

PUBLICATIONS 155



LIST OF TABLES

Table No.		Page
2.1	Medicinal uses of several <i>Litsea</i> species	7
2.2	The selected species of the genus <i>Litsea</i>	8
2.3	Medicinal uses of several <i>Beilschmiedia</i> species	9
2.4	The selected species of the genus <i>Beilschmiedia</i>	11
2.5	The selected species of the genus <i>Cryptocarya</i>	12
2.6	Properties and uses of the essential oils	15
2.7	Major components identified from the essential oils of several <i>Litsea</i> species	16
2.8	Major components identified from the essential oils of <i>Litsea cubeba</i>	32
2.9	Major components identified from the essential oils of several <i>Beilschmiedia</i> species	36
2.10	Major components identified from the essential oils of several <i>Cryptocarya</i> species	41
2.11	Biological activities of several <i>Litsea</i> essential oils	45
2.12	Biological activities of several <i>Beilschmiedia</i> essential oils	48
2.13	Biological activities of several <i>Cryptocarya</i> essential oils	52
2.14	Alkaloids isolated from several <i>Litsea</i> species	54
2.15	Lignans and neolignans isolated from several <i>Beilschmiedia</i> species	56
2.16	Alkaloids isolated from several <i>Cryptocarya</i> species	59
3.1	The details of the selected species	61
4.1	The percentage yield and appearance of the essential oils	76
4.2	Chemical components identified from <i>Litsea</i> essential oils	77





4.3	Chemical components identified from <i>Beilschmiedia</i> essential oils	85
4.4	Chemical components identified from <i>Cryptocarya</i> essential oils	92
4.5	The percentage yield and appearance of the extracts	101
4.6	^1H and ^{13}C NMR spectral data of compound (164) and literature	120
4.7	^1H and ^{13}C NMR spectral data of compound (165) and literature	127
4.8	^1H and ^{13}C NMR spectral data of compound (166) and literature	133
4.9	DPPH free radical scavenging assay of the essential oils and extracts	139





LIST OF FIGURES

Figure No.		Page
3.1	Flow chart for the isolation process of <i>B. insignis</i> extracts	66
4.1A	GC-MS chromatogram of <i>L. costalis</i> essential oil	79
4.1B	GC-MS chromatogram of <i>L. machilifolia</i> essential oil	80
4.1C	GC-MS chromatogram of <i>L. globularia</i> essential oil	80
4.2	PCA (A) and HCA (B) analysis of the components of <i>Litsea</i> essential oils	84
4.3A	GC-MS chromatogram of <i>B. kunstleri</i> essential oil	88
4.3B	GC-MS chromatogram of <i>B. pahangensis</i> essential oil	88
4.3C	GC-MS chromatogram of <i>B. insignis</i> essential oil	89
4.4	PCA (A) and HCA (B) analysis of the components of <i>Beilschmiedia</i> essential oils	91
4.5A	GC-MS chromatogram of <i>C. infectoria</i> essential oil	95
4.5B	GC-MS chromatogram of <i>C. impressa</i> essential oil	95
4.5C	GC-MS chromatogram of <i>C. rugulosa</i> essential oil	96
4.6	PCA (A) and HCA (B) analysis of the components of <i>Cryptocarya</i> essential oils	100
4.7A	IR spectrum of vanillic acid (160)	104
4.7B	¹ H NMR spectrum of vanillic acid (160)	105
4.7C	¹³ C NMR spectrum of vanillic acid (160)	105
4.7D	DEPT spectra of vanillic acid (160)	106
4.7E	HMBC spectrum of vanillic acid (160)	106
4.7F	MS spectrum of vanillic acid (160)	107
4.8A	IR spectrum of vanillin (161)	108





4.8B	¹ H NMR spectrum of vanillin (161)	109
4.8C	¹³ C NMR spectra of vanillin (161)	109
4.8D	DEPT spectra of vanillin (161)	110
4.8E	HMQC spectrum of vanillin (161)	110
4.8F	MS spectrum of benzyl benzoate (161)	111
4.9A	IR spectrum of benzyl benzoate (162)	112
4.9B	¹ H NMR spectrum of benzyl benzoate (162)	113
4.9C	¹³ C NMR spectrum of benzyl benzoate (162)	113
4.9D	DEPT spectra of benzyl benzoate (162)	114
4.9E	MS spectrum of benzyl benzoate (162)	114
4.10A	IR spectrum of benzoic acid (163)	116
4.10B	¹ H NMR spectrum of benzoic acid (163)	116
4.10C	¹³ C NMR spectrum of benzoic acid (163)	117
4.10D	MS spectrum of benzoic acid (163)	117
4.11A	IR spectrum of betulin (164)	122
4.11B	¹ H NMR spectrum of betulin (164)	122
4.11C	¹³ C NMR spectrum of betulin (164)	123
4.11D	DEPT spectra of betulin (164)	123
4.11E	COSY spectrum of betulin (164)	124
4.11F	HMQC spectrum of betulin (164)	124
4.11G	MS spectrum of betulin (164)	125
4.12A	IR spectrum of β -sitosterol (165)	129
4.12B	¹ H NMR spectrum of β -sitosterol (165)	129
4.12C	¹³ C NMR spectrum of β -sitosterol (165)	130
4.12D	DEPT spectra of β -sitosterol (165)	130





4.12E	MS spectrum of β -sitosterol (165)	131
4.13A	IR spectrum of β -sitostenone (166)	135
4.13B	^1H NMR spectrum of β -sitostenone (166)	135
4.13C	^{13}C NMR spectrum of β -sitostenone (166)	136
4.13D	DEPT spectra of β -sitostenone (166)	136
4.13E	GC-MS spectrum of β -sitostenone (166)	137





LIST OF SCHEME

Scheme No.		Page
4.1	Mass fragmentation pattern of compound (160)	104





ABBREVIATIONS

α	Alpha
Abs	Absorbance
β	Beta
br	broad
^{13}C	Carbon-13
CC	Column Chromatography
CDCl_3	Deuterated chloroform
CHCl_3	Chloroform
cm^{-1}	Per centimeter
COSY	Correlation spectroscopy
DCM	Dichloromethane
1D	1 Dimension
2D	2 Dimension
δ	chemical shift
d	doublet
dd	doublet of doublets
DEPT	Distortionless Enhancement by Polarization Transfer
EIMS	Electron Impact Mass Spectrometry
Et_2O	Diethyl ether
GC	Gas Chromatography
GC-MS	Gas Chromatography-Mass Spectrometry
^1H	Proton
HMBC	Heteronuclear Multiple Bond Correlation





HMQC	Heteronuclear Multiple Quantum Coherence
Hz	Hertz
IR	Infrared
J	Coupling constant
KBr	Potassium bromide
KI	Kovats Index
L	Liter
m	multiplet
M^+	Molecular ion
MeOH	Methanol
MHz	Megahertz
m/z	Mass to charge ion
mg	milligram
m.p	Melting point
$MgSO_4$	Magnesium sulphate
mL	milliliter
mm	millimeter
MS	Mass Spectrometer
NMR	Nuclear Magnetic Resonance
nm	nanometer
R_f	Retention factor
s	singlet
SiO_2	Silica gel
t	triplet
TLC	Thin Layer Chromatography



CHAPTER 1

INTRODUCTION

Plants are believed to have existed before the era of humankind. Humans have been using plants for their benefit since the early beginning of human history. Plants have so many usages as they are the source of food, shelter, and utensil as well as treatment for discomfort and diseases (Newman et al., 2000). Today, plants still serve as the main supplier for human development and ensure our survival as they are the main source of oxygen on the planet. Over the last few decades, many types of research have been carried on using plants as the main ingredient to produce alternative medicine (Ali et al., 2015).

Medicinal plants are the local heritage in many developing countries. The traditional medicinal system and drugs are being widely performed commonly in



India and China, as well as East Asian and African countries. They are also important as raw materials of several chemical drugs and also used for antimicrobials, anti-inflammatory, antituberculosis, antiviral treatments (Kumar et al., 1997). Modern drug-based medicine that is widely used nowadays proposed a variety of side effects, hence people have to find an alternative way to treat them by using traditional medicine from plants. Presently, most of the clinical drugs are from plant extracts and their derivatives (Igdir et al., 2013).

In Malaysia, many medicinal plants have been used as traditional forms of Malay, Chinese and Indian medicine. These plants can be found in herbal products and as part of the traditional Malaysian health care system because of their therapeutic efficacy (Alsarhan et al., 2014). The most popular Malaysian medicinal plants are *Aloe vera* (lidah buaya), *Labisia pumila* (kacip fatimah), *Ficus deltoidea* (mas cotek), *Centella asiatica* (pegaga) and *Piper sarmentosum* (kaduk). Several Malaysian medicinal plants are used for health preparations. The roots of *Eurycoma longifolia* (tongkat ali) is one of the main ingredients in preparing these mixtures. *Andrographis paniculata* (hempedu bumi), a mixture of buds from several types of *lime* and *kancing baju* are used to treat diabetes, whereas *Orthosiphon grandifloras* (misai kucing) are used for hypertension (Burkill, 1966; Ahmad, 2015).

Currently, numerous researchers are currently striving to discover more plants which have medicinal qualities and have the capability to be marketed as herbal remedies. Lauraceae is one of the families of plants that are considered to have elevated therapeutic benefits due to its extensive used in various alternative medicines.





1.2 Problem Statement

Due to their limited range of phytochemicals and biological properties that have been documented, medicinal plants from the Lauraceae family seem to be of considerable significance. However, several Malaysian species of Lauraceae have not been extensively studied, both chemically and biologically. Nine species from the Lauraceae family have been selected for this study. Among them, six species was not reported yet on essential oils composition, whereas seven species was not described yet on phytochemistry. Taking into account the significance of the traditional uses of this genus in the management of several diseases, it is clear that there is a need to explore a wider range on the studies of essential oils, phytochemistry and their biological activity. Thus, studies on investigation concerning the extraction of essential oils, the isolation of phytochemicals, and the biological activity of the species selected. The research findings will contribute to the development in nutraceutical and potential in the pharmaceutical industry.

1.3 Objectives of Study

The objectives of the study are:

1. To investigate the chemical compositions of the essential oils from nine species of the Lauraceae family.
2. To isolate the phytochemicals from *B. insignis* extracts and characterized using spectroscopic techniques.
3. To determine the antioxidant activity of the essential oils and extracts.





1.4 Scopes of Study

The study was separated into three sections. The first section was the extraction of essential oils using the hydrodistillation method from the leaves of *L. costalis*, *L. machilifolia*, *L. ferruginea*, *B. kunstleri*, *B. insignis*, *B. pahangensis*, *C. impressa*, *C. infectoria*, and *C. rugulosa*. The essential oil composition were examined using GC, GC-MS, and Kovats Indices in order to classify the chemical components. The second section was the isolation of the phytochemicals from *B. insignis* extracts using various chromatography methods such as column chromatography and preparative thin layer chromatography. The structures of the isolated phytochemicals were elucidated spectroscopically using IR, NMR, and MS. Lastly, the antioxidant evaluation was carried out using DPPH free radical scavenging method on the essential oils and crude

