



UNIVERSITI PUTRA MALAYSIA

IDENTIFICATION OF MALAYSIAN LEMON MYRTLE (*BACKHOUSIA CITRIODORA F. MUELL*) CHEMICAL CONSTITUENTS RESPONSIBLE FOR INSECTICIDAL ACTIVITY USING GC-MC-BASED METABOLOMICS

JAMILA GARBA

FS 2016 65



IDENTIFICATION OF MALAYSIAN LEMON MYRTLE (*Backhousia citriodora* F. MUELL) CHEMICAL CONSTITUENTS RESPONSIBLE FOR INSECTICIDAL ACTIVITY USING GC-MS-BASED METABOLOMICS

By

JAMILA GARBA

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science

September 2016

All material contained within the thesis, including without limitation text, logos, icons, photographs and all other artwork, is copyright material of Universiti Putra Malaysia unless otherwise stated. Use may be made of any material contained within the thesis for non-commercial purposes from the copyright holder. Commercial use of material may only be made with the express, prior, written permission of Universiti Putra Malaysia.

Copyright © Universiti Putra Malaysia



DEDICATION

To my beloved parents, brothers and sisters;
For their love, trust and confidence in me.



Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

IDENTIFICATION OF MALAYSIAN LEMON MYRTLE (*Backhousia citriodora* F. MUELL) CHEMICAL CONSTITUENTS RESPONSIBLE FOR INSECTICIDAL ACTIVITY USING GC-MS-BASED METABOLOMICS

By

JAMILA GARBA

September 2016

Chairman : Nur Kartinee Bt Kassim, PhD
Faculty : Science

Anti-feedant and larvicidal activities of the essential oil, hexane, ethyl acetate and methanol extracts of *Backhousia citriodora* (grown in Malaysia) were studied using leaf disc no-choice and leaf dip methods respectively, against second instar larvae of *S. litura* and *C. binotalis*. *Backhousia citriodora*, commonly known as lemon myrtle, is a native Australian plant which belongs to the Myrtaceae family. Lemon myrtle steam-distilled essential oil has been reported to exhibit effective repellent properties against mosquitoes (domestic insects); making lemon myrtle a valuable exploratory source of novel insecticides for the management of agricultural insects. *Spodoptera litura* and *Crociodolomia binotalis* are dangerous agricultural insects. While *S. litura* is already resistant to many classes of synthetic insecticides, insecticides used in controlling *C. binotalis* are lethal to living organisms and also contaminate the environment. Therefore this study was carried out to investigate the insecticidal potential of lemon myrtle plant extracts against *S. litura* and *C. binotalis*.

Lemon myrtle hexane extract showed maximum larvicidal activity of 100% at 5.0% (w/v) concentration with a lethal concentration (LC₅₀) value of 1.8% (w/v), against *C. binotalis*. At 5.0% (w/v) concentration, the hexane extract killed 80.0 ± 2.9% of second instar *S. litura* larvae after 72 hours. The active hexane extract was subjected to Vacuum Liquid Chromatography (VLC) which afforded four major fractions namely LM1, LM4, LM5 and LM7. When tested against *C. binotalis*, fraction LM4 displayed maximum larvicidal activity of 100%, at a concentration of 1.4% (w/v) with an LC₅₀ of 0.8% (w/v). At the same concentration, the fraction LM4 also completely inhibited the feeding activity of *C. binotalis* larvae, thus indicating good anti-feedant properties. Gas Chromatography-Mass Spectrometry (GC-MS) and Orthogonal Partial Least Squares (OPLS) were employed to investigate the chemical constituents of the different fractions. The compounds responsible for the insecticidal activity of lemon myrtle were identified as epoxy-linalool oxide, isopropyl 4-methyl-3-methylene-4-pentenoate,

neric acid and citral. The results of this study indicated that lemon myrtle leaf extract, particularly the hexane extract, possesses remarkable insecticidal properties and could therefore, serve as a viable source for the development of a safer and efficient insecticide for crop protection.



Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

**PENGENALAN LEMON MYRTLE (*Backhousia citriodora* F. MUELL)
MALAYSIA KANDUNGAN KIMIA BERTANGGUNGJAWAB BAGI ACTIVITI
INSEKTISIDAL, MENGGUNAKAN ANALISIS METABOLOMIC
BESASASKAN KROMATOGRAFI GAS SPEKTRUM JISIM**

Oleh

JAMILA GARBA

September 2016

Pengerusi : Nur Kartinee Bt Kassim, PhD
Fakulti : Sains

Aktiviti anti-feedan dan larvisidal aktiviti minyak pati, ekstrak heksana, etil asetat dan metanol daripada *lemon myrtle* yang (ditanam di Malaysia) telah dikaji dengan menggunakan kaedah cakera daun tiada pilihan dan kaedah celupan daun terhadap larva instar kedua *S. litura* dan *C. binotalis*. *Backhousia citriodora* biasanya dikenali sebagai *Lemon myrtle*, tumbuhan asli Australia daripada keluarga Myrtaceae. Minyak pati *lemon myrtle* yang diperoleh melalui penyulingan telah dilaporkan dalam mempamerkan sifat repelan berkesan terhadap nyamuk (perosak domestik); menjadikan *lemon myrtle* sebagai sumber berharga bagi penemuan novel racun serangga dalam pengurusan perosak pertanian. *Spodoptera litura* dan *Crociodolomia binotalis* adalah serangga perosak pertanian berbahaya akan menyebabkan kemusnahan hasil tanaman dan kerugian ekonomi yang besar. Manakala *S. litura* telah rentan terhadap banyak racun serangga sintetik yang digunakan dalam mengawal *C. binotalis* adalah berbahaya kepada organisma hidup dan mencemari alam sekitar. Oleh itu, kajian ini dijalankan untuk menyiasat potensi serangga tumbuhan *lemon myrtle* ekstrak terhadap *S. litura* dan *C. binotalis*.

Lemon myrtle ekstrak heksana menunjukkan aktiviti larvisidal yang maksimum, 100% pada kepekatan 5.0% (w/v) dengan nilai kepekatan kematian (LC₅₀) sebanyak 1.8% (w/v), terhadap *C. binotalis*. Pada 5.0% (w/v) kepekatan ekstrak heksana, peratus kematian adalah 80.0 ± 2.9% daripada instar kedua bagi larva *S. litura* selepas 72 jam. Ekstrak heksana yang aktif daripada kajian bioasai adalah tertakluk kepada Kromatografi Cecair Vakum (VLC) yang memberikan empat fraksi utama LM1, LM4, LM5 dan LM7. Fraksi LM4 memaparkan kesan aktiviti larvisidal yang maksimum sebanyak 100% pada kepekatan 1.4% (w/v) dengan nilai kepekatan kematian (LC₅₀) sebanyak 0.8% (w/v). Analisis Kromatografi Gas Berspektrum Jisim (GC-MS) dan Ortogonal Separa Kuasa dua Terkecil (OPLS) telah digunakan untuk mengkaji

kandungan kimia daripada fraksi yang berbeza. Sebatian yang bertanggungjawab ke atas aktiviti serangga telah dikenal pasti sebagai epoksi-linalool oksida, isopropil 4-metil-3-metilena-4-pentenoat, asid nerolik dan sitral. Keputusan kajian ini mendapati bahawa daun ekstrak *lemon myrtle*, terutama ekstrak heksana, mempunyai ciri-ciri insectisidal luar biasa dan oleh itu boleh menjadi sumber berpotensi untuk pembangunan produk racun serangga yang lebih selamat untuk perlindungan tanaman.



ACKNOWLEDGEMENTS

All praises are due to Allah (S.W.T) for His abundant blessings and favours during the period of this study and throughout my life. I would like to express my deepest appreciation to my supervisor, Dr. Nur Kartinee Bt. Kassim, whose excellent supervision, encouragement and confidence in me strengthened me this far. May Allah (S.W.T) reward her kindness and tolerance towards me. My immense gratitude goes to my co-supervisors, Dr. Mohd Shukri Bin Mat Ali Ibrahim, Dr. Siti Mariam Bt Mohd Nor and Prof. Madya Dr. Intan Safinar Ismail, for their valuable comments and excellent guidance. I feel thankful to have worked under the most insightful supervisory team.

I am indebted to my family at Natural Products Lab 405, for their helpfulness and providing a friendly environment to work in; and to the chemistry department, faculty of science, UPM, for providing working facilities. The staff at the Insectary, MARDI and those at spectroscopy lab, IBS, UPM, are greatly appreciated. I sincerely appreciate Pn Siti Noor Aishikin Abdul Hamid and Aunty Fatima both of MARDI, and brother Safwan of IBS for dedicating huge time and effort to guide me through. I am also grateful to En cik Zainal Abidin bin Kassim of GC-MS lab, faculty of science, UPM for his assistance with GC-MS analysis.

I would like to express my warm appreciation to my parents, Alh. Garba Suleiman and Haj. Lantana Maryam, and all members of my immediate and extended family for their invaluable prayers and moral support. Last but not the least, many thanks to all my friends and well-wishers, for their prayers and support.

I certify that a Thesis Examination Committee has met on 22 September 2016 to conduct the final examination of Jamila Garba on her thesis entitled "Identification of Malaysian Lemon Myrtle (*Backhousia citriodora* F. Muell) Chemical Constituents Responsible for Insecticidal Activity using Gc-MS-Based Metabolomics" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

Members of the Thesis Examination Committee were as follows:

Norhazlin binti Zainuddin, PhD

Senior Lecturer
Faculty of Science
Universiti Putra Malaysia
(Chairman)

Faridah binti Abas, PhD

Associate Professor
Faculty of Food Science and Technology
Universiti Putra Malaysia
(Internal Examiner)

Idris Abd Ghani, PhD

Professor
Universiti Kebangsaan Malaysia
Malaysia
(External Examiner)



NOR AINI AB. SHUKOR, PhD
Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 27 December 2016

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science.

The members of the Supervisory Committee were as follows:

Nur Kartinee Bt. Kassim, PhD

Senior Lecturer
Faculty of Science
Universiti Putra Malaysia
(Chairman)

Intan Safinar Ismail, PhD

Associate Professor
Institute of Bioscience
Universiti Putra Malaysia
(Member)

Siti Mariam Bt Mohd Nor, PhD

Senior Lecturer
Faculty of Science
Universiti Putra Malaysia
(Member)

Mohd Shukri Bin Mat Ali Ibrahim, PhD

Principal Research Officer
Strategic Resources Research Centre
Malaysian Agriculture Research & Development Institute
(Member)

ROBIAH BINTI YUNUS, PhD

Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

Declaration by graduate student

I hereby confirm that:

- this thesis is my original work;
- quotations, illustrations and citations have been duly referenced;
- this thesis has not been submitted previously or concurrently for any other degree at any other institutions;
- intellectual property from the thesis and copyright of thesis are fully-owned by Universiti Putra Malaysia, as according to the Universiti Putra Malaysia (Research) Rules 2012;
- written permission must be obtained from supervisor and the office of Deputy Vice-Chancellor (Research and Innovation) before thesis is published (in the form of written, printed or in electronic form) including books, journals, modules, proceedings, popular writings, seminar papers, manuscripts, posters, reports, lecture notes, learning modules or any other materials as stated in the Universiti Putra Malaysia (Research) Rules 2012;
- there is no plagiarism or data falsification/fabrication in the thesis, and scholarly integrity is upheld as according to the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) and the Universiti Putra Malaysia (Research) Rules 2012. The thesis has undergone plagiarism detection software.

Signature: _____ Date: _____

Name and Matric No.: Jamila Garba, GS41742

Declaration by Members of Supervisory Committee

This is to confirm that:

- the research conducted and the writing of this thesis was under our supervision;
- supervision responsibilities as stated in the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) are adhered to.

Signature: _____
Name of Chairman of
Supervisory
Committee: _____

Signature: _____
Name of Member of
Supervisory
Committee: _____

Signature: _____
Name of Member of
Supervisory
Committee: _____

Signature: _____
Name of Member of
Supervisory
Committee: _____

TABLE OF CONTENTS

	Page
ABSTRACT	i
ABSTRAK	iii
ACKNOWLEDGEMENTS	v
APPROVAL	vi
DECLARATION	viii
LIST OF TABLES	xiv
LIST OF FIGURES	xv
LIST OF ABBREVIATIONS	xvi
CHAPTER	
1 INTRODUCTION	1
1.1 General Introduction	1
1.2 Problem statement	2
1.3 Significance of study	2
1.4 Objectives	2
2 LITERATURE REVIEW	3
2.1 The Family Myrtaceae	3
2.2 The Genus <i>Backhousia</i>	3
2.2.1 Traditional Medicinal Use of <i>Backhousia</i>	4
2.2.2 Phytochemical Studies on <i>Backhousia</i>	5
2.2.3 Bioactivity Studies on <i>Backhousia</i>	8
2.3 Terpene Biosynthesis	10
2.4 Agricultural Crop Insects	13
2.4.1 <i>Crocidolomia binotalis</i> Zeller (Lepidoptera: Pyralidae)	13
2.4.2 <i>Spodoptera litura</i> Fabricius (Lepidoptera: Noctuidae)	14
2.5 Insecticides	15
2.5.1 Synthetic Insecticides	15
2.5.2 Botanical Insecticides	15
2.6 Anti-feedant and Larvicidal Assays	17
2.7 Metabolomics	18
2.7.1 GC-MS-Based Metabolomics	19
2.7.2 Multivariate Data Analysis	20
3 MATERIALS AND METHODS	21
3.1 Experimental Design	21
3.2 Materials	22
3.2.1 Plant Material	22
3.2.2 Silica Gel	22
3.2.3 Botanical Standard	23
3.3 Instruments	23
3.3.1 UltraViolet (UV)	23
3.3.2 Gas-Chromatography Mass Spectrometry (GC-MS)	23

3.3.3	Infrared (IR) Spectroscopy	23
3.3.4	Nuclear Magnetic Resonance (NMR) Spectroscopy	23
3.4	Chromatography methods	24
3.4.1	Vacuum Liquid Chromatography (VLC)	24
3.4.2	Thin Layer Chromatography (TLC)	24
3.5	Extraction and Fractionation	24
3.5.1	Essential Oil Extraction by Hydrodistillation	24
3.5.2	Solvent Crude Extraction by Maceration	24
3.5.3	Isolation of Active Fraction LM4 from Hexane Crude	25
3.6	Screening of Insecticidal Activity	25
3.6.1	Insects Species	25
3.6.2	Larvicidal Assay	26
3.6.3	Anti-feedant Assay	27
3.7	XCMS Analysis	28
3.8	Statistical Analysis	28
4	RESULTS AND DISCUSSION	30
4.1	Extraction and Isolation of Active Fraction LM4	30
4.2	Insecticidal Activities	31
4.2.1	Larvicidal and Anti-feedant Activity of Extracts against <i>S. litura</i>	31
4.2.2	Larvicidal and Anti-feedant Activity of Extracts against <i>C. binotalis</i>	32
4.2.3	Lethal Concentration (LC ₅₀) of Hexane Extract against <i>C. binotalis</i>	33
4.2.4	Larvicidal and Anti-feedant Activity of Hexane Fractions against <i>C. binotalis</i>	35
4.2.5	Lethal Concentration (LC ₅₀) of Active Fraction LM4 against <i>C. binotalis</i>	36
4.3	GC-MS Analysis of Hexane Fractions	38
4.4	Multivariate data analysis	40
4.5	Identification of Active Compounds	43
4.6	Spectral Analysis of Active Fraction LM4	46
4.6.1	Infrared Spectral analysis	46
4.6.2	¹ H-NMR and APT Spectral Analysis	47
4.6.3	HMQC and HMBC Spectral Analysis	51
5	CONCLUSION AND RECOMMENDATIONS	55
5.1	Conclusion	55
5.2	Recommendations for Future Studies	56
	REFERENCES	57
	APPENDIX	66
	BIODATA OF STUDENT	67

LIST OF TABLES

Table		Page
2.1	Phytochemicals from leaf oils of nine <i>Backhousia</i> species	6
2.2	Biological activities of <i>Backhousia</i> species	10
2.3	Comparison between synthetic and botanical insecticides	17
2.4	Classification of metabolomics	18
2.5	Comparison between analytical tools in metabolomics	19
4.1	Mean percentage mortality of <i>Spodoptera litura</i> treated with 5.0% (w/v) lemon myrtle extracts	31
4.2	Mean percentage mortality of <i>Crocidolomia binotalis</i> treated with 5.0% (w/v) lemon myrtle extracts	32
4.3	Mean percentage mortality of various concentrations of hexane extract against 2 nd instar <i>Crocidolomia binotalis</i>	34
4.4	Mean percentage mortality of <i>Crocidolomia binotalis</i> treated with 1.4% (w/v) hexane fractions	35
4.5	Mean percentage mortality of various concentrations of active fraction LM4 against <i>Crocidolomia binotalis</i>	37
4.6	Chemical constituents of active fraction LM4	39
4.7	Insecticidal compounds identified in fractions obtained from lemon myrtle hexane extract	45
4.8	IR Spectral data of active fraction LM4	47
4.9	¹ H-NMR (CDCl ₃ , 600 MHz) Spectral data of active fraction LM4	49
4.10	APT (CDCl ₃ , 150 MHz) Spectral data of active fraction LM4	51

LIST OF FIGURES

Figure	Page
2.1 Australian lemon myrtle (a) leaves (b) tree	4
2.2 Malaysian lemon myrtle (a) leaves (b) tree	4
2.3 Mevalonic Acid (MVA) pathway	11
2.4 MethylErythritol Phosphate (MEP) pathway	12
2.5 Larva of <i>Crocidolomia binotalis</i>	13
2.6 Larva of <i>Spodoptera litura</i>	14
2.7 Natural insecticides	16
3.1 Flow chart for the identification of insecticidal constituents of Malaysian lemon myrtle, using gc-ms metabolomics	22
3.2 Second instar larvae of <i>Spodoptera litura</i> and <i>Crocidolomia binotalis</i>	26
3.3 Assay method for larvicidal activity	26
3.4 Flow chart for multivariate data analysis	29
4.1 Schematic diagram for results of extraction and fractionation	30
4.2 Dead 2 nd instar <i>Spodoptera litura</i> larvae treated with 5.0 % (w/v) hexane extract	32
4.3 Dead 2 nd instar <i>C. binotalis</i> larvae after treatment with 5.0 % (w/v) hexane extract	33
4.4 LC ₅₀ of hexane extract when tested against 2 nd instar <i>Crocidolomia binotalis</i>	34
4.5 2 nd instar <i>Crocidolomia binotalis</i> larvae treated (a) acetone and (b) neem oil, alive and growing into next developmental stage	36
4.6 Dead larvae of 2 nd instar <i>C. binotalis</i> after treatment with 1.4 % (w/v) active fraction LM4	37
4.7 LC ₅₀ of fraction LM4 when tested against 2 nd instar <i>Crocidolomia binotalis</i>	38
4.8 Total Ion Chromatogram (TIC) of fraction LM4	39
4.9 PCA Score plot of different fractions of lemon myrtle hexane extract	40
4.10 OPLS Score scatter plot of lemon myrtle hexane fractions	41
4.11 OPLS Loading scatter plot of lemon myrtle hexane fractions in the range 0.02-0.12 labelled according to peak numbers on GC-MS chromatogram	41

4.12	Observed versus predicted insecticidal activity of all lemon myrtle hexane fractions	43
4.13	Loading column plot of lemon myrtle hexane fractions	44
4.14	IR spectrum of active fraction LM4	47
4.15	¹ H-NMR spectrum of active fraction LM4	48
4.16	APT spectrum of active fraction LM4	50
4.17	Expanded HMQC spectrum of fraction LM4 in the range of 1.0-2.7 ppm	52
4.18	Selected HMBC (↷) correlation of citral showing correlation between carbonyl proton H-1 and carbon atom C-2	53
4.19	Expanded HMQC spectrum of fraction LM4 in the range 3.5 – 6.0 ppm	54

LIST OF ABBREVIATIONS

δ	Chemical shift in ppm
ANTF	Anti-feedant
APT	Attached Proton Test
CDCl ₃	Deuterated chloroform
CDF	Computable Document Format
cm ⁻¹	Per centimetre
¹³ C-NMR	Carbon-Nuclear Magnetic Resonance
<i>d</i>	Doublet
DMAPP	Dimethylallyl diphosphate
g/mol	gram per mole
GC-MS	Gas Chromatography-Mass Spectrometry
HMBC	Heteronuclear Multiple Bond Connectivity
¹ H-NMR	Proton-Nuclear Magnetic Resonance
HMQC	Heteronuclear Multiple Quantum Coherence
IPP	Isopentenyl diphosphate
IR	Infra-red
<i>J</i>	Coupling constant
LC	Lethal Concentration
LVCD	Larvicidal
<i>m</i>	Multiplet
<i>m/z</i>	Mass per charge
MEP	Methylerythritol phosphate
MVA	Mevalonic acid
NIST	National Institute of Standards and Technology
NMR	Nuclear Magnetic Resonance
OPLS	Orthogonal Partial Least Squares
PCA	Principal Component Analysis
RT	Retention Time
<i>s</i>	Singlet
SE	Standard Error
TIC	Total Ion Chromatogram

TLC	Thin Layer Chromatography
UV	Ultra-violet
VIP	Variable Importance of Projection
VLC	Vacuum Liquid Chromatography
w/v	Weight per volume



CHAPTER 1

INTRODUCTION

1.1 General Introduction

Backhousia citriodora (F. Muell) is an Australian plant commonly known as lemon myrtle. It belongs to the family Myrtaceae and the genus *Backhousia*. Phytochemical analysis of essential oil of Australian lemon myrtle and that of lemon myrtle grown in Malaysia, revealed that both essential oils contain predominantly citral (Kean et al., 2013; Brophy et al., 1995). Citral is a monoterpenoid obtained from plants and has been proven to be poisonous to insects (Adorjan & Buchbauer, 2010; Sforcin et al., 2009). Hence, lemon myrtle could serve as a potential source for the development of an efficient and safe insecticide. Important agricultural crops such as cruciferous vegetables e.g. cabbage, widely cultivated in diverse parts of the world are often attacked by insects such as the cotton leafworm, *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae); the cabbagehead caterpillar (CHC), *Crociodolomia binotalis* (Zeller) (Lepidoptera: Pyralidae) and the cabbage webworm (CWW), *Hellula undalis* (Guenee) (Lepidoptera: Pyralidae) (Reddy, 2011). In recent years, several plants with insecticidal potentials have generated significant amount of interest amongst researchers, for the development of insecticides (Ojebode et al., 2016; Arivoli & Tennyson, 2012; Baskar et al., 2011).

In this study, bioassay and Gas Chromatography-Mass Spectrometry (GC-MS)-based metabolomics were used to identify the bioactive compounds of lemon myrtle (grown in Malaysia). Solvent crudes and essential oil were first extracted from the leaves of lemon myrtle via maceration and hydro distillation, respectively. All extracts were preliminary screened for insecticidal potential against two important agricultural insect species, i.e. *Spodoptera litura* and *Crociodolomia binotalis*. In order to separate the most effective extract into different fractions, it was subjected to fractionation using Vacuum Liquid Chromatography (VLC). All fractions were screened against the target insect species via the anti-feedant and larvicidal assays and subjected to GC-MS analysis. GC-MS analysis was conducted to identify the chemical constituents of the different fractions. Subsequently, multivariate data analysis specifically Principal Component Analysis (PCA) and Orthogonal Partial Least Squares (OPLS) was employed to discriminate between sample groups and to correlate between chemical constituents of the different fractions and the observed bioactivity pattern displayed against target insects. Also in this study, the chemical constituents responsible for the insecticidal activity of lemon myrtle were identified. The active constituents were further confirmed using Infrared (IR) and Nuclear Magnetic Resonance (NMR) spectroscopic techniques.

1.2 Problem statement

Insects attack and destroy numerous species of important crops, consequently, decreasing marketability and causing economic loss. Currently, synthetic insecticides are applied to agricultural fields for protection against defoliating insects. While some of the insect species are relatively susceptible to the insecticides (Rattan, 2010), other species for example *S. litura* have developed resistance to numerous groups of synthetic insecticides such as the organophosphates, organochlorines, carbamates, pyrethroids and the newer chemistry insecticides for example indoxacarb, spinosad; resulting from the large dependence of crop protection on synthetic insecticides (Tong et al., 2013; Ahmad et al., 2008). Despite the benefits they present to agricultural crop protection, the use of synthetic insecticides accounts for undesirable effects such as disruption of natural ecosystem, contamination of air and water bodies, killing of non-target organisms and chemical residual effects on harvested food produce (de Oliveira et al., 2014; Ebadollahi, 2013). Therefore, due to the raising concerns over insect resistance to synthetic insecticides and the environmental and health hazards posed by synthetic insecticides, the search for eco-friendly, safer and efficient alternatives becomes imperative. Citral, a terpenoid present in the oils of several plants has been extensively investigated for its insecticidal properties and has been shown to demonstrate effective anti-feedant properties. Lemon myrtle essential oil has been reported to contain over 90% citral and therefore could serve as a potent insecticide (Kean et al., 2013; Brophy et al., 1995).

1.3 Significance of study

This study would result in the discovery of a safer, eco-friendly and an efficient insecticide. The insecticide would primarily be employed in the field of agriculture, by spraying on crops, to prevent insects from feeding on crops, to kill insects and to mitigate against insect resistance. Furthermore, results of this study could find application in the field of forestry where insecticides are needed to control insects and promote wild life; and in public health where disease-transmitting insects can be killed or minimized through the application of insecticides.

1.4 Objectives

The objectives of this study are:

1. to investigate the insecticidal properties of lemon myrtle extracts and essential oil against important agricultural insect species *Spodoptera litura* and *Crocidolomia binotalis*.
2. to identify the plant constituents responsible for the insecticidal activity of lemon myrtle using GC-MS metabolomics.
3. to confirm the active insecticidal constituents using IR and NMR spectroscopic techniques.

REFERENCES

- Adeyemi, M. M. H. (2010). The potential of secondary metabolites in plant material as deterrents against insect pests: A review. *African Journal of Pure and Applied Chemistry*, 4(11), 243–246.
- Adorjan, B., & Buchbauer, G. (2010). Biological properties of essential oils: an updated review. *Flavour and Fragrance Journal*, 25(6), 407–426. <http://doi.org/10.1002/ffj.2024>
- Aggio, R., Mayor, A., Reade, S., Probert, C., & Ruggiero, K. (2014). Identifying and quantifying metabolites by scoring peaks of GC-MS data. *BMC Bioinformatics*, 15(1), 374. <http://doi.org/10.1186/s12859-014-0374-2>
- Ahmad, M., Arif, M. I., & Ahmad, M. (2007). Occurrence of insecticide resistance in field populations of *Spodoptera litura* (Lepidoptera: Noctuidae) in Pakistan. *Crop Protection*, 26(6), 809–817. <http://doi.org/10.1016/j.cropro.2006.07.006>
- Ahmad, M., Sayyed, A. H., Saleem, M. A., & Ahmad, M. (2008). Evidence for field evolved resistance to newer insecticides in *Spodoptera litura* (Lepidoptera: Noctuidae) from Pakistan. *Crop Protection*, 27(10), 1367–1372. <http://doi.org/10.1016/j.cropro.2008.05.003>
- Amer, A., & Mehlhorn, H. (2006). Repellency effect of forty-one essential oils against *Aedes*, *Anopheles*, and *Culex* mosquitoes. *Parasitology Research*, 99(4), 478–490. <http://doi.org/10.1007/s00436-006-0184-1>
- Ang, P. Y., Yajun, M. A., & Heng, S. Z. (2005). Adulticidal activity of five essential oils against *Culex pipiens quinquefasciatus*. *Journal of Pesticide Science*, 30(2), 84–89.
- Arivoli, S., & Tennyson, S. (2012). Antifeedant activity of plant extracts against *Spodoptera litura* (Fab.) (Lepidoptera: Noctuidae). *American-Eurasian Journal of Agricultural and Environmental Sciences*, 12(6), 764–768. <http://doi.org/10.5829/idosi.aejaes.2012.12.06.63178>
- Atkinson, N., & Brice, H. E. (1955). Antibacterial substances produced by flowering plants: 2. the antibacterial action of essential oils from some Australian plants. *Australian Journal of Experimental Biology and Medical Science*, 33(5), 547–554.
- Barchet, G. (2007). A brief overview of metabolomics: What it means, how it is measured, and its utilization. *The Science Creative Quarterly*, 8, 1–3.
- Baskar, K., & Ignacimuthu, S. (2012). Antifeedant, larvicidal and growth inhibitory effects of ononitol monohydrate isolated from *Cassia tora* L. against *Helicoverpa armigera* (Hub.) and *Spodoptera litura* (Fab.) (Lepidoptera: Noctuidae). *Chemosphere*, 88(4), 384–388. <http://doi.org/10.1016/j.chemosphere.2012.02.051>
- Baskar, K., Kingsley, S., Vendan, S. E., Paulraj, M. G., Duraipandiyan, V., & Ignacimuthu, S. (2009). Antifeedant, larvicidal and pupicidal activities of *Atalantia monophylla* (L) Correa against *Helicoverpa armigera* Hubner

- (Lepidoptera : Noctuidae). *Chemosphere*, 75(3), 355–359.
<http://doi.org/10.1016/j.chemosphere.2008.12.034>
- Baskar, K., Maheshwaran, R., Kingsley, S., & Ignacimuthu, S. (2011). Bioefficacy of plant extracts against Asian army worm *Spodoptera litura* Fab. (Lepidoptera : Noctuidae). *Journal of Agricultural Technology*, 7(1), 123–131.
- Baumwollwurm, A., & Baumwollwurm, A. (1996). *EPPO data sheets on quarantine pests: Spodoptera littoralis and Spodoptera Litura*. European Plant Pest Organization: Wallingford, UK.
- Biffin, E., Lucas, E. J., Craven, L. A., Da Costa, I. R., Harrington, M. G., & Crisp, M. D. (2010). Evolution of exceptional species richness among lineages of fleshy-fruited Myrtaceae. *Annals of Botany*, 106(1), 79–93.
<http://doi.org/10.1093/aob/mcq088>
- Birch, A. J. (1951). 670. β -Triketones. Part I. The structures of angustione, dehydroangustione, calythrone, and flavaspidic acid. *Journal of the Chemical Society*, 3026–3030. <http://doi.org/10.1039/JR9510003026>
- Bohlmann, J., & Keeling, C. I. (2008). Terpenoid biomaterials. *The Plant Journal*, 54, 656–669. <http://doi.org/10.1111/j.1365-313X.2008.03449.x>
- Brophy, J. J., Clarkson, J. R., & Fookes, C. J. R. (1989). Angustifolenone, a ketone from *Backhousia angustifolia*. *Phytochemistry*, 28(4), 1259–1261.
- Brophy, J. J., Goldsack, R. J., Fookes, C. J. R., & Forster, P. I. (1995). Leaf oils of the Genus *Backhousia* (Myrtaceae). *Journal of Essential Oil Research*, 7(3), 237–254. <http://doi.org/10.1080/10412905.1995.9698514>
- Burke, B. E., Baillie, J. E., & Olson, R. D. (2004). Essential oil of Australian lemon myrtle (*Backhousia citriodora*) in the treatment of molluscum contagiosum in children. *Biomedicine and Pharmacotherapy*, 58(4), 245–247.
<http://doi.org/10.1016/j.biopha.2003.11.006>
- Cahn, R. S., Gibson, S. C., Penfold, A. R., & Simonsen, J. L. (1931). XXXVIII.—The essential oil of *Backhousia angustifolia*. Part III. The constitutions of angustione and dehydroangustione. *Journal of the Chemical Society*, 286–294. <http://doi.org/10.1039/JR9310000286>
- Chan, E. W. C., Lim, Y. Y., Chong, K. L., Tan, J. B. L., & Wong, S. K. (2010). Antioxidant properties of tropical and temperate herbal teas. *Journal of Food Composition and Analysis*, 23(2), 185–189.
<http://doi.org/10.1016/j.jfca.2009.10.002>
- Claudio, L., Barbosa, A., Silva, C. J., Teixeira, R. R., Maria, R., & Alves, S. (2013). Chemistry and biological activities of essential oils from *Melaleuca L.* Species. *Agriculturae Conspectus Scientificus (ACS)*, 78(1), 11–23.
- Cock., I. (2013). Antimicrobial activity of *Backhousia citriodora* (lemon myrtle) methanolic extracts. *Pharmacognosy Communications*, 3(2), 58–63.
<http://doi.org/10.5530/pc.2013.2.12>

- Cozzolino, D., Cynkar, W. U., Shah, N., & Smith, P. (2011). Multivariate data analysis applied to spectroscopy: Potential application to juice and fruit quality. *Food Research International*, 44(7), 1888–1896. <http://doi.org/10.1016/j.foodres.2011.01.041>
- Dadang, & Ohsawa, K. (2001). Efficacy of plant extracts for reducing larval populations of the diamondback moth, *Plutella xylostella* L. (Lepidoptera: Yponomeutidae) and cabbage webworm, *Crociodolomia binotalis* Zeller (Lepidoptera: Pyralidae), and evaluation of cabbage damage. *Applied Entomology and Zoology*, 36(1), 143–149. <http://doi.org/10.1303/aez.2001.143>
- de Oliveira, J. L., Campos, E. V. R., Bakshi, M., Abhilash, P. C., & Fraceto, L. F. (2014). Application of nanotechnology for the encapsulation of botanical insecticides for sustainable agriculture: Prospects and promises. *Biotechnology Advances*, 32(8), 1550–1561. <http://doi.org/10.1016/j.biotechadv.2014.10.010>
- Deng, X., Liao, Q., Xu, X., Yao, M., Zhou, Y., Lin, M., ... Xie, Z. (2014). Analysis of essential oils from *cassia* bark and *cassia* twig samples by GC-MS combined with multivariate data analysis. *Food Analytical Methods*, 7(9), 1840–1847. <http://doi.org/10.1007/s12161-014-9821-y>
- Dettmer, K., Aronov, P. A., & Hammock, B. D. (2007). Mass spectrometry-based metabolomics. *Wiley InterScience*, 26(12), 51–78. <http://doi.org/10.1002/mas>
- Do, T. K. T., Hadji-Minaglou, F., Antoniotti, S., & Fernandez, X. (2014). Secondary metabolites isolation in natural products chemistry: Comparison of two semipreparative chromatographic techniques (high pressure liquid chromatography and high performance thin-layer chromatography). *Journal of Chromatography A*, 1325, 256–260. <http://doi.org/10.1016/j.chroma.2013.11.046>
- Dubey, N. K., Shukla, R., Kumar, A., Singh, P., & Prakash, B. (2010). Prospects of botanical pesticides in sustainable agriculture. *Current Science*, 98(4), 479–480.
- Dupont, S., Caffin, N., Bhandari, B., & Dykes, G. A. (2006). *In vitro* antibacterial activity of Australian native herb extracts against food-related bacteria. *Food Control*, 17(11), 929–932. <http://doi.org/10.1016/j.foodcont.2005.06.005>
- Duraipandiyar, V., Ignacimuthu, S., & Paulraj, M. G. (2011). Antifeedant and larvicidal activities of Rhein isolated from the flowers of *Cassia fistula* L. *Saudi Journal of Biological Sciences*, 18(2), 129–133. <http://doi.org/10.1016/j.sjbs.2010.12.009>
- Ebadollahi, A. (2013). Essential oils isolated from Myrtaceae family as natural insecticides. *Annual Review and Research in Biology*, 3(3), 148–175.
- El-Wakeil, N. E. (2013). Botanical pesticides and their mode of action. *Gesunde Pflanzen*, 65(4), 125–149. <http://doi.org/10.1007/s10343-013-0308-3>
- Fagoonee, I. (1980). The life-cycle, bionomics and control of the cabbage webworm *Crociodolomia binotalis* Zell. (Lepidoptera, Pyralidae). *Revue Agricole et*

Sucriere de l'Île Maurice, 59(2), 57–62.

- Fagoonee, I., & Lauge, G. (1981). Noxious effects of neem extracts *Crocidolomia binotalis*. *Phytoparasitica*, 9(2), 111–118.
- Fiehn, O. (2002). Metabolomics - the link between genotypes and phenotypes. *Plant Molecular Biology*, 48, 155–171.
- Fukusaki, E., & Kobayashi, A. (2005). Plant metabolomics: potential for practical operation. *Journal of Bioscience and Bioengineering*, 100(4), 347–354. <http://doi.org/10.1263/jbb.100.347>
- Gahukar, R. T. (2014). Factors affecting content and bioefficacy of neem (*Azadirachta indica* A. Juss.) phytochemicals used in agricultural pest control: A review. *Crop Protection*, 62, 93–99. <http://doi.org/10.1016/j.cropro.2014.04.014>
- Gibson, C. S., Penfold, A. R., & Simonsen, J. L. (1930). CXLIX.—The essential oil of *Backhousia angustifolia*. Part II. The isolation of naturally occurring β -diketones: angustione and dehydroangustione. *Journal of the Chemical Society*, 1184–1201. <http://doi.org/10.1039/JR9300001184>
- Greive, K. A., Staton, J. A., Miller, P. F., Peters, B. A., & Oppenheim, V. M. J. (2010). Development of *Melaleuca* oils as effective natural-based personal insect repellents. *Australian Journal of Entomology*, 49(1), 40–48. <http://doi.org/10.1111/j.1440-6055.2009.00736.x>
- Guo, Y., Sakulnarmrat, K., & Konczak, I. (2014). Anti-inflammatory potential of native Australian herbs polyphenols. *Toxicology Reports*, 1, 385–390. <http://doi.org/10.1016/j.toxrep.2014.06.011>
- Hall, R. D. (2006). Plant metabolomics: From holistic hope, to hype, to hot topic. *New Phytologist*, 169(3), 453–468. <http://doi.org/10.1111/j.1469-8137.2005.01632.x>
- Hayes, A., & Markovic, B. (2002). Toxicity of Australian essential oil *Backhousia citriodora* (lemon myrtle). Part 1. Antimicrobial activity and *in vitro* cytotoxicity. *Food and Chemical Toxicology*, 40(4), 535–543. [http://doi.org/10.1016/S0278-6915\(01\)00103-X](http://doi.org/10.1016/S0278-6915(01)00103-X)
- Huang, S., & Han, Z. (2007). Mechanisms for multiple resistances in field populations of common cutworm, *Spodoptera litura* (Fabricius) in China. *Pesticide Biochemistry and Physiology*, 87(1), 14–22. <http://doi.org/10.1016/j.pestbp.2006.05.002>
- Ibanez, S., Gallet, C., & Després, L. (2012). Plant insecticidal toxins in ecological networks. *Toxins*, 4(4), 228–243. <http://doi.org/10.3390/toxins4040228>
- Isman, M. B. (2008). Perspective Botanical Insecticide: for richer, for poorer. *Pest Management Science*, 64(1), 8–11.
- Isman, M. B., & Akhtar, Y. (2007). Plant natural products as a source for developing environmentally acceptable insecticides. *Insecticides Design Using Advanced Technologies*, 235–248. http://doi.org/10.1007/978-3-540-46907-0_10

- Isman, M. B., & Grieneisen, M. L. (2014). Botanical insecticide research: Many publications, limited useful data. *Trends in Plant Science*, 19(3), 140–145. <http://doi.org/10.1016/j.tplants.2013.11.005>
- Isman, M. B., Miresmaili, S., & MacHial, C. (2011). Commercial opportunities for pesticides based on plant essential oils in agriculture, industry and consumer products. *Phytochemistry Reviews*, 10(2), 197–204. <http://doi.org/10.1007/s11101-010-9170-4>
- Isman, M. B., Wilson, J. A., & Bradbury, R. (2008). Insecticidal activities of commercial rosemary oils (*Rosmarinus officinalis*) against larvae of *Pseudaletia unipuncta* and *Trichoplusia ni* in relation to their chemical compositions. *Pharmaceutical Biology*, 46(1-2), 82–87. <http://doi.org/10.1080/13880200701734661>
- Jeyasankar, A., Raja, N., & Ignacimuthu, S. (2011). Insecticidal compound isolated from *Syzygium lineare* Wall. (Myrtaceae) against *Spodoptera litura* (Lepidoptera: Noctuidae). *Saudi Journal of Biological Sciences*, 18(4), 329–332. <http://doi.org/10.1016/j.sjbs.2011.01.003>
- Jones, O. A. H., & Cheung, V. L. (2007). An introduction to metabolomics and its potential application in veterinary science. *Comparative Medicine*, 57(5), 436–442.
- Kaneria, M., & Chanda, S. (2011). Phytochemical and pharmacognostic evaluation of leaves of *Psidium guajava* L. (Myrtaceae). *Pharmacognosy Journal*, 3(23), 41–45. <http://doi.org/10.5530/pj.2011.23.6>
- Katajamaa, M., & Orešič, M. (2007). Data processing for mass spectrometry-based metabolomics. *Journal of Chromatography A*, 1158(1-2), 318–328. <http://doi.org/10.1016/j.chroma.2007.04.021>
- Kaur, T., Vasudev, A., Sohal, S. K., & Manhas, R. K. (2014). Insecticidal and growth inhibitory potential of *Streptomyces hydrogenans* DH16 on major pest of India, *Spodoptera litura* (Fab.) (Lepidoptera: Noctuidae). *BMC Microbiology*, 14(1), 1–9. <http://doi.org/10.1186/s12866-014-0227-1>
- Kean, O. B., Yusoff, N., Ali, N. A. M., Subramaniam, V., & Yee, S. K. (2013). Chemical composition and antioxidant properties of *Backhousia citriodora* volatile oil. In *The Open Conference Proceedings Journal* (Vol. 4, No. 1).
- Koek, M. M., van der Kloet, F. M., Kleemann, R., Kooistra, T., Verheij, E. R., & Hankemeier, T. (2011). Semi-automated non-target processing in GC/GC-MS metabolomics analysis: Applicability for biomedical studies. *Metabolomics*, 7(1), 1–14. <http://doi.org/10.1007/s11306-010-0219-6>
- Konczak, I., Zabarás, D., Dunstan, M., & Aguas, P. (2010). Antioxidant capacity and phenolic compounds in commercially grown native Australian herbs and spices. *Food Chemistry*, 122(1), 260–266. <http://doi.org/10.1016/j.foodchem.2010.03.004>
- Kostic, M., Popovic, Z., Brkic, D., Milanovic, S., Sivcev, I., & Stankovic, S. (2008). Larvicidal and antifeedant activity of some plant-derived compounds to *Lymantria dispar* L. (Lepidoptera: Limantriidae). *Bioresource Technology*,

99(16), 7897–7901. <http://doi.org/10.1016/j.biortech.2008.02.010>

- Kurekci, C., Padmanabha, J., Bishop-Hurley, S. L., Hassan, E., Al Jassim, R. A. M., & McSweeney, C. S. (2013). Antimicrobial activity of essential oils and five terpenoid compounds against *Campylobacter jejuni* in pure and mixed culture experiments. *International Journal of Food Microbiology*, 166(3), 450–457. <http://doi.org/10.1016/j.ijfoodmicro.2013.08.014>
- Lei, Z., Huhman, D. V., & Sumner, L. W. (2011). Mass spectrometry strategies in metabolomics. *Journal of Biological Chemistry*, 286(29), 25435–25442. <http://doi.org/10.1074/jbc.R111.238691>
- Lim, G. S., Sivapragasam, A., & Loke, W. H. (1997). Crucifer insect pest problems: trends, issues and management strategies. In *The management of diamondback moth and other crucifer pests*. MARDI, Malaysia.
- Lingathurai, S., Vendan, S. E., Paulraj, M. G., & Ignacimuthu, S. (2011). Antifeedant and larvicidal activities of *Acalypha fruticosa* Forssk. (Euphorbiaceae) against *Plutella xylostella* L. (Lepidoptera : Yponomeutidae) larvae. *Journal of King Saud University - Science*, 23(1), 11–16. <http://doi.org/10.1016/j.jksus.2010.05.012>
- Lv, M. Y., Sun, J. B., Wang, M., Fan, H. Y., Zhang, Z. J., & Xu, F. G. (2016). Comparative analysis of volatile oils in the stems and roots of *Ephedra sinica* via GC-MS-based plant metabolomics. *Chinese Journal of Natural Medicines*, 14(2), 0133–0140. [http://doi.org/http://dx.doi.org/10.1016/S1875-5364\(16\)60006-7](http://doi.org/http://dx.doi.org/10.1016/S1875-5364(16)60006-7)
- Maham, M., Akbari, H., & Delazar, A. (2013). Chemical composition and antinociceptive effect of the essential oil of *Dracocephalum moldavica* L. *Pharmaceutical Sciences*, 18(4), 187–192.
- McChesney, J. D., & Rodenburg, D. L. (2014). Preparative chromatography and natural products discovery. *Current Opinion in Biotechnology*, 25, 111–113. <http://doi.org/10.1016/j.copbio.2013.11.002>
- Moco, S., Vervoort, J., Moco, S., Bino, R. J., De Vos, R. C. H., & Bino, R. (2007). Metabolomics technologies and metabolite identification. *TrAC - Trends in Analytical Chemistry*, 26(9), 855–866. <http://doi.org/10.1016/j.trac.2007.08.003>
- Moorthy, P. N. K., & Kumar, N. K. K. (2000). Efficacy of neem seed kernel powder extracts on cabbage pests. *Pest Management in Horticultural Ecosystems*, 6(1), 27–31.
- Ojebode, M. E., Olaiya, C. O., Adegbite, A. E., Karigidi, K. O., & Ale, T. O. (2016). Efficacy of some plant extracts as storage protectants against *Callosobruchus maculatus*. *Journal of Biotechnology & Biomaterials*, 6(1), 1–4. <http://doi.org/10.4172/2155-952X.1000217>
- Ooi, P. A. C., & Kelderman, W. (1979). The biology of three common pests of cabbages in Cameron Highlands, Malaysia. *Malaysian Journal of Agriculture*, 52(1), 85–101.

- Oyedele, A. O., Gbolade, A. A., Sosan, M. B., Adewoyin, F. B., Soyelu, O. L., & O, O. (2002). Formulation of an effective mosquito-repellent topical product from Lemongrass oil. *Phytomedicine*, 9(3), 259–262.
- Pasikanti, K. K., Ho, P. C., & Chan, E. C. Y. (2008). Gas chromatography/mass spectrometry in metabolic profiling of biological fluids. *Journal of Chromatography B: Analytical Technologies in the Biomedical and Life Sciences*, 871(2), 202–211. <http://doi.org/10.1016/j.jchromb.2008.04.033>
- Pavia, D. L., Lampman, G. M., Kriz, G. S., & Vyvyan, J. R. (2001). *Spectroscopy* (4th ed.). Carlifonia: Cengage Learning.
- Pavunraj, M., Muthu, C., Ignacimuthu, S., Janarthanam, S., Duraipandiyam, V., Raja, N., & Vimalraj, S. (2011). Antifeedant activity of a novel 6-(4,7-hydroxyheptyl) quinone from the leaves of the milkweed *Pergularia daemia* on the cotton bollworm *Helicoverpa armigera* (Hub.) and the tobacco armyworm *Spodoptera litura* (Fab.). *Phytoparasitica*, 39(2), 145–150. <http://doi.org/10.1007/s12600-010-0141-5>
- Peter, C., Iqbal, S., ChannaBasavanna, G. P., Suman, C. L., & Krishnaiah, K. (1988). Loss estimation in cabbage due to leaf webber *Crociodolomia binotalis* (Lepidoptera: Pyralidae). *Journal of the Bombay Natural History Society*, 85(3), 642–644.
- Potts, K. T., & Roy, S. K. (1965). Triterpenoid Constituents of *Backhousia angustifolia* F. Muell. *Australian Journal of Chemistry*, 18(5), 767–768.
- Rahman, A., Choudhary, M. I., & Thomson, W. (2005). *Bioassay techniques for drug development*. Taylor & Francis.
- Rajalahti, T., & Kvalheim, O. M. (2011). Multivariate data analysis in pharmaceuticals: A tutorial review. *International Journal of Pharmaceutics*, 417(1-2), 280–290. <http://doi.org/10.1016/j.ijpharm.2011.02.019>
- Rattan, R. S. (2010). Mechanism of action of insecticidal secondary metabolites of plant origin. *Crop Protection*, 29(9), 913–920. <http://doi.org/10.1016/j.cropro.2010.05.008>
- Raut, J. S., & Karuppayil, S. M. (2014). A status review on the medicinal properties of essential oils. *Industrial Crops & Products*, 62, 250–264. <http://doi.org/10.1016/j.indcrop.2014.05.055>
- Reddy, G. V. (2011). Comparative effect of integrated pest management and farmers' standard pest control practice for managing insect pests on cabbage (*Brassica* spp.). *Pest Management Science*, 67(8), 980–985. <http://doi.org/10.1002/ps.2142>
- Reynertson, K. A., Yang, H., Jiang, B., Basile, M. J., & Kennelly, E. J. (2008). Quantitative analysis of antiradical phenolic constituents from fourteen edible Myrtaceae fruits. *Food Chemistry*, 109(4), 883–890. <http://doi.org/10.1016/j.foodchem.2008.01.021>
- Richardson, M. (2009). Principal component analysis. <http://centaur.reading.ac.uk/5209/>. Retrieved 20 April 2016.

- Ringnér, M. (2008). What is principal component analysis? *Nature Biotechnology*, 26(3), 303–304.
- Rupasinghe, E. J. R., Jones, A., Shalliker, R. A., & Cekic, S. P. (2016). A rapid screening analysis of antioxidant compounds in native Australian food plants using multiplexed detection with active flow technology columns. *Molecules*, 21(118), 1–14. <http://doi.org/10.3390/molecules21010118>
- Sakulnarmrat, K., Fenech, M., Thomas, P., & Konczak, I. (2013). Cytoprotective and pro-apoptotic activities of native Australian herbs polyphenolic-rich extracts. *Food Chemistry*, 136(1), 9–17. <http://doi.org/10.1016/j.foodchem.2012.07.089>
- Sakulnarmrat, K., & Konczak, I. (2012). Composition of native Australian herbs polyphenolic-rich fractions and *in vitro* inhibitory activities against key enzymes relevant to metabolic syndrome. *Food Chemistry*, 134(2), 1011–1019. <http://doi.org/10.1016/j.foodchem.2012.02.217>
- Sasidharan, S., Chen, Y., Saravanan, D., Sundram, K. M., & Yoga Latha, L. (2011). Extraction, isolation and characterization of bioactive compounds from plants' extracts. *African Journal of Traditional, Complementary and Alternative Medicines*, 8(1), 1–10. <http://doi.org/10.4314/ajtcam.v8i1.60483>
- Schneider, R. (2011). Why drink lemon myrtle tea? http://www.naturaltherapypages.com.au/article/why_drink_lemon_myrtle_tea. Retrieved 15 May 2016.
- Sforcin, J. M., Amaral, J. T., A. F. J., Sousa, J. P. B., & Bastos, J. K. (2009). Lemongrass effects on IL-1 β and IL-6 production by macrophages. *Natural Product Research*, 12(15), 1151–1159. <http://doi.org/10.1080/14786410902800681>
- Shlens, J. (2014). A tutorial on principal component analysis. *ArXiv*, 1–13. <http://doi.org/10.1.1.115.3503>
- Tholl, D. (2015). Biosynthesis and biological functions of terpenoids in plants. *Advances in Biochemical Engineering Biotechnology*, 148, 63–106. http://doi.org/10.1007/10_2014_295
- Thornhill, A. H., Popple, L. W., Carter, R. J., Ho, S. Y. W., & Crisp, M. D. (2012). Are pollen fossils useful for calibrating relaxed molecular clock dating of phylogenies? A comparative study using Myrtaceae. *Molecular Phylogenetics and Evolution*, 63(1), 15–27. <http://doi.org/10.1016/j.ympev.2011.12.003>
- Tian, J., Shi, C., Gao, P., Yuan, K., Yang, D., Lu, X., & Xu, G. (2008). Phenotype differentiation of three *E. coli* strains by GC-FID and GC-MS based metabolomics. *Journal of Chromatography B*, 871(2), 220–226. <http://doi.org/10.1016/j.jchromb.2008.06.031>
- Tong, H., Su, Q., Zhou, X., & Bai, L. (2013). Field resistance of *Spodoptera litura* (Lepidoptera: Noctuidae) to organophosphates, pyrethroids, carbamates and four newer chemistry insecticides in Hunan, China. *Journal of Pest Science*, 86(3), 599–609. <http://doi.org/10.1007/s10340-013-0505-y>

- Ueese, A., Ridland, P. M., Stouthamer, R., He, Y. rong, Ang, G., Zalucki, M. P., & Furlong, M. J. (2014). *Trichogramma chilonis* Ishii: A potential biological control agent of *Crocidolomia pavonana* in Samoa. *Biological Control*, 73, 31–38. <http://doi.org/10.1016/j.biocontrol.2014.03.011>
- Venkataramani, M., & Chinnagounder, S. (2012). Preliminary phytochemical screening and GC-MS profiling of *Hiptage benghalensis* (L.) kurz, 5(5), 2895–2899.
- Wilkinson, J. M., Hipwell, M., Ryan, T., & Cavanagh, H. M. A. (2003). Bioactivity of *Backhousia citriodora*: antibacterial and antifungal activity. *Journal of Agricultural and Food Chemistry*, 51(1), 76–81. <http://doi.org/10.1021/jf0258003>
- Xu F., Zou, L., & Ong, C. N. (2010). Experiment-originated variations, and multi-peak and multi-origination phenomena in derivatization-based GC-MS metabolomics. *Trends in Analytical Chemistry*, 29(3), 269-280.
- Ye, Y., Li, X. Q., Tang, C. P., & Yao, S. (2013). Natural products chemistry research: progress in China in 2011. *Chinese Journal of Natural Medicines*, 11(2), 97–109. [http://doi.org/10.1016/S1875-5364\(13\)60036-9](http://doi.org/10.1016/S1875-5364(13)60036-9)
- Yuliana, N. D., Verpoorte, R., & Choi, Y. H. (2011). Metabolomics : A new bioactivity screening method for plants, adenosine A1 receptor binding compounds in *Orthosiphon stamineus* Benth. *Analytical Chemistry*, 83(17), 6902–6906.
- Zandi, S. N., Hojjati, M., & Carbonell-Barrachina, Á. A. (2013). Insecticidal and repellent activities of the essential oil of *Callistemon citrinus* (Myrtaceae) against *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae). *Neotropical Entomology*, 42(1), 89–94. <http://doi.org/10.1007/s13744-012-0087-z>

BIODATA OF STUDENT

The student was born in Kaduna state, Nigeria. She had her elementary education in her hometown and obtained a Bachelor degree in Chemistry at Ahmadu Bello University, Zaria, Nigeria. In 2014, she enrolled in a Master program at the Chemistry Department of Universiti Putra Malaysia, under the Natural Products Field. She has a passion for natural products discovery and hopes to further in this field by Allah's grace.





UNIVERSITI PUTRA MALAYSIA

STATUS CONFIRMATION FOR THESIS / PROJECT REPORT AND COPYRIGHT

ACADEMIC SESSION : _____

TITLE OF THESIS / PROJECT REPORT :

IDENTIFICATION OF MALAYSIAN LEMON MYRTLE (*Backhousia citriodora* F. MUELL)

CHEMICAL CONSTITUENTS RESPONSIBLE FOR INSECTICIDAL ACTIVITY USING GC-MS-BASED METABOLOMICS

NAME OF STUDENT: JAMILA GARBA

I acknowledge that the copyright and other intellectual property in the thesis/project report belonged to Universiti Putra Malaysia and I agree to allow this thesis/project report to be placed at the library under the following terms:

1. This thesis/project report is the property of Universiti Putra Malaysia.
2. The library of Universiti Putra Malaysia has the right to make copies for educational purposes only.
3. The library of Universiti Putra Malaysia is allowed to make copies of this thesis for academic exchange.

I declare that this thesis is classified as :

*Please tick (v)

CONFIDENTIAL

(Contain confidential information under Official Secret Act 1972).

RESTRICTED

(Contains restricted information as specified by the organization/institution where research was done).

OPEN ACCESS

I agree that my thesis/project report to be published as hard copy or online open access.

This thesis is submitted for :

PATENT

Embargo from _____ until _____
(date) (date)

Approved by:

(Signature of Student)
New IC No/ Passport No.:

Date :

(Signature of Chairman of Supervisory Committee)
Name:

Date :

[Note : If the thesis is CONFIDENTIAL or RESTRICTED, please attach with the letter from the organization/institution with period and reasons for confidentially or restricted.]