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

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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

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

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September 2016

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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 *Crocidolomia 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 (LC_{50}) 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 LC_{50} 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

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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 *Crocidolomia 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 (LC_{50}) 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 (LC_{50}) 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.

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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.

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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.

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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
d	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
J	Coupling constant
LC	Lethal Concentration
LVCD	Larvicidal
m	Multiplet
m/z	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
s	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), *Crocidolomia 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 *Crocidolomia 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.

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