THE ESSENTIAL OILS AND ISOLATED CONSTITUENTS OF *Neolitsea kedahense*, *Neolitsea coccinea* AND *Neolitsea zeylanica* AND THEIR BIOACTIVITIES

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A thesis submitted in fulfilment of the
requirements for the award of the degree of
Doctor of Philosophy

Faculty of Science
Universiti Teknologi Malaysia

DECEMBER 2017

This work I dedicate to:

My father
Jani Omar

My mother
Khtijah Giman

My siblings
Syed Mahadir
Norhanizah
Muhammad Jaafarudin
Nurhamna
Nor Syafawati
Nur Hidayu

ACKNOWLEDGEMENT

All praises to Allah the Almighty for giving me strength and His blessing in completing this research project. Special appreciation goes to my supervisor, Prof. Dr. Hasnah Mohd Sirat for her knowledge, guidance, motivation and patience throughout this research. I would also like to thank my co-supervisor, Prof. Dr. Farediah Ahmad for her support and suggestion.

My acknowledgement also goes to all lecturers and staffs of Department of Chemistry, UTM especially Dr. Shajarahtunnur Jamil, Dr. Norazah Basar, Mr. Azmi, Mr. Rasyidi and Mr. Subre for their valuable assistance and cooperation. A special thank also goes to Dr. Nor Azah Mohamad Ali, Mrs. Mailina Jamil and Mr. Muhd Hafizi Zainal for their assistance during my attachment period at Forest Research Institute of Malaysia (FRIM), Kepong. In addition, I would also like to acknowledge the Department of Chemistry, UTM for facilities and the Ministry of Higher Education (MOHE) and Universiti Teknologi MARA (UiTM) for my doctoral scholarship.

Sincere thanks to my fellow colleagues, Dr. Nurul Iman, Dr. Salam, Dr. Nuzul, Dr. Ernieyanti, Mrs. Athirah, Ms. Iryani, Mrs. Zafneza, Mrs. Awanis, Mrs. Atiqah, Mrs. Edelin and Mr. Saidu for their friendship and moral support. Last but not the least, my deepest gratitude goes to my parents, siblings and my friends for their prayers and encouragement during my study.

ABSTRACT

Three Malaysian species of *Neolitsea*, *N. kedahense* Gamble, *N. coccinea* B. C. Stone, and *N. zeylanica* (Nees) Merr. have been investigated. The aims of this study were to analyse the chemical composition of the essential oils, isolate and identify the pure compounds, and screen the antibacterial, α-glucosidase inhibitory and antioxidant activities. The essential oils were extracted by hydrodistillation and analysed by capillary gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS). The pure compounds were isolated by sequential maceration followed by fractionation and purification using several chromatographic techniques. The structures of the pure compounds were elucidated by spectroscopic methods. δ-Cadinene (17.4%) was the main compound of the stem oil of *N. kedahense*, while β-caryophyllene (18.9%) and bicyclogermacrene (18.6%) were the major compounds of the leaf oil. The main compounds in the stem and leaf oils of *N. coccinea* were δ-cadinene (21.2%) and selin-11-en-4-α-ol (26.8%), respectively. The bark and the heartwood oils of *N. zeylanica* were found to be rich in α-cadinol (15.7%) and τ-cadinol (21.4%), respectively, while its leaf oil was dominated by β-caryophyllene (38.4%). Twenty nine compounds, comprising of twelve sesquiterpenoids, six triterpenes, six flavonoids and five alkaloids were isolated from three *Neolitsea* species. Two new sesquiterpenoids, kedahensane and coccinine, together with another three new alkaloids, kedahensines A-C, were successfully identified. Kedahensane, kedahensines A and B, taraxerone, taraxerol, pseudoneoliacine, daibucarboline A and linderaggrine A were obtained from the stem extract of *N. kedahense*. Purification of the stem and leaf extracts of *N. coccinea* afforded coccinine, kedahensine C, linderane, linderalactone, pseudoneolinderane, linderanlide C, linderanine A, epicatechin, (-)-taxifolin, astilbin, L-quercitrin and afzelin. Isolation of compounds from the bark and leaf extracts of *N. zeylanica* yielded zeylanidine, zeylanine, lupenone, epifriedelanol, lupeol, 29-norlupan-3,20-dione, genkwanin, zeylaninone, acutotrinol, afzelin, astilbin and L-quercitrin. The leaf oil of *N. coccinea* demonstrated moderate antibacterial activity against *Bacillus subtilis* with MIC value of 250 μg/mL. The stem oil of *N. kedahense* (IC_{50} 16.76 μg/mL), linderalactone (IC_{50} 12.72 μM) and linderanlide C (IC_{50} 12.10 μM) showed the strongest activity against α-glucosidase enzyme. The ethyl acetate extract of the stem of *N. coccinea* showed the highest total phenolic content at 501.42 mg GAE/g and also displayed good DPPH radical scavenging activity (IC_{50} 21.11 μg/mL). The presence of the new compounds in *N. kedahense* and *N. coccinea* could be used as chemotaxonomic markers for classification of the *Neolitsea* species.

ABSTRAK

Tiga spesies *Neolitsea* Malaysia, *N. kedahense* Gamble, *N. coccinea* B. C. Stone, dan *N. zeylanica* (Nees) Merr. telah dikaji. Tujuan kajian ini ialah untuk menganalisis komposisi kimia minyak pati, mengasingkan dan mengenalpasti sebatian tulen, dan menyaring aktiviti antibakteria, perencatan α-glukosidase, dan antioksidan. Minyak pati diekstrak menggunakan penyulingan hidro dan dianalisis menggunakan kromatgrafi gas (GC) kapilari dan kromatografi gas-spektrometri jisim (GC-MS). Sebatian tulen telah dipisahkan secara rendaman berturutan diikuti dengan pemeringkatan dan penulenan menggunakan pelbagai teknik kromatografi. Struktur sebatian tulen ditentukan dengan kaedah spektroskopi. δ-Kadinena (17.4%) merupakan sebatian utama minyak batang *N. kedahense*, manakala β-kariofilena (18.9%) dan bisiklogermakrena (18.6%) merupakan sebatian utama minyak daun. Sebatian utama minyak batang dan minyak daun *N. coccinea* masing-masing adalah δ-kadinena (21.2%) dan selin-11-en-4-α-ol (26.8%). Minyak kulit batang dan minyak teras kayu *N. zeylanica* masing-masing didapati kaya dengan α-kadinol (15.7%) dan τ-kadinol (21.4%), manakala minyak daunnya didominasi oleh β-kariofilena (38.4%). Dua puluh sembilan sebatian, terdiri daripada dua belas seskuiterpenoid, enam triterpena, enam flavonoid dan lima alkaloid telah diasingkan daripada tiga spesies *Neolitsea*. Dua seskuiterpenoid baharu, kedahensana dan kokinina, bersama-sama dengan tiga alkaloid baharu, kedahensina A-C, telah berjaya dikenalpasti. Kedahensana, kedahensina A dan B, tarakseron, tarakserol, pseudoneoliasina, daibukarbolina A dan linderaggrina A telah diperolehi daripada ekstrak batang *N. kedahense*. Penulenan ekstrak batang dan daun *N. coccinea* menghasilkan kokinina, kedahensina C, linderana, linderalakton, pseudoneolinderana, linderanlida C, linderanina A, epikatekin, (-)-taxifolin, astilbin, L-kuersitrin dan afzelin. Pengasingan sebatian daripada ekstrak kulit batang dan daun *N. zeylanica* menghasilkan zeylanidina, zeylanina, lupenon, epifriedelanol, lupeol, 29-norlupan-3,20-dion, genkwanin, zeylaninon, akutotrinol, afzelin, astilbin dan L-kuersitrin. Minyak daun *N. coccinea* mempamerkan aktiviti antibakteria yang sederhana terhadap *Bacillus subtilis* dengan nilai MIC 250 μg/mL. Minyak batang *N. kedahense* (IC_{50} 16.76 μg/mL), linderalakton (IC_{50} 12.72 μM) dan linderanlida C (IC_{50} 12.10 μM) menunjukkan aktiviti yang paling kuat terhadap enzim α-glukosidase. Ekstrak etil asetat batang *N. coccinea* menunjukkan jumlah kandungan fenolik yang tertinggi pada 501.42 mg GAE/g dan juga mempamerkan aktiviti perencatan radikal DPPH yang baik (IC_{50} 21.11 μg/mL). Kehadiran sebatian baharu dalam *N. kedahense* dan *N. coccinea* boleh digunakan sebagai penanda kemotaksonomi bagi pengelasan spesies *Neolitsea*.

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LIST OF ABBREVIATIONS

α	-	alpha
AA	-	Ascorbic Acid
Abs	-	Absorbance
$AlCl_3$	-	Aluminium trichloride
APCIMS	-	Atmospheric Pressure Chemical Ionization Mass Spectrometry
ATP	-	Adenosine triphosphate
ATR	-	Attenuated Total Reflection
β	-	beta
$BaCl_2.2H_2O$	-	Barium chloride dihydrate
BHT	-	Butylated hydroxytoluene
br	-	broad
^{13}C	-	Carbon-13
c	-	concentration
Calcd.	-	Calculated
CC	-	Column Chromatography
$CDCl_3$	-	Deuterated chloroform
CD_3COCD_3	-	Deuterated acetone
CD_3OD	-	Deuterated methanol
$(CD_3)_2SO$	-	Deuterated dimethyl sulfoxide
CFU	-	Colony Forming Unit
$CHCl_3$	-	Chloroform
cm	-	centimeter
cm^{-1}	-	per centimeter
CO_2	-	Carbon dioxide
COSY	-	Correlation Spectroscopy
δ_H	-	chemical shift for proton

δ_C	-	chemical shift for carbon
1D	-	1 Dimension
2D	-	2 Dimension
DAD	-	Diode Array Detector
d	-	doublet
dd	-	doublet of doublets
ddd	-	doublet of doublet of doublets
dddd	-	doublet of doublet of doublet of doublets
ddq	-	doublet of doublet of quartets
DEPT	-	Distortionless Enhancement by Polarization Transfer
DMSO	-	Dimethyl sulfoxide
D_2O	-	Deuterated water
DPPH	-	2,2-Diphenyl-1-picrylhydrazyl
dq	-	doublet of quartets
dt	-	doublet of triplets
EIMS	-	Electron Impact Mass Spectrometry
ESI	-	Electrospray Ionization
ESIMS	-	Electrospray Ionization Mass Spectrometry
Et_2O	-	Diethyl ether
EtOAc	-	Ethyl acetate
γ	-	gamma
g	-	gram
GC	-	Gas Chromatography
GC-MS	-	Gas Chromatography-Mass Spectrometry
h	-	Hour(s)
^1H	-	Proton
H_2O	-	Water
H_2SO_4	-	Sulphuric acid
HCl	-	Hydrochloric acid
HMBC	-	Heteronuclear Multiple Bond Correlation
HMQC	-	Homonuclear Multiple Bond Correlation
HPLC	-	High Performance Liquid Chromatography
HRAPCIMS	-	High Resolution Atmospheric Pressure Chemical

		Ionization Mass Spectrometry
HRESIMS	-	High Resolution Electrospray Ionization Mass Spectrometry
Hz	-	Hertz
IC_{50}	-	Inhibition Concentration at 50%
i.d	-	internal diameter
int.	-	intensity
IR	-	Infrared
J	-	coupling constant
KBr	-	Potassium bromide
kg	-	kilogram
λ	-	lambda
lit.	-	literature
m	-	multiplet
mp	-	melting point
m/z	-	mass to charge ratio
M^+	-	Molecular ion
M	-	Molar
m.a.s.l	-	meters above sea level
Me_2CO	-	Acetone
MeOH	-	Methanol
mg	-	milligram
$MgSO_4$	-	Magnesium sulphate
MHz	-	Megahertz
MIC	-	Minimum Inhibition Concentration
min	-	Minute (s)
mL	-	mililiter
mM	-	milimolar
mm	-	milimeter
MPa	-	Megapascal
MS	-	Mass Spectrometry
n	-	subsample size
NA	-	Nutrient Agar

NaCl	-	Sodium chloride
NADPH	-	Reduced nicotinamide adenine dinucleotide phosphate
NaOH	-	Sodium hydroxide
NB	-	Nutrient Broth
n-hex	-	*n*-Hexane
nm	-	nanometer
NMR	-	Nuclear Magnetic Resonance
NOESY	-	Nuclear Overhauser Effect Spectroscopy
O_2	-	Oxygen
p	-	*para*
PE	-	Petroleum ether
PI	-	Percentage inhibition
PNPG	-	*p*-Nitrophenyl-α-D-glucopyranoside
ppm	-	parts per million
PTLC	-	Preparative Thin Layer Chromatography
q	-	quartet
Ref	-	Reference
rel.	-	relative
R_f	-	Retention factor
RI	-	Retention Index
s	-	singlet
SD	-	Standard deviation
sec.	-	Second(s)
sh	-	shoulder
SiO_2	-	Silica gel
S_N1	-	Substitution, Nucleophilic, unimolecular
τ	-	tau
t	-	triplet
td	-	triplet of doublets
TLC	-	Thin Layer Chromatography
TPC	-	Total Phenolic Content
t_R	-	Retention time
µg	-	microgram

μL	-	microliter
μm	-	micrometer
μM	-	micromolar
UV	-	Ultraviolet
Vis	-	Visible
VLC	-	Vacuum Liquid Chromatography
W-M	-	Wagner-Meerwein

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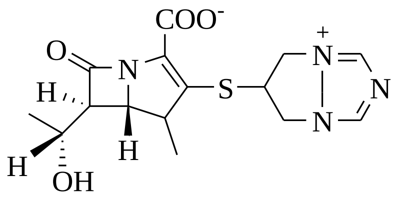
CHAPTER 1

INTRODUCTION

1.1 General Introduction

For thousands of years, natural products derived from animals, plants and microbes have been used as traditional medicines and natural poisons in treating human diseases [1]. Primary and secondary metabolites are two classes of natural products which are categorized based on their metabolism pathways and biological functions. Primary metabolites such as amino acids, proteins, nucleic acids, lipids and carbohydrates are compounds that are involved in the fundamental metabolic pathways. These compounds are utilized by the organisms and cells for their own growth and development [2].

In contrast, secondary metabolites are excreting compounds that do not involved in the process of building and maintaining living cells, but are useful for protection against predators and pathogens. Secondary metabolites include glycosides, alkaloids, volatile oils, flavonoids, phenolics and terpenoids. These secondary metabolites have attracted attention of many researchers due to their variety and unique structures as well as interesting bioactivities such as antibacterial, antifungal, antivirus and antitumor activities [2]. Biapenem **(1)**, nitisinone **(2)** and apomorphine **(3)** are several secondary metabolites derived from natural sources which have been applied as drugs in medicinal industry [3].



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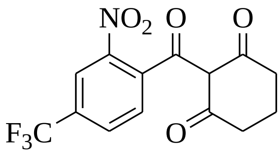

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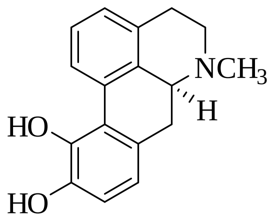

(3)

The occurrence of the secondary metabolites is often restricted to specific family and hence useful in chemotaxonomic classification. For instance, three subfamily of Apocynaceae i.e. Cerberoideae, Echitoideae and Plumerioideae can be differentiated by analysing their secondary metabolites, in which piperidine derivatives can only be detected in Cerberoideae, cardiac glycosides in Echitoideae and indole alkaloids in Plumerioideae. The presence of the secondary metabolites can give additional information to botanists to solve taxonomical problems especially in classification of the family and subfamily of plants [2].

1.2 Medicinal Plants

Plants are the major contributors of natural products and are extremely important as sources of medicinal agents and models for the design, synthesis, and semisynthesis of novel substances for treating human ailments [4]. The Chinese was the earliest people using medicinal herbs to heal diseases followed by the Egyptian [2]. Approximately, up to 50,000 to 70,000 plant species are used in traditional and modern medicinal systems throughout the world [5].

Globally, almost two third of population in the developing countries relies on medicinal plants and traditional practitioners for their primary healthcare, income generation and livelihood improvement [5]. At the same time, there is an increasing interest on plant medications especially herbs due to the assumption that the plant preparations will produce less incidence of adverse reactions compared to synthetic pharmaceuticals [6]. Several available synthetic drugs are toxic [7] and cause adverse effects such as allergic reactions, skin rashes, fever, hepatitis and gastrointestinal toxicity [8]. Secondary metabolites derived from plants are good candidates for drug development since they often perceived as showing more "drug-likeness and biological friendliness" than synthetic drugs [1]. A rapid increase has been observed in the number of global market for drug originated from plant in USA. According to the most recent report by BCC Research, the global plant-derived drug market was valued at US$ 22.1 billion in 2012 and sales are estimated to grow up to US$ 26.6 billion by 2017 at a compound annual growth rate (CAGR) of 3.7% [9].

Besides plant-natural drugs, plants essential oils or volatile oils also have been applied in medicinal and pharmaceutical industries. Their uses as aromatherapy products, traditional systems of medicines and in complementary systems of medicines are growing up steadily in USA, Europe, Africa as well as Asian countries including Malaysia. Anise seed (*Pimpinella anisum*), peppermint (*Mentha piperita*), clove (*Syzygium aromaticum*) and sweet basil (*Ocimum basilicum*) are examples of plant essential oils which are applied in medicinal applications [7].

Malaysia is one among other countries which have an extensive variety of plant species and traditional medical systems. Out of 20,000 species of angiosperms and 600 species of ferns in Malaysia, more than 1,082 species and 76 species, respectively, have been claimed to have medicinal properties [10]. In line with these findings, Malaysian herbal product market is experiencing a tremendous growth. The Ministry of the Natural Resources and Environment estimated the herbal local market to grow at a rate of 15% per annum from RM7 billion in 2010 to around RM29 billion by 2020 [11].

Examples of popular herbs which undergoing extensive research and given special attention under the first Entry Point Project (EPP1) for the nation's Agriculture National Key Economic Areas (NKEA) are tongkat ali (*Eurycoma longifolia*), kacip fatimah (*Labisia pumila*), misai kucing (*Orthosiphon stamineus*), hempedu bumi (*Andrographis paniculata*) and dukung anak (*Phyllanthus niruri*) [11]. These herbs are well known for their ability as traditional remedy and have potential to be commercialized as herbal based products in industrial applications. Apart from these herbs and more than 1,158 plants in Malaysia which possess medicinal values, over 19,000 of remaining plants [10] including *Neolitsea* species are not fully exploited and discovered by the researchers. It is believed that this genus also have potential to be used for medicinal purposes due to its uses in traditional health care systems.

1.3 Lauraceae Family

Lauraceae or Laurel, a large family of woody plants (except for the herbaceous parasite *Cassytha*) are distributed throughout tropical and subtropical latitudes mostly in American, Asian tropics, Australia and Madagascar [12]. The family consists of about 67 genera with more than 2500 species throughout the world [13]. A total of 16 genera and around 213 species of Lauraceae family are found in Peninsular Malaysia which occurred from lowland up to montane forests [14]. *Cinnamomun*, *Litsea*, *Lindera*, *Nothaphoebe* [15] and *Neolitsea* [16] are several genera found in Malaysia. The members of Lauraceae family known as Medang or Tejur by the Malays [17] are characterized by their spiral, alternate, opposite, sub-opposite, whorled, entire and leathery leaves as well as small, regular, greenish white or yellow, fragrant or rancid smell, bisexual or unisexual and united with six sepals in two rows flowers [18]. The classification of Lauraceae is unsettled and being investigated until today. The system of Kostermans is currently the one most widely employed for the family **(Table 1.1)**. According to the system, this family is comprises of two sub-families, five tribes and eight subtribes [19].

Several members of Lauraceae family are used as sources of medicine, timber, nutritious fruits, spices and perfumes. The essential oils of *Cinnamomum* species such as *C. camphora*, *C. glanduliferum* and *C. parthenoxylon* are used to make perfumes and medicines, while the bark of *C. cassia* and the root of *Lindera aggregata* are applied in traditional Chinese medicines [18]. In Asian countries, *C. zeylanicum* or locally known as cinnamon have been used traditionally as stomachic, carminative, diaphoretic, astringent, analgesic and antipyretic [20]. On the other hand, the leaf of *Laurus nobilis* has been used by European people to treat rheumatism and stomachic as well as antiseptic [21]. Some of Lauraceae species have been claimed to exhibit vary and interesting bioactivities such as anti-inflammatory, antimicrobial, antioxidant, antimite, antiplatelet aggregation and antitumour activities [22].

Table 1.1: Subfamilies, Tribes, Subtribes and Genus of the Lauraceae Family

Subfamily	Tribe	Subtribe	Genus
Lauroideae	Perseae	Perseineae	*Persea* *Machilus* *Notaphoebe* *Alseodaphne* *Phoebe*
		Beilschmiediineae	*Apollonias* *Beilschmiedia* *Dehaasia* *Endiandra* *Hexapora* *Mezilaurus* *Potameia*
	Cinnamomeae	Cinnamomineae	*Actinodaphne* *Cinnamomum* *Ocotea* *Nectandra* *Dicypellium* *Pleurothyrium* *Sassafras* *Umbellularia*
		Anibineae	*Aniba* *Aiouea* *Endlicheria* *Licaria* *Phyllostemodaphne* *Systemonodaphne* *Urbanodendron*
	Litseeae	Litseineae	*Litsea* *Neolitsea*
		Laurineae	*Laurus* *Lindera*
	Cryptocaryeae	Eusideroxylineae	*Eusideroxylon*
		Cryptocaryineae	*Cryptocarya* *Ravensara*
	Hypodaphneae		*Hypodaphnis*
Cassythoideae			*Cassytha*

Most of the members of Lauraceae family produce essential oils which consist of monoterpenes and sesquiterpenes as the main group components [23]. Alkaloids are found to be the major secondary metabolites present in the genus of this family [13, 24-28], followed by lignans [26], butanolides [26, 27], endiandric acids [29], sesquiterpenes [24, 25, 27, 28, 30] and flavonoids [24-26, 28]. Majority of the alkaloids existed in this family are isoquinolines, primarily aporphines and benzylisoquinolines groups. Aporphines are predominant in genera *Ocotea*, *Cassytha*, *Lindera*, *Litsea*, *Phoebe*, *Neolitsea* and *Beilschmiedia*, while benzylisoquinolines are found particularly in *Aniba* and *Cryptocarya* [13, 24-28]. Other than that, lignans are occurred mainly in *Beilschmiedia*, *Cinnamomum* and *Machilus* [26], butanolides are existed frequently in *Litsea* [26, 27] and *Lindera* [26], while endiandric acids derivatives are commonly present in *Beilschmiedia* and *Endiandra* [29].

The skeletons of sesquiterpenes in Laurel plants vary in their structure, which include aliphatic, monocyclic, bicyclic and tricyclic sesquiterpenes and their oxygenated derivatives as well as sesquiterpene lactones. *Litsea* [27], *Neolitsea* and *Lindera* species contain a great number of these sesquiterpenes [24, 25, 28, 30]. The existence of sesquiterpene lactones in the form of furanogermacrane skeleton is unique to *Neolitsea* [24, 25, 28, 30] and *Lindera* species [30]. Flavonols and their glycosides are the most representative class of flavonoids found in *Cassytha*, *Cinnamomum*, *Cryptocarya*, *Lindera*, *Litsea*, *Machilus* [26] and *Neolitsea* [24-26, 28].

1.4 *Neolitsea* Genus

Neolitsea is an evergreen shrub which comprises approximately 100 species especially in tropical regions of Asia particularly in the East, South and Southwest China with 45 species (eight varieties) were reported to date [24, 28]. These species can also be found in America [25] and Australia [31]. *Neolitsea* species can be recognized by its clustered, six anthers, triplinerved leaves, sessile sub-umbels, the latter frequently borne on the bare, inter-leaf cluster stem regions and dimerous flowers [32].

Some of *Neolitsea* species are used traditionally to relieve several ailments especially by people in subtropical regions of Asia. For examples, the leaf of *N. cambodiana* are applied externally to cure furuncle and carbuncle; the bark and root of *N. aurata* have been used to alleviate edema by inducing diuresis [24]; and the bark and leaf of *N. cassia* are useful in treating fractures [33]. Besides, a few of *Neolitsea* species such as *N. sericea* is exploited as an ornamental tree in public gardens due to its graceful shape and religious connotation [34].

N. kedahense Gamble **(Figure 1.1)** is a rare and endemic plant of Malaysia which can be found in montane forest at about 1000 m.a.s.l. This small tree can reach 6 meter tall and confined to Gunung Jerai, Kedah. Its leaf often in pseudowhorls and can be characterised by elliptic to lanceolate shape, blade leathery, velvety hairy below, apex pointed and base cuneate. The flower usually in sessile umbellules in clusters from leaf axils or from twigs and have enclosed in hairy bracts and densely hair perianth [16].

Figure 1.1: Leaf of *N. kedahense* Gamble

N. coccinea B. C. Stone **(Figure 1.2)**, an endemic species of Malaysia can only be found from montane forest at Gunung Ulu Kali, Pahang. It grows at about 1600 m.a.s.l. in the form of a shrub to 3 meter tall. The leaf of this rare species are spirally arranged, ovate to elliptic shape, blade thickly leathery, apex bluntly pointed and base broadly cuneate to rounded shape. Its flower often in axillary clusters of umbellules having perianth tube densely hairy [16].

Figure 1.2: Leaf of *N. coccinea* B. C. Stone

N. zeylanica (Nees) Merr. **(Figure 1.3)** locally known as '*teja pasir'* or '*medang pasir*' [15] is widely distributed in India, Malaysia, Burma, Sri Lanka, Borneo, Philippines [16] as well as Australia [15]. This shrub or small tree can grow up from coasts to inland forests including limestone to 1000 meter altitude. Its timber is used for house building and planks, while its root is applied traditionally in the treatment of eruptions on fingers [15] and rheumatic arthralgia [24]. The leaf of *N. zeylanica* are alternate, ovate to lanceolate shape, blade thinly to thickly leathery, apex bluntly pointed or blunt, glabrous and base acute or rounded. Its flower usually in sessile umbellules in clusters or short racemes from leaf axils or from twigs and have perianth lobes hairy at outside [16].

Figure 1.3: Leaf of *N. zeylanica* (Nees) Merr.

1.5 Problem Statement

Phytochemical studies on *Neolitsea* species originated from India, Taiwan, Japan, Korea, Australia and Vietnam led to the isolation and identification of various classes of secondary metabolites such as terpenoids (sesquiterpenoids and triterpenoids), alkaloids, flavonoids, lignans and essential oils [24]. The distribution of these secondary metabolites is scientifically significant for chemotaxonomic classification of this species. So far, approximately 26 species had been investigated for their phytochemistry and biological studies [24]. However, the essential oils and chemical constituents of *Neolitsea* species such as *N. kedahense*, *N. coccinea* and *N. zeylanica* from Malaysia have not been studied, and only one report on the isolation of sesquiterpene lactones from the root of *N. zeylanica* of India [35] was found. Thus, there is a requirement to investigate the presence of secondary metabolites from the three *Neolitsea* species of Malaysia. Furthermore, there is still insufficient information regarding the essential oils and chemical constituents on the other plant parts of *N. zeylanica* such as leaf and bark.

Plants of *Neolitsea* species have been used in traditional medicine for the treatment of various illnesses including furuncle, carbuncle, edema, fractures, eruptions on fingers and rheumatic arthralgia [15, 24, 33]. *Neolitsea* species had been found to show interesting medicinal value that leads to intensive pharmacological and biological properties such as antibacterial, antifungal, anti-inflammatory and cytotoxicity activities [24]. However, among the previous studies, none of them reported the bioactivity of *N. kedahense*, *N. coccinea* and *N. zeylanica*. Hence, more attention on the bioactivity screenings of these three species are required.

1.6 Objectives of Research

The objectives of this study were;

1. To extract and analyse the chemical composition of the essential oils from the fresh samples of *N. kedahense*, *N. coccinea* and *N. zeylanica*.

2. To isolate and identify the structures of pure constituents from the dried samples of *N. kedahense*, *N. coccinea* and *N. zeylanica*.
3. To screen the bioactivities of the essential oils, crude extracts and pure isolated constituents for antibacterial, α-glucosidase inhibitory and antioxidant activities.

1.7 Significance of Research

The presence of the secondary metabolites in plant such as phenolics, terpenes, alkaloids, flavonoids, glycosides and volatile oils are very useful for chemotaxonomic classification due to their specific distribution in plants. Therefore, the findings which obtained from the chemical composition of the essential oils and isolated constituents from *N. kedahense*, *N. coccinea* and *N. zeylanica* may be used to verify the chemotaxonomic relationship between the related species of *Neolitsea* and the related genus of Lauraceae as well as providing the phytochemical databases for *Neolitsea* species. Furthermore, plant secondary metabolites also play a significant role for treating diseases either as a single compound or as a mixture. Hence, the results obtained from the bioactivity screenings of *N. kedahense*, *N. coccinea* and *N. zeylanica* may give additional knowledge to the pharmaceutical field.

1.8 Scope of Research

This research was focused on three *Neolitsea* of Malaysia, *N. kedahense*, *N. coccinea* and *N. zeylanica*. Study on the chemical composition of the essential oils covered the stems and leaves of *N. kedahense* and *N. coccinea* as well as the bark, heartwood and leaf of *N. zeylanica*. The fresh samples were extracted using hydrodistillation technique to afford the essential oils. The essential oils were analysed using GC and GC-MS to identify the chemical composition of the oils.

The dried samples of *N. kedahense* (stem), *N. coccinea* (stem and leaf) and *N. zeylanica* (bark and leaf) were powdered and soaked successively using solvents with different polarities to give the crude extracts. The crude extracts were fractionated using vacuum liquid chromatography (VLC). Purification of the fractions were conducted using chromatographic methods including gravity column chromatography (CC) over SiO_2 and Sephadex LH-20, preparative TLC (PTLC) and recycling preparative HPLC as well as recrystallization techniques to obtain the pure constituents. Structures of pure constituents were elucidated by extensive spectroscopic analysis which include NMR (1D and 2D), IR, UV, MS spectrometry and X-ray crystallography. Finally, three bioactivities namely antibacterial, α-glucosidase inhibitory and antioxidant activities were performed on the essential oils, crude extracts and isolated constituents.

REFERENCES

1. Koehn, F. E. and Carter, G. T. The Evolving Role of Natural Products in Drug Discovery. *Nat. Rev. Drug Discov*. 2005. 4(3): 206-220.
2. Ahmad, F. and Raji, H. *Kimia Hasilan Semulajadi dan Tumbuhan Ubatan*. Kuala Lumpur: Dewan Bahasa dan Pustaka. 1993.
3. Chin, Y. W., Balunas, M. J., Chai, H. B. and Kinghorn, A. D. Drug Discovery from Natural Sources. *AAPS J.* 2006. 8(2): E239-E253.
4. Balandrin, M. F., Kinghorn, A. D. and Farnsworth, N. R. Plant-Derived Natural Products in Drug Discovery and Development: An Overview. In: Kinghorn, A. D. and Balandrin, M. F. *Human Medicinal Agents from Plants*. Washington, DC: American Chemical Society. 2-12; 1993.
5. Polat, R and Satil, F. An Ethnobotanical Survey of Medicinal Plants in Edremit Gulf (Balıkesir-Turkey). *J. Ethnopharmacol*. 2012. 139: 626-641.
6. Kamazeri, T. S. A. T., Samah, O. A., Taher, M., Susanti, D. and Qaralleh, H. Antimicrobial Activity and Essential Oils of *Curcuma aeruginosa*, *Curcuma mangga* and *Zingiber cassumunar* from Malaysia. *Asian Pac. J. Trop. Med.* 2012. 5(3): 202-209.
7. Raut, J. S. and Karuppayil, S. M. A Status Review on the Medicinal Properties of Essential Oils. *Ind. Crops. Prod*. 2014. 62: 250-264.
8. de Souza, M. R., de Paula, C. A., de Resende, M. L. P., Grabe-Guimarães, A., de Souza Filho, J. D. and Saúde-Guimarães, D. A. Pharmacological Basis for Use of *Lychnophora trichocarpha* in Gouty Arthritis: Anti-hyperuricemic and Anti-inflammatory Effects of Its Extract, Fraction and Constituents. *J. Ethnopharmacol*. 2012. 142: 845-850.
9. Lawson, K. *Botanical and Plant-Derived Drugs: Global Markets*. Wellesley: BCC Research. 2013.
10. Noor Rain, A., Khozirah, S., Mohd Ridzuan, M. A. R., Ong, B. K., Rohaya, C., Rosilawati, M., Hamdino, I., Badrul, A. and Zakiah, I. Antiplasmodial

Properties of Some Malaysian Medicinal. *Trop. Biomed*. 2007. 24(1): 29-35.

11. Ahmad, F., Zaidi, M. A. S., Sulaiman, N. and Majid, F. A. A. Issues and Challenges in the Development of the Herbal Industry in Malaysia. *Proceedings of the Persidangan Kebangsaan Ekonomi Malaysia Ke-10*. September 18-20 2015. Melaka: Fakulti Ekonomi dan Pengurusan Universiti Kebangsaan Malaysia. 2015. 227-238.
12. Van Der Werff, H. and Richter, H. G. Toward an Improved Classification of Lauraceae. *Ann. Mo. Bot. Gard*. 1996. 83: 409-418.
13. Custódio, D. L. and da Veiga Junior, V. F. Lauraceae Alkaloids. *RSC Adv*. 2014. 4: 21864-21890.
14. Khamis, S. and Nizam, M. S. Distribution of *Litsea* Complex (Lauraceae) and Their Association with Soil Properties in Different Forest Types of Peninsular Malaysia. *AIP Conf. Proc*. 2013. 1571: 363-367.
15. Burkill, I. H. *A Dictionary of Economic Products of the Malay Peninsula* Vol 2. Kuala Lumpur: Ministry of Agriculture and Co-operatives. 1966.
16. Ng, F. S. P. and Phil, D. *Tree Flora of Malaya: A Manual for Foresters*. Kuala Lumpur: Longman. 1989.
17. Khong, H. Y., Din, L. B., Zakaria, Z., Ismail, N. H., Ismail, N. and Yarmo, M. A. A Qualitative Analysis of *Litsea fulva* Essential Oils Using Comprehensive Two-dimensional Gas Chromatography Coupled with Time-of-Flight Mass Spectrometry. *Sains Malays*. 2013. 42(7): 943-948.
18. Li, X. W., Li, J., Huang, P. H., Wei, F. N., Cui, H. B. and van der Werff, H. Lauraceae. In: Wu, Z. Y., Raven, P. H. and Hong, D. Y. (Eds.). *Flora of China*. Beijing, China: Science Press and St. Louis, MO, USA: Missouri Botanical Garden Press. 102; 2008.
19. Kostermans, A. J. G. H. Lauraceae. *Reinwardtia*. 1957. 4(2): 193-256.
20. Yazaki, K. and Okuda, T. *Cinnamomum cassia* Blume (Cinnamon): *In Vitro* Culture and the Production of Condensed Tannins. In: Bajaj, Y. P. S. *Medicinal and Aromatic Plants V: Biotechnology in Agriculture and Forestry*. Heidelberg: Springer-Verlag. 122; 1993.
21. Wiart, C. *Ethnopharmacology of Medicinal Plants: Asia and the Pacific*. New Jersey: Humana Press. 2006.
22. Lin, C. T., Chu, F. H., Tseng, Y. H., Tsai, J. B., Chang, S. T. and Wang, S. Y. Bioactivity Investigation of Lauraceae Trees Grown in Taiwan. *Pharm. Biol*.

2007. 45(8): 638-644.

23. Joshi, S. C., Padalia, R. C., Bisht, D. S. and Mathela, C. S. Terpenoid Diversity in the Leaf Essential Oils of Himalayan Lauraceae Species. *Chem. Biodivers*. 2009. 6: 1364-1373.

24. Wang, W. Y., Ma, P., Xu, L. J., Peng, Y. and Xiao, P. G. Chemical Constituents and Biological Activities of Plants from the Genus *Neolitsea*. *Chem. Biodivers*. 2014. 11: 55-72.

25. Qing, X., Zhao, T., Guo, R. X., Wu, Y. B., Shi, Q. W., Gu, Y. C. and Kiyota, H. Chemical Constituents of Plants from the Genus *Neolitsea*. *Heterocycl. Commun*. 2014. 20(2): 61-75.

26. Chang, H. S. and Chen, I. S. Chemical Constituents and Bioactivity of Formosan Lauraceous Plants. *J. Food Drug Anal*. 2016. 24: 247-263.

27. Wang, Y. S., Wen, Z. Q., Li, B. T., Zhang, H. B. and Yang, J. H. Ethnobotany, Phytochemistry and Pharmacology of the Genus *Litsea*: An Update. *J. Ethnopharmacol*. 2016. 181: 66-107.

28. Cao, Y., Gao, X. L., Su, G. Z., Yu, X. L., Tu, P. F. and Chai, X. Y. The Genus *Neolitsea* of Lauraceae: A Phytochemical and Biological Progress. *Chem. Biodivers*. 2015. 12: 1443-1465.

29. Lenta, B. N., Chouna, J. R., Nkeng-Efouet, P. A. and Sewald, N. Endiandric Acid Derivatives and Other Constituents of Plants from the Genera *Beilschmiedia* and *Endiandra* (Lauraceae). *Biomolecules*. 2015. 5: 910-942.

30. Hayashi, N and Komae, H. Chemistry and Distribution of Sesquiterpene Furans in Lauraceae. *Biochem. Syst. Ecol*. 1980. 8(4): 381-383.

31. Brophy, J. J., Goldsack, R. J., Fookes, C. J. R. and Forster, P. I. The Leaf Oils of the Australian Species of *Neolitsea* (Lauraceae). *J. Essent. Oil Res*. 2002. 14: 191-195.

32. Li, L., Li, J., Conran, J. G. and Li, X. W. Phylogeny of *Neolitsea* (Lauraceae) Inferred from Bayesian Analysis of nrDNA ITS and ETS Sequences. *Pl. Syst. Evol*. 2007. 269: 203-221.

33. de Silva, S. S. M., Kumar, N. S. and Aman, P. Structural Studies of an Arabinoxylan Isolated from the Leaves of *Neolitsea cassia*. *Carbohydr. Res.* 1986. 152: 229-236.

34. Wang, Z. S., An, S. Q., Liu, H., Leng, X., Zheng, J. W. and Liu, Y. H. Genetic Structure of the Endangered Plant *Neolitsea sericea* (Lauraceae)

from the Zhoushan Archipelago Using RAPD Markers. *Ann. Bot.* 2005. 95: 305-313.

35. Joshi, B. S., Kamat, V. N. and Govindachari, T. R. Sesquiterpenes of *Neolitsea zeylanica* Merr. I: Isolation of Some Constituents. *Tetrahedron*. 1967. 23: 261-265.

36. Dewick, P. M. *Medicinal Natural Products: A Biosynthetic Approach*. 6th. ed. UK: John Wiley and Sons Inc. 2009.

37. John, A. J., George, V., Pradeep, N. S. and Sethuraman, M. G. Chemical Composition and Antibacterial Activity of the Leaf, Bark and Fruit Oils of *Neolitsea fischeri* Gamble. *J. Essent. Oil Res*. 2008. 20: 279-282.

38. John, A. J., Vrujesh, P. K., Dan, M., Pradeep, N. S., Sethuraman, M. G. and George, V. Chemical Composition and Antibacterial Activity of Leaf and Bark Oils of *Neolitsea scrobiculata* (Meisn.) Gamble. *Indian Perfumer*. 2006. 50(3): 61-64.

39. Ho, C. L., Liao, P. C., Wang, E. I. C. and Su, Y. C. Composition and Antifungal Activities of the Leaf Essential Oil of *Neolitsea parvigemma* from Taiwan. *Nat. Prod. Commun*. 2011. 6(9): 1357-1360.

40. John, A. J., Karunakaran, V. P., George, V., Pradeep, N. S. and Sethuraman, M. G. Chemical Composition and Antibacterial Activity of Leaf Oil of *Neolitsea foliosa* (Nees) Gamble var. *caesia* (Meisner) Gamble. *J. Essent. Oil Res*. 2007. 19: 498-500.

41. Padalia, R. C., Chanotiya, C. S., Thakuri, B. C. and Mathela, C. S. Germacranolide Rich Essential Oil from *Neolitsea pallens*. *Nat. Prod. Commun*. 2007. 2(5): 591-593.

42. Yoon, W. J., Moon, J. Y., Kang, J. Y., Kim, G. O., Lee, N. H. and Hyun, C. G. *Neolitsea sericea* Essential Oil Attenuates LPS-induced Inflammation in RAW 264.7 Macrophages by Suppressing NF-κB and MAPK Activation. *Nat. Prod. Commun*. 2010. 5(8): 1311-1316.

43. Thang, T. D., Dai, D. N., Thai, T. H. and Ogunwande, I. A. Essential Oils of *Phoebe angustifolia* Meisn., *Machilus velutina* Champ. ex Benth. and *Neolitsea polycarpa* Liou (Lauraceae) from Vietnam. *Rec. Nat. Prod*. 2013. 7(3): 192-200.

44. Ouyang, S., Xie, P., Yang, S. L., Feng, Y. L. and Yan, Y. Chemical Constituents of Essential Oils from Fruit of *Neolitsea confertifolia*. *Zhong*

Cao Yao (Chin. Tradit. Herb Drugs). 2007. 38(10): 1470-1471.

45. Su, Y. C., Hsu, K. P., Wang, E. I. C. and Ho, C. L. Composition and *In vitro* Anticancer Activities of the Leaf Essential Oil of *Neolitsea variabillima* from Taiwan. *Nat. Prod. Commun*. 2013. 8(4): 531-532.
46. Gunde, B. G. and Hilditch, T. P. The Seed and Fruit-Coat Fats of *Neolitsea involucrata*. *J. Chem. Soc*. 1938: 1610-1614.
47. Nakasato, T. and Nomura, S. Studie on the Alkaloids of Lauraceous Plants. I. Alkaloids of *Neolitsea sericea* (Blume) Koidz. (1). *Yakugaku Zasshi*. 1957. 77: 816-817.
48. Seaman, F. C. Sesquiterpene Lactones as Taxonomic Characters in the Asteraceae. *Bot. Rev*. 1982. 48(2): 121-594.
49. Adio, A. M. Germacrenes A-E and Related Compounds: Thermal, Photochemical and Acid Induced Transannular Cyclizations. *Tetrahedron*. 2009. 65: 1533-1552.
50. Wong, S. L., Chang, H. S., Wang, G. J., Chiang, M. Y., Huang, H. Y., Chen, C. H., Tsai, S. C., Lin, C. H. and Chen, I. S. Secondary Metabolites from the Roots of *Neolitsea daibuensis* and Their Anti-inflammatory Activity. *J. Nat. Prod*. 2011. 74: 2489-2496.
51. Wu, S. L. and Li, W. S. Chemical Constituents from the Roots of *Neolitsea hiiranensis*. *J. Chin. Chem. Soc*. 1995. 42: 555-560.
52. Chen, K. S., Chang, F. R., Jong, T. T. and Wu, Y. C. Two Novel Sesquiterpenes from *Neolitsea parvigemma*. *J. Nat. Prod*. 1996. 59: 704-706.
53. Chen, K. S. and Wu, Y. C. Sesquiterpenoids from *Neolitsea parvigemma*: Isolation, Oxidation Products and Antiplatelet Actions. *Tetrahedron*. 1999. 55: 1353-1366.
54. Li, W. S. and McChesney, J. D. Chemical Constituents of *Neolitsea parvigemma*. *J. Nat. Prod*. 1990. 53(6): 1581-1584.
55. Li, W. S. Sesquiterpene Lactones from the Root of *Neolitsea acutotrinervia*. *J. Nat. Prod*. 1992. 55(11): 1614-1619.
56. Wu, S. L. and Li, W. S. Terpenoids from *Neolitsea buisanensis*. *Phytochemistry*. 1991. 30(12): 4160-4162.
57. Chen, K. S., Hsieh, P. W., Hwang, T. L., Chang, F. R. and Wu, Y. C. Anti-inflammatory Furanogermacrane Sesquiterpenes from *Neolitsea parvigemma*. *Nat. Prod. Res*. 2005. 19(3): 283-286.

58. Wu, X., Vogler, B., Jackes, B. R. and Setzer, W. N. Terpenoids from *Neolitsea dealbata*. *Nat. Prod. Commun*. 2008. 3(2): 129-132.

59. Li, W. S. and Duh, C. Y. Sesquiterpene Lactones from *Neolitsea villosa*. *Phytochemistry*. 1993. 32(6): 1503-1507.

60. Chen, K. S., Chang, F. R., Chia, Y. C., Wu, T. S. and Wu, Y. C. Chemical Constituents of *Neolitsea parvigemma* and *Neolitsea konishii*. *J. Chin. Chem. Soc*. 1998. 45: 103-110.

61. Boruah, P., Bhuyan, P. D., Dutta, S. C., Mohan, S. and Mathur, R. K. Isolation of Two Antifungal Compounds from *Neolitsea cuipala* and Their Activity against Some Plant Pathogenic Fungi. *Folia Microbiol. (Prague)*. 1999. 44(4): 385-387.

62. Liou, B. J., Chang, H. S., Wang, G. J., Chiang, M. Y., Liao, C. H., Lin, C. H. and Chen, I. S. Secondary Metabolites from the Leaves of *Neolitsea hiiranensis* and the Anti-inflammatory Activity of Some of Them. *Phytochemistry*. 2011. 72: 415-422.

63. Daisuke, T., Hiroyuki, T., Hiroshi, N. and Mitsuru, N. Structures of Three Germacranolide Sesquiterpene Dilactones from *Neolitsea aciculata* Koid. *Nat. Prod. Lett*. 1993. 3(3): 203-208.

64. Chang, F. R., Hsieh, T. J., Huang, T. L., Chen, C. Y., Kuo, R. Y., Chang, Y. C., Chiu, H. F. and Wu, Y. C. Cytotoxic Constituents of the Stem Bark of *Neolitsea acuminatissima*. *J. Nat. Prod*. 2002. 65: 255-258.

65. Hamid, K., Alqahtani, A., Kim, M. S., Cho, J. L., Cui, P. H., Li, C. G., Groundwater, P. W. and Li, G. Q. Tetracyclic Triterpenoids in Herbal Medicines and Their Activities in Diabetes and Its Complications. *Curr. Top. Med. Chem*. 2015. 15: 2406-2430.

66. Xu, R., Fazio, G. C. and Matsuda, S. P. T. On the Origins of Triterpenoid Skeletal Diversity. *Phytochemistry*. 2004. 65: 261-291.

67. Patočka, J. Biologically Active Pentacyclic Triterpenes and Their Current Medicine Signification. *J. Appl. Biomed*. 2003. 1:7-12.

68. Yano, K., Akihisa, T., Kawaguchi, R., Tamura, T. and Matsumoto, T. 24-Methylene-25-methylcycloartanol and 24α-Ethyl-5α-cholestan-3α-ol from *Neolitsea sericea*. *Phytochemistry*. 1992. 31(5): 1741-1746.

69. Sharma, M. C., Ohira, T. and Yatagai, M. Sericeol, A Cycloartane Triterpene from *Neolitsea sericea*. *Phytochemistry*. 1993. 33(3): 721-722.

70. Yano, K., Akihisa, T., Tamura, T. and Matsumoto, T. Four 4α-Methylsterols and Triterpene Alcohols from *Neolitsea aciculata*. *Phytochemistry*. 1992. 31(6): 2093-2098.

71. Sharma, M. C., Ohira, T. and Yatagai, M. Lanostane Triterpenes from the Bark of *Neolitsea sericea*. *Phytochemistry*. 1994. 37(1): 201-203.

72. Aniszewski, T. *Alkaloids-Secrets of Life*: *Alkaloid Chemistry, Biological Significance, Applications and Ecological Role*. UK: Elsevier. 2007.

73. Lee, S. S. and Yang, H. S. Isoquinoline Alkaloids from *Neolitsea konishii*. *J. Chin. Chem. Soc*. 1992. 39(2): 189-194.

74. Lee, S. S., Lai, Y. C., Chen, C. K., Tseng, L. H. and Wang, C. Y. Characterization of Isoquinoline Alkaloids from *Neolitsea sericea* var. *aurata* by HPLC-SPE-NMR. *J. Nat. Prod*. 2007. 70: 637-642.

75. Tran, T. D., Pham, N. B., Fechner, G. and Quinn, R. J. Chemical Investigation of Drug-Like Compounds from the Australian Tree, *Neolitsea dealbata*. *Bioorg. Med. Chem. Lett*. 2010. 20: 5859-5863.

76. Buchanan, M. S., Carroll, A. R., Pass, D. and Quinn, R. J. Aporphine Alkaloids from the Chinese Tree *Neolitsea aurata* var. *paraciculata*. *Nat. Prod. Commun*. 2007. 2(3): 255-259.

77. Kim, S. S., Hyun, C. G., Choi, Y. H. and Lee, N. H. Tyrosinase Inhibitory Activities of the Compounds Isolated from *Neolitsea aciculata* (Blume) Koidz. *J. Enzyme Inhib. Med. Chem*. 2013. 28(4): 685-689.

78. Kumar, S. and Pandey, A. K. Chemistry and Biological Activities of Flavonoids: An Overview. *Scientific World J*. 2013. 2013: 1-16.

79. Lam, S. H., Chen, C. K., Wang, J. S. and Lee, S. S. Investigation of Flavonoid Glycosides from *Neolitsea sericea* var. *aurata via* the General Method and HPLC-SPE-NMR. *J. Chin. Chem. Soc*. 2008. 55: 449-455.

80. Yu, E. J., Kim, T. H., Kim, K. H. and Lee, H. J. Characterization of Aroma-active Compounds of *Abies nephrolepis* (Khingan fir) Needles Using Aroma Extract Dilution Analysis. *Flavour Fragr. J*. 2004. 19: 74-79.

81. Clery, R. A., Cason, J. R. L. and Zelenay, V. Constituents of Cypriol Oil (*Cyperus scariosus* R.Br.): N-Containing Molecules and Key Aroma Components. *J. Agric. Food Chem*. 2016. 64: 4566-4573.

82. Gauvin, A. and Smadja, J. Essential Oil Composition of Four *Psiadia* Species from Reunion Island: A Chemotaxanomic Study. *Biochem. Syst. Ecol*. 2005.

33: 705-714.

83. Adam, R. P. *Identification of Essential Oil Components by Gas Chromatography/Mass Spectroscopy*. Carol Stream IL, USA: Allured Publishing Corporation. 2007.

84. Schossler, P., Schneider, G. L., Wunsch, D., Soares, G. L. G. and Zini, C. A. Volatile Compounds of *Baccharis punctulata*, *Baccharis dracunculifolia* and *Eupatorium laevigatum* Obtained Using Solid Phase Microextraction and Hydrodistillation. *J. Braz. Chem. Soc*. 2009. 20(2): 277-287.

85. Kurose, K., Okamura, D. and Yatagai, M. Composition of the Essential Oils from the Leaves of Nine *Pinus* Species and the Cones of Three of *Pinus* Species. *Flavour Fragr. J*. 2007. 22: 10-20.

86. Robinson, C. J. *A New Essential Oil-Agonis Fragrans, A Report for the Rural Industries Research and Development Corporation*. Barton ACT, Australia: Rural Industries Research and Development Corporation. 2006.

87. Leffingwell, J. C., Alford, E. D., Leffingwell, D. and Peen, R. Identification of the Volatile Constituents of Cyprian Latakia Tobacco by Dynamic and Static Headspace Analyses. *Leffingwell Reports*. 2013. 5(2): 1-29.

88. Bittencourt, M. L. F., Ribeiro, P. R., Franco, R. L. P., Hilhorst, H. W. M., de Castro, R. D. and Fernandez, L. G. Metabolite Profiling, Antioxidant and Antibacterial Activities of Brazilian Propolis: Use of Correlation and Multivariate Analyses to Identify Potential Bioactive Compounds. *Food Res. Int*. 2015. 76: 449-457.

89. Xu, F., Zhao, X., Yang, L., Wang, X. and Zhao, J. A New Cycloartane-Type Triterpenoid Saponin Xanthine Oxidase Inhibitor from *Homonoia riparia* Lour. *Molecules*. 2014. 19: 13422-13431.

90. Ragasa, C. Y. and Cornelio, K. B. Triterpenes from *Euphorbia hirta* and Their Cytotoxicity. *Chin. J. Nat. Med*. 2013. 11(5): 0528-0533.

91. Sakurai, N., Yaguchi, Y. and Inoue, T. Triterpenoids from *Myrica rubra*. *Phytochemistry*. 1987. 26(1): 217-219.

92. Takeda, K., Horibe, I., Teraoka, M. and Minato, H. Sesquiterpenes of Lauraceae Plants. Part 1. Components of *Neolitsea aciculata* Koidz. *J. Chem. Soc. C.* 1970. 7: 973-980.

93. Eshimbetov, A. G. and Tulyaganov, T. S. Theoretical and UV Spectral Study of Isomeric 1-(Quinolinyl)-β-Carbolines Conformations. *Spectrochim. Acta*

Mol. Biomol. Spectrosc. 2007. 67: 1139-1143.

94. Kuo, P. C., Li, Y. C., Hwang, T. L., Ma, G. H., Yang, M. L., Lee, E. J. and Wu, T. S. Synthesis and Structural Characterization of an Anti-inflammatory Principle Purified from *Lindera aggregata*. *Tetrahedron Lett*. 2014. 55: 108-110.

95. Qiang, Y., Yang, Z. D., Yang, J. L. and Gao, K. Sesquiterpenoids from the Root Tubers of *Lindera aggregata*. *Planta Med*. 2011. 77: 1610-1616.

96. Choi, J. Y., Seo, C. S., Zheng, M. S., Lee, C. S. and Son, J. K. Topoisomerase I and II Inhibitory Constituents from the Bark of *Tilia amurensis*. *Arch. Pharm. Res*. 2008. 31(11): 1413-1418.

97. Antonelli Ushirobira, T. M., Yamaguti, E., Uemura, L. M., Nakamura, C. V., Dias Filho, B. P. and Palazzo de Mello, J. C. Chemical and Microbiological Study of Extract from Seeds of Guaraná (*Paullinia cupana* var. *sorbilis*). *Lat. Am. J. Pharm*. 2007. 26(1): 5-9.

98. Ban, J. Y., Jeon, S. Y., Bae, K. W., Song, K. S. and Seong, Y. H. Catechin and Epicatechin from *Smilacis chinae* Rhizome Protect Cultured Rat Cortical Neurons against Amyloid β Protein (25-35)-Induced Neurotoxicity Through Inhibition of Cytosolic Calcium Elevation. *Life Sci.* 2006. 79: 2251-2259.

99. Nonaka, G. I., Goto, Y., Kinjo, J. E., Nohara, T. and Nishioka, I. Tannins and Related Compounds. LII. Studies on the Constituents of the Leaves of *Thujopsis dolabrata* SIEB. Et ZUCC. *Chem. Pharm. Bull*. 1987. 35(3): 1105-1108.

100. Markham, K. R. *Techniques of Flavonoid Identification*. New York, London: Academic Press. 1982.

101. Joo, S. J., Park, H. J., Park, J. H., Cho, J. G., Kang, J. H., Jeong, T. S., Kang, H. C., Lee, D. Y., Kim, H. S., Byun, S. Y. and Baek, N. I. Flavonoids from *Machilus japonica* Stems and Their Inhibitory Effects on LDL Oxidation. *Int. J. Mol. Sci*. 2014. 15: 16418-16429.

102. Ishiguro, K., Nagata, S., Fukumoto, H., Yamaki, M., Takagi, S and Isoi, K. A Flavanonol Rhamnoside from *Hypericum japonicum*. *Phytochemistry*. 1991. 30(9): 3152-3153.

103. Slowing, K., Sollhuber, M., Carretero, E. and Villar, A. Flavonoid Glycosides from *Eugenia jambos*. *Phytochemistry*. 1994. 37(1): 255-258.

104. Masuda, T., Jitoe, A., Kato, S. and Nakatani, N. Acetylated Flavonol

Glycosides from *Zingiber zerumbet*. *Phytochemistry*. 1991. 30(7): 2391-2392.

105. Prachayasittikul, S., Saraban, P., Cherdtrakulkiat, R., Ruchirawat, S. and Prachayasittikul, V. New Bioactive Triterpenoids and Antimalarial Activity of *Diospyros rubra* Lec. *EXCLI J*. 2010. 9: 1-10.

106. Van Kiem, P., Van Minh, C., Huong, H. T., Nam, N. H., Lee, J. J. and Kim, Y. H. Pentacyclic Triterpenoids from *Mallotus apelta*. *Arch. Pharm. Res*. 2004. 27(11): 1109-1113.

107. Ghosh, P., Mandal, A., Chakraborty, M. and Saha, A. Triterpenoids from *Quercus suber* and Their Antimicrobial and Phytotoxic Activities. *J. Chem. Pharm. Res.* 2010. 2(4): 714-721.

108. Begum, F., Haque, M. R., Nahar, K. S. and Rashid, M. A. Secondary Metabolites from Different Extractives of *Stereospermum suaveolens*. *Dhaka Univ. J. Pharm. Sci*. 2014. 13(1): 31-36.

109. Kundu, J. K., Rouf, A. S. S., Hossain, M. N., Hasan, C. M. and Rashid, M. A. Antitumor Activity of Epifriedelanol from *Vitis trifolia*. *Fitoterapia*. 2000. 71: 577-579.

110. Cole, B. J. W., Bentley, M. D., Hua, Y. and Bu, L. Triterpenoid Constituents in the Outer Bark of *Betula alleghaniensis* (Yellow Birch). *J. Wood Chem. Tech*. 1991. 11(2): 209-223.

111. Zahid, M., Ishrud, O., Pan, Y., Asim, M., Riaz, M. and Ahmad, V. U. Flavonoid Glycosides from *Salvia moorcroftiana* Wall. *Carbohydr. Res*. 2002. 337: 403-407.

112. Mishra, M. P., Rath, S., Swain, S. S., Ghosh, G., Das, D. and Padhy, R. N. *In Vitro* Antibacterial Activity of Crude Extracts of 9 Selected Medicinal Plants Against UTI Causing MDR Bacteria. *J. King Saud. Univ. Sci*. 2017. 29: 84-95.

113. Costa, A. R., de Lima Silva, J., Lima, K. R. R., Rocha, M. I., Barros, L. M., da Costa, J. G. M., Boligon, A. A., Kamdem, J. P., Carneiro, J. N. P., Leite, N. F., de Menezes, I. A., Duarte, A. E., Morais-Braga, M. F. B. and Coutinho, H. D. M. *Rhaphiodon echinus* (Nees & Mart.) Schauer: Chemical, Toxicological Activity and Increased Antibiotic Activity of Antifungal Drug Activity and Antibacterial. *Microb. Pathog.* 2017. 107: 280-286.

114. Nascimento, G. G. F., Locatelli, J., Freitas, P. C. and Silva, G. L. Antibacterial Activity of Plant Extracts and Phytochemicals on Antibiotic-

resistant Bacteria. *Braz. J. Microbiol*. 2000. 31: 247-256.

115. Wright, G. D. Bacterial Resistance to Antibiotics: Enzymatic Degradation and Modification. *Adv. Drug Deliv. Rev*. 2005. 57: 1451-1470.

116. Ahmad, I., Mehmood, Z. and Mohammad, F. Screening of Some Indian Medicinal Plants for Their Antimicrobial Properties. *J. Ethnopharmacol*. 1998. 62: 183-193.

117. Valgas, C., de Souza, S. M., Smânia, E. F. A. and Smânia, Jr., A. Screening Methods to Determine Antibacterial Activity of Natural Products. *Braz. J. Microbiol*. 2007. 38: 369-380.

118. Balouiri, M., Sadiki, M. and Ibnsouda, S. K. Methods for *In vitro* Evaluating Antimicrobial Activity: A Review. *J. Pharm. Anal*. 2016. 6: 71-79.

119. Eloff, J. N. A Sensitive and Quick Microplate Method to Determine the Minimal Inhibitory Concentration of Plant Extracts for Bacteria. *Planta Med*. 1998. 64: 711-713.

120. Ríos, J. L. and Recio, M. C. Medicinal Plants and Antimicrobial Activity. *J. Ethnopharmacol*. 2005. 100: 80-84.

121. Holetz, F. B., Pessini, G. L., Sanches, N. R., Cortez, D. A. G., Nakamura, C. V. and Filho, B. R. D. Screening of Some Plants Used in the Brazilian Folk Medicine for the Treatment of Infectious Diseases. *Mem. Inst. Oswaldo Cruz*. 2002. 97(7): 1027-1031.

122. Dorman, H. J. D. and Deans, S. G. Antimicrobial Agents from Plants: Antibacterial Activity of Plant Volatile Oils. *J. Appl. Microbiol*. 2000. 88: 308-316.

123. Sousa, R. M. F., de Morais, S. A. L., Vieira, R. B. K., Napolitano, D. R., Guzman, V. B., Moraes, T. S., Cunha, L. C. S., Martins, C. H. G., Chang, R., de Aquino, F. J. T., do Nascimento, E. A. and de Oliveira, A. Chemical Composition, Cytotoxic and Antibacterial Activity of the Essential Oil from *Eugenia calycina* Cambess. Leaves against Oral Bacteria. *Ind. Crops. Prod*. 2015. 65: 71-78.

124. Ud-Daula, A. F. M. S., Demirci, F., Salim, K. A, Demirci, B., Lim, L. B. L., Baser, K. H. C. and Ahmad, N. Chemical Composition, Antioxidant and Antimicrobial Activities of Essential Oils from Leaves, Aerial Stems, Basal Stems and Rhizomes of *Etlingera fimbriobracteata* (K.Schum.) R.M.Sm. *Ind. Crops. Prod*. 2016. 84: 189-198.

125. Magina, M. D. A., Dalmarco, E. D., Wisniewski, Jr. A., Simionatto, E. L., Dalmarco, J. B., Pizzolatti, M. G. and Brighente, I. M. C. Chemical Composition and Antibacterial Activity of Essential Oils of *Eugenia* Species. *J. Nat. Med*. 2009. 63: 345-350.

126. Kim, K. Y., Nam, K. A., Kurihara, H. and Kim, S. M. Potent α-Glucosidase Inhibitors Purified from the Red Alga *Grateloupia elliptica*. *Phytochemistry*. 2008. 69: 2820-2825.

127. Basak, S. S. and Candan, F. Chemical Composition and *In vitro* Antioxidant and Antidiabetic Activities of *Eucalyptus Camaldulensis* Dehnh. Essential Oil. *J. Iran. Chem. Soc*. 2010. 7(1): 216-226.

128. Oboh, G., Akinbola, I. A., Ademosun, A. O., Sanni, D. M., Odubanjo, O. V., Olasehinde, T. A. and Oyeleye, S. I. Essential Oil from Clove Bud (*Eugenia aromatica* Kuntze) Inhibit Key Enzymes Relevant to the Management of Type-2 Diabetes and Some Pro-oxidant Induced Lipid Peroxidation in Rats Pancreas *In vitro*. *J. Oleo Sci*. 2015. 64(7): 775-782.

129. Kim, S. D. α-Glucosidase Inhibitor from *Buthus martensi* Karsch. *Food Chem*. 2013. 136: 297-300.

130. Lee, S. Y., Mediani, A., Nur Ashikin, A. H., Azliana, A. B. S. and Abas, F. Antioxidant and α-Glucosidase Inhibitory Activities of the Leaf and Stem of Selected Traditional Medicinal Plants. *Int. Food. Res. J*. 2014. 21(1): 165-172.

131. Sarikurkcu, C., Zengin, G., Oskay, M., Uysal, S., Ceylan, R. and Aktumsek, A. Composition, Antioxidant, Antimicrobial and Enzyme Inhibition Activities of Two *Origanum vulgare* Subspecies (subsp. *vulgare* and subsp. *hirtum*) Essential Oils. *Ind. Crop Prod*. 2015. 70: 178-184.

132. Zhang, L., Hogan, S., Li, J., Sun, S., Canning, C., Zheng, S. J. and Zhou, K. Grape Skin Extract Inhibits Mammalian Intestinal α-Glucosidase Activity and Suppresses Postprandial Glycemic Response in Streptozocin-treated Mice. *Food Chem*. 2011. 126: 466-471.

133. Wilson, K. and Walker, J. *Principles and Techniques of Practical Biochemistry*. 5th. ed. UK: Cambridge University Press. 2000.

134. Kim, S. H., Jo, S. H., Kwon, Y. I. and Hwang, J. K. Effects of Onion (*Allium cepa* L.) Extract Administration on Intestinal α-Glucosidases Activities and Spikes in Postprandial Blood Glucose Levels in SD Rats Model. *Int. J. Mol.*

Sci. 2011. 12: 3757-3769.

135. Chai, T. T., Chiam, M. J., Lau, C. H., Ismail, N. I. M., Ong, H. C., Manan, F. A. and Wong, F. C. Alpha-Glucosidase Inhibitory and Antioxidant Activity of Solvent Extracts and Fractions of *Typha domingensis* (Typhaceae) Fruit. *Trop. J. Pharm. Res*. 2015. 14: 1983-1990.

136. Elya, B., Malik, A., SeptiMahanani, P. I. and Loranza, B. Antidiabetic Activity Test by Inhibition of α-Glucosidase and Phytochemical Screening from the Most Active Fraction of Buni (*Antidesma bunius* L.) Stem Barks and Leaves. *Int. J. PharmTech*. *Res*. 2012. 4(4): 1667-1671.

137. Reddy, P. P., Tiwari, A. K., Rao, R. R., Madhusudhana, K., Rao, V. R. S., Ali, A. Z., Babu, K. S. and Rao, J. M. New Labdane Diterpenes as Intestinal α-Glucosidase Inhibitor from Antihyperglycemic Extract of *Hedychium spicatum* (Ham. Ex Smith) Rhizomes. *Bioorg. Med. Chem. Lett*. 2009. 19: 2562-2565.

138. Tsai, F. S., Lin, L. W. and Wu, C. R. Lupeol and Its Role in Chronic Diseases. In: Gupta, S. C., Prasad, S. and Aggarwal, B. B. *Drug Discovery from Mother Nature*. Switzerland: Springer International Publishing. 160; 2016.

139. Tadera, K., Minami, Y., Takamatsu, K. and Matsuoka, T. Inhibition of α-Glucosidase and α-Amylase by Flavonoids. *J. Nutr. Sci. Vitaminol*. 2006. 52: 149-153.

140. Wang, H., Du, Y. J. and Song, H. C. α-Glucosidase and α-Amylase Inhibitory Activities of Guava Leaves. *Food Chem*. 2010. 123: 6-13.

141. Rahal, A., Kumar, A., Singh, V., Yadav, B., Tiwari, R., Chakraborty, S. and Dhama, K. Oxidative Stress, Prooxidants and Antioxidants: The Interplay. *BioMed. Res. Int*. 2014: 1-19.

142. Nordberg, J. and Arnér, E. S. J. Reactive Oxygen Species, Antioxidants and the Mammalian Thioredoxin System. *Free Radic. Biol. Med*. 2001. 31(11): 1287-1312.

143. Pham-Huy, L. A., He, H. and Pham-Huy, C. Free Radicals, Antioxidants in Disease and Health. *Int. J. Biomed. Sci*. 2008. 4(2): 89-96.

144. Nićiforović, N., Mihailović, V., Mašković, P., Solujić, S., Stojković, A. and Pavlović Muratspahić, D. Antioxidant Activity of Selected Plant Species; Potential New Sources of Natural Antioxidants. *Food Chem. Toxicol*. 2010.

48: 3125-3130.

145. Shahidi, F. and Zhong, Y. Novel Antioxidants in Food Quality Preservation and Health Promotion. *Eur. J. Lipid Sci. Technol*. 2010. 112: 930-940.

146. Akbarirad, H., Gohari Ardabili, A., Kazemeini, S. M. and Mousavi Khaneghah, A. An Overview on Some of Important Sources of Natural Antioxidants. *Int. Food. Res. J*. 2016. 23(3): 928-933.

147. Ainsworth, E. A. and Gillespie, K. M. Estimation of Total Phenolic Content and Other Oxidation Substrates in Plant Tissues Using Folin-Ciocalteu Reagent. *Nat. Protoc*. 2007. 2(4): 875-877.

148. Ismail, A., Marjan, Z. M. and Foong, C. W. Total Antioxidant Activity and Phenolic Content in Selected Vegetables. *Food Chem*. 2004. 87: 581-586.

149. Bobo-García, G., Davidov-Pardo, G., Arroqui, C., Vírseda, P., Marín-Arroyo, M. R. and Navarro, M. Intra-laboratory Validation of Microplate Methods for Total Phenolic Content and Antioxidant Activity on Polyphenolic Extracts and Comparison with Conventional Spectrophotometric Methods. *J. Sci. Food Agric*. 2015. 95: 204-209.

150. Babbar, N., Oberoi, H. S., Sandhu, S. K. and Bhargav, V. K. Influence of Different Solvents in Extraction of Phenolic Compounds from Vegetable Residues and Their Evaluation as Natural Sources of Antioxidants. *J. Food Sci. Technol*. 2014. 51(10): 2568-2575.

151. Gangwar, M., Gautam, M. K., Sharma, A. K., Tripathi, Y. B., Goel, R. K. and Nath, G. Antioxidant Capacity and Radical Scavenging Effect of Polyphenol Rich *Mallotus philippenensis* Fruit Extract on Human Erythrocytes: An *In vitro* Study. *Scientific World J*. 2014: 1-12.

152. Brand-Williams, W., Cuvelier, M. E. and Berse, C. Use of a Free Radical Method to Evaluate Antioxidant Activity. *Lebensm. Wiss. Technol*. 1995. 28: 25-30.

153. Andrade, M. A., das Graças Cardoso, M., de Andrade, J., Silva, L. F., Teixeira, M. L., Valério Resende, J. M., da Silva Figueiredo, A. C. and Barroso, J. G. Chemical Composition and Antioxidant Activity of Essential Oils from *Cinnamodendron dinisii* Schwacke and *Siparuna guianensis* Aublet. *Antioxidants*. 2013. 2: 384-397.

154. Zengin, H. and Baysal, A. H. Antibacterial and Antioxidant Activity of Essential Oil Terpenes against Pathogenic and Spoilage-Forming Bacteria

and Cell Structure-Activity Relationships Evaluated by SEM Microscopy. *Molecules*. 2014. 19: 17773-17798.

155. Ozsoy, N., Can, A., Yanardag, R. and Akev, N. Antioxidant Activity of *Smilax excelsa* L. Leaf Extracts. *Food Chem*. 2008. 110: 571-583.

156. Smith, J. E., Tucker, D., Watson, K. and Jones, G. L. Identification of Antibacterial Constituents from the Indigenous Australian Medicinal Plant *Eremophila duttonii* F. Muell. (Myoporaceae). *J. Ethnopharmacol*. 2007. 112: 386-393.

157. Chapin, K. C. and Lauderdale, T. Reagents, Stains and Media: Bacteriology. In: Murray, P. R., Baron, E. J., Jorgensen, J. H., Pfaller, M. A. and Yolken, R. H. *Manual of Clinical Microbiology*. 8th. ed. Washington, D.C.: ASM Press. 358; 2003.

158. Perumal, S., Pillai, S., Lee, W. C., Mahmud, R. and Ramanathan, S. Determination of Minimum Inhibitory Concentration of *Euphorbia hirta* (L.) Extracts by Tetrazolium Microplate Assay. *J. Nat. Prod*. 2012. 5: 68-76.

159. Kassim, N. K., Rahmani, M., Ismail, A., Sukari, M. A., Ea, G. C. L., Nasir, N. M. and Awang, K. Antioxidant Activity-Guided Separation of Coumarins and Lignan from *Melicope glabra* (Rutaceae). *Food Chem*. 2013. 139: 87-92.