UNIVERSITI PUTRA MALAYSIA

PHYTOCHEMICAL, BIOACTIVITY AND LC-DAD-MS/MS ANALYSES OF NAM-NAM (Cynometra cauliflora L.) AND TAMPANG BESI (Callicarpa maingayi K. & G.) LEAF EXTRACTS

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By

MUHAMMAD ABUBAKAR ADO

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfiliment of the Requirements for the Degree of Doctor of Philosophy

September 2016

DEDICATION

This thesis is dedicated to my parents and family

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the Degree of Doctor of Philosophy

PHYTOCHEMICAL, BIOACTIVITY AND LC-DAD-MS/MS ANALYSES OF NAM-NAM (*Cynometra cauliflora* L.) AND TAMPANG BESI (*Callicarpa maingayi* K. & G.) LEAF EXTRACTS

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September 2016

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Institute : Biosciences

Current research indicates that radical oxygen species (ROS) liberated along with some other components in the body are capable of destroying cellular constituents and act as secondary messengers for some chronic diseases, such as diabetes, Alzheimer`s disease (AD), coronary heart diseases, skin disease and inflammation. Because many researchers have demonstrated that most of the drugs and additives used in the treatments of AD, diabetes, or skin diseases may result in toxic effects and causes other serious diseases, chemical and biological studies on medicinal and edible plants have been investigated to discover new active compounds. Therefore, the main objective of this study was to evaluate the effect of methanol extract and different polarity fractions of two Malaysian medicinal plants *Cynometra cauliflora* (nam-nam) and *Callicarpa maingayi* (tampang besi) leaves for their antioxidant, cholinesterase, tyrosinase and α-glucosidase inhibitory activities. In addition, the identification of the bioactive compounds from the active fractions was performed using the LC-DAD-ESIMS/MS and spectroscopic techniques.

The methanolic leaf extract of *C. cauliflora* exhibited potent inhibition against all three enzymes and high antioxidant activity. The bioactivity was found to be concentrated in the EtOAc and *n*-BuOH fractions. Applying LC-DAD-ESIMS/MS to the two active fractions led to the identification of total 18 metabolites. These compounds includes isomer of procyanidin; procyanidin tetramer (**139**), procyanidin trimer (**141**) and procyanidin hexamer (**149**), catechin (**140**), isomer of taxifolin pentoside (**142**), vitexin (**143**), isovitexin (**144**), kaempferol hexoside (**145**), isomer of quercetin pentoside (**146**), quercetin hexoside (**147**), apigenin 6-*C*-glucose-8-*C*-glucose (**148**), kaempferol–coumaroyl-hexoside (**150**) and isorhamnetin hexoside (**151**). The phytochemical investigation of the EtOAc and *n*-BuOH fractions of the plant led to the isolation of four different compounds. Through a combination of spectroscopy (1D and 2D NMR) and mass spectrometry, these compounds were identified as apigenin 8-*C*-glucoside (**143**), apigenin 6-*C*-glucoside (**144**), taxifolin 3-*O*-arabinofuranoside (**142**) and acacetin 7-*O*-*β*-glucoside (**152**). All the compounds exhibited good to weak DPPH radical scavenging and α-glucosidase inhibitory activities and relatively moderate to weak inhibitory

activity against acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) when compared to positive control.

Similarly for *C. cauliflora*, LC-DAD-ESIMS/MS was used to analyze the most bioactive fractions (EtOAc and *n*-BuOH) of *Callicarpa maingayi*. Cistanoside F (**109**), apigenin 6-*C*-glucoside-8-*C*-glucoside (**148**), *β*-OH-forsythoside B (**132**) and campneoside II (**112**), isocampneoside I (**122**), rhamnazin 3-*O*-rutinoside (**153**), forsythoside B (**26**), campneoside I (**118**), aceteoside (**27**), isoaceteoside (**24**), eukovoside (**123**), acacetin diglucuronide (**126**), apigenin 7-*O*-rutinoside (**154**), 2'-acetylacteoside (**28**), 2'-acetylacteoside isomer (**28**), acacetin 7-*O*-glucoronide (**130**), β-OH-poliumoside (**114**), kaempferol 3-sulfate-7-arabinopyranoside (**155**) and poliumoside (**29**), were tentatively identified based on their UV spectra and MS/MS data. All these compounds are reported in this species for the first time. Phytochemical investigations of the DCM and hexane fractions of *C. maingayi* were also carried out. Eight known compounds namely euscaphic acid (**51**), arjunic acid (**156**), ursolic acid (**20**), apigenin (**131**), acacetin (**157**), stigmasterol 3-*O*-*β*-glycopyranoside (**158**) and sitosterol 3-*O*-*β*-glycopyranoside (**159**) were isolated from the DCM fraction, while *n*-hexacosanoic acid (**160**) from the hexane fraction. Compounds **51**, **156**, **20**, **131**, **157** and **160** were isolated for the first time from the *C. maingayi*. Furthermore, the triterpenoid effects on AChE and α-glucosidase enzymes were also investigated. Ursolic acid (**20**) was found to display moderate inhibition against AChE, whereas euscaphic acid (**51**) and arjunic acid (**156**) demonstrated moderate α-glucosidase inhibitory activity. The biological activities of the crude extracts of *C. cauliflora* and *C. maingayi* and the pure compounds were in alignment with their ethno pharmacological uses. In conclusion, this study have validated the use of these plants in traditional medicinal practice and suggested that they may have potential applications in the treatment of various free radical mediated diseases.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Doktor Falsafah

FITOKIMIA, BIOAKTIVITI DAN LC-DAD-MS/MS ANALISIS EKSTRAK DAUN NAM-NAM (*Cynometra cauliflora* L.) DAN TAMPANG BESI (*Callicarpa maingayi* K. & G.)

Oleh

MUHAMMAD ABUBAKAR ADO

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Kajian terkini menunjukkan bahawa radikal oksigen spesies (ROS) dibebaskan bersama-sama dengan beberapa komponen lain dalam badan mampu memusnahkan juzuk sel dan bertindak sebagai utusan sekunder untuk beberapa penyakit kronik seperti diabetes, penyakit Alzheimer`s (AD), penyakit jantung koronari, penyakit kulit dan keradangan. Oleh kerana ramai penyelidik telah menunjukkan bahawa kebanyakan ubat-ubatan dan bahan tambahan yang digunakan dalam rawatan AD, kencing manis, atau penyakit kulit boleh menyebabkan kesan toksik dan menyebabkan penyakit serius yang lain, kajian kimia dan biologi ke atas tumbuh-tumbuhan ubatan dan boleh dimakan adalah cara alternatif untuk mencari sebatian aktif yang baru. Oleh itu, objektif utama kajian ini adalah untuk menilai kesan ekstrak metanol dan fraksi kekutuban berbeza daripada dua tumbuhan ubatan Malaysia iaitu *Cynometra cauliflora* (nam-nam) dan *Callicarpa maingayi* (tampang besi) terhadap aktiviti antioksidan, perencatan asetilkolinesterase, tirosinase dan α-glukosidase. Di samping itu, pengenalpastian sebatian bioaktif daripada fraksi aktif dilakukan dengan menggunakan teknik LC-DAD-ESIMS/MS dan spektroskopi.

Ekstrak metanol daun *C. cauliflora* menunjukan perencatan kuat terhadap ketiga-tiga enzim dan aktiviti antioksidan yang tinggi. Bioaktiviti itu ditemui tertumpu pada fraksi EtOAc dan *n*-BuOH. Analisis LC-DAD-ESIMS/MS terhadap dua fraksi aktif ini membawa kepada pengenalpastian 18 metabolit. Sebatian-sebatian ini termasuk isomer daripada prosianidin; prosianidin tetramer (**139**), prosianidin trimer (**141**) dan prosianidin heksamer (**149**), catechin (**140**), isomer daripada taksifolin pentosida (**142**), viteksin (**143**), isoviteksin (**144**), kaempferol heksosida (**145**), isomer kuersetin pentosida (**146**), kuersetin heksosida (**147**), apigenin 6-*C*-glukosa-8-*C*-glukosa (**148**), kaempferol-koumaroil-heksosida (**150**) dan isorhamnetin heksosida (**151**). Kajian fitokimia terhadap fraksi EtOAc dan *n*-BuOH membawa kepada pemencilan empat sebatian yang berbeza. Melalui gabungan analisis spektroskopi (1D dan 2D NMR) dan spektrometri jisim, sebatian ini telah dikenal pasti sebagai apigenin 8-*C*-glukosida (**143**), apigenin 6-*C*-glukosida (**144**), taksifolin 3-*O*-arabinofuranosida (**142**) dan akasetin 7-*O*-

β-glukosida (**152**). Semua sebatian mempamerkan aktiviti pemerangkap radikal DPPH dan perencatan α-glukosidase dari bak hingga baik lemah dan aktiviti perencatan asetikolinesterase (AChE) dan butirilkolinesterase (BChE) dari sederhana hingga lemah berbanding kawalan positif.

Sama seperti *C. cauliflora*, LC-DAD-ESIMS/MS telah digunakan untuk menganalisis fraksi paling aktif (EtOAc dan *n*-BuOH) daripada *Callicarpa maingayi*. Cistanosida F (**109**), apigenin 6-*C*-glukosida-8-*C*-glukosida (**148**), *β*-OH-forsitosida B (**132**) dan campneosida II (**112**), isocampneosida I (**122**), rhamnazin 3-*O*-rutinosida (**153**), forsitosida B (**26**), campneosida I (**118**), aseteosida (**27**), isoaseteosida (**24**), eukovosida (**123**), akasetin diglukuronida (**126**), apigenin 7-*O*-rutinosida (**154**), 2'-asetilakteosida (**28**), isomer 2'-asetilakteosida (**28**), akasetin 7-O-glukuronida (**130**), *β*-OH-poliumosida (**114**), kaempferol 3-sulfat-7-arabinopiranosida (**155**) dan poliumosida (**29**), telah dikenal pasti secara tentatif berdasarkan data MS/MS dan spektrum UV. Semua sebatian dilaporkan dalam spesies ini buat kali pertama. Kajian fitokimia terhadap fraksi DCM dan heksana *C. maingayi* juga telah dijalankan. Lapan sebatian yang dikenali sebagai asid euskapik (**51**), asid arjunik (**156**), asid ursolik (**20**), apigenin (**131**), akasetin (**157**), stigmasterol 3-*O*-*β*-glikopiranosida (**158**) dan sitosterol 3-*O*-*β*-glikopiranosida (**159**) telah berjaya dipencilkan daripada fraksi DCM, manakala asid n-heksakosanoik (**160**) daripada fraksi heksana. Sebatian **51**, **156**, **20**, **131**, **157** dan **160** tersebut telah dipencilkan buat kali pertama dari *C. maingayi*. Tambahan pula, kesan triterpenoid terhadap enzim AChE dan α-glukosidase turut disiasat. Asid ursolik (**20**) didapati mempamerkan perencatan sederhana terhadap enzim AChE, manakala asid euskapik (**51**) dan asid arjunik (**156**) menunjukkan aktiviti perencatan sederhana terhadap enzim α-glukosidase. Aktiviti biologi ekstrak mentah *C. cauliflora* dan *C. maingayi* dan sebatian tulen adalah selari mengikut kegunaan etnofarmakologi. Kesimpulannya, kajian ini telah mengesahkan penggunaan tumbuh-tumbuhan ini dalam amalan perubatan tradisional dan mencadangkan bahawa tumbuhan tersebut berpotensi digunakan dalam rawatan pelbagai penyakit yang disebabkan pengantara radikal bebas.

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I certify that a Thesis Examination Committee has met on 19 September 2016 to conduct the final examination of Muhammad Abubakar Ado on his thesis entitled "Phytochemical, Bioactivity and LC-DAD-MS/MS Analyses of Nam-Nam (*Cynometra cauliflora* L.) and Tampang Besi (*Callicarpa maingayi* K. & G.) Leaf Extracts" 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 Doctor of Philosophy.

Members of the Thesis Examination Committee were as follows:

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This thesis was submitted to the Senate of the Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree Doctor of Philosophy. The members of the Supervisory Committee were as follows:

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I hereby confirm that:

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This is to confirm that:

- the research conducted and the writing of this thesis was under our supervision;
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Name of Member of Supervisory Committee: Associate Professor Dr. Intan Safinar binti Ismail

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

^{1}H-NMR	Proton Nuclear Magnetic Resonance Spectroscopy
^{13}C-NMR	Carbon-13 Nuclear Magnetic Resonance
BHT	Butylated Hydroxytoluene
CC	Column Chromatography
CHCL3	Chloroform
d	Doublet
DAD	Diode Array Detector
dd	Doublet of Doublet
ddd	Doublet of Double of Doublet
DPPH	Diphenylpicrylhdrazyl
ESI	Electrospray Ionization
g	Gram
GAE	Gallic Acid Equivalent
gCOSY	Gradient Correlation Spectroscopy
gHMBC	Gradient Heteronuclear Multiple Bond Correlation
gHSQC	Gradient Heteronuclear Single-Quantum Coherence
HPLC	High Performance Liquid Chromatography
HREIMS	High Resolution Electron Impact Mass Spectroscopy
Hz	Hertz
IC_{50}	Inhibition Concentration at 50 percent
L	Litre
LC-MS	Liquid Chromatography–Mass Spectrometry
m	Multiplet
m/z	Mass per Charge

MeOH	Methanol
MHz	Megahertz
MHz	Megahertz
mL	Milliliter
MS	Mass Spectrometry
℃	Degree in Celsius
ppm	Part Per Million
ROS	Reactive Oxygen Species
s	Singlet
TLC	Thin Layer Chromatography
TPC	Total Phenolic Contents
UV	Ultraviolet
UV/VIS	Ultravoilet/visible
δ	Chemical Shift in ppm
µg	Microgram
µL	Microliter
mg	Milligram
LC-DAD-ESIMS/MS	Liquid Chromatography–Diode Array Detector–Electrospray Ionization–Tandem Mass Spectrometry
e.g	Example
DTNB	Dithio-bis-(2-nitrobenzoic Acid)
ATCI	Acetylthiocholine Iodide
n-BuOH	Butanol
DCM	Dichloromethane
EtOAc	Ethyl acetate
Aq	Aqueous

TPC	Total Phenolic Content
RSA	Radical-scavenging Activity
FRAP	Ferric-reducing Antioxidant Power Assay
TCA	Trichloroacetic Acid
µM	Micro molar
RSA	Radical-scavenging Activity
FRAP	Ferric-reducing Antioxidant Power Assay
TCA	Trichloroacetic Acid
µM	Micro molar
DMSO	Dimethyl Sulfoxide
p-NPG	4-Nitrophenyl β-D-glucuropyranoside
WHO	World Health Organization

CHAPTER 1

INTRODUCTION

1.1 General

Medicinal plants and their derived products have been an important medicinal source for several thousands of years worldwide (McChesney *et al*., 2007). In the early 1900s, i.e. prior to the "Synthetic Era", approximately 80% of all medicines used were obtained from medicinal plants or natural products (Singh *et al*., 2014). In the past few years, there has been a drastic increase in research in the field of natural product-based drugs because of their ready availability, cost effectiveness, relatively less side effects, structural diversity and better tolerability (Singh *et al*., 2014). Twenty-five years ago, it was estimated that approximately 70% of new chemical entities used in medicinal practice were obtained directly or indirectly from natural products. Even the World Health Organization has recognized the importance of traditional medicine and has established guidelines, strategies and standards for the use of medicinal plants (Baker *et al*., 2007; McChesney *et al*., 2007; Singh *et al*., 2014).

Medicinal plants occupy a prominent position in meeting the primary health care needs of many people, particularly in developing countries because they are inexpensive, effective, safe and readily available (Farnsworth *et al*., 1985; Agara *et al*., 2007; Cragg and Newman, 2013). Plant-based systems continue to play an important part in healthcare, and their use by various cultures has been widely documented. A survey of plant-derived pure compounds used as drugs in countries hosting WHO-Traditional Medicine Centers indicated that of 122 compounds identified, 80% were used for the same or related ethnomedical purposes and were derived from only 94 plant species (Cragg and Newman, 2013). Furthermore, the increased costs of prescription drugs for the maintenance of health have fueled interest in medicinal plants to obtain new plant-derived drugs (Hoareau and DaSilva, 1999). Historically, all medicinal preparations were obtained from plants, in the simple form of plant parts or by combining different parts as crude extracts (Ayyanar and Ignacimuthu, 2011).

The use of medicinal plants as sources of therapeutic agents in drug discovery occurs via four pathways: a) using the whole plant or plant parts as herbal remedies, e.g., echinaceas, feverfew, garlic, cranberry, and *Ginkgo biloba*; b) isolating bioactive compounds from plants for direct use as drugs, e.g., digitoxin, morphine, reserpine, digoxin, taxol, vinblastine, and vincristine; or c) identifying bioactive compounds of new or known structures that will guide the production of semi-synthetic products for use as patentable drugs with higher activity and lower toxicity, e.g., nabilone, oxycodone, metformin, and taxotere: and/or d) using the agents for clinical, pharmacological, and chemical studies e.g., lysergic acid diethylamide, mescaline, and yohimbine (Fabricant and Farnsworth, 2001).

Innovations in the use of medicinal plants in different parts of the world are vital to establishing new directions for the propagation of alternative medicine with better economic and social benefits. In Malaysia, medicinal plants play an important role in the treatment of various ailments. The use of plant preparations for such purposes has been well documented (Herbal Medicine Research Centre, 2002; Zaidan *et al*., 2005). More than one hundred plant species in Malaysia are reported to have medicinal properties (Zaidan *et al*., 2005). Indeed, it has been reported that more than 250,000 species of higher plants in Malaysia with therapeutic potential, only 5–15% have been studied. Thus, there is enormous potential to identify plant resources with useful phytochemicals (Chew *et al*., 2011).

1.2 Plant Secondary Metabolites

For many decades, humans have used various secondary metabolites (phytochemicals) produced by higher plants (Yazaki, 2005). These bioactive constituents of plants can be classified into three different categories based on their biosynthetic origins: alkaloids, terpenoids, and phenylpropanoids and allied phenolic compounds (Wolfender *et al*., 2003; Edeoga *et al*., 2005). Several of these compounds have shown various biological activities such as the inhibition of protein synthesis, cardiac activity, the inhibition of the nerve system, and the modulation of microtubule structure. These bioactive secondary metabolites from plants have been used as natural medicines, and the plants containing these metabolites have frequently been utilized as medicinal plants and identified in numerous recipes for crude drugs (Wolfender *et al*., 2003; Yazaki 2005).

Secondary metabolites from medicinal plants with unknown pharmacological activities have been extensively examined as sources of medicinal agents for the treatment and prevention of different ailments. For example, more than 80% of drug substances were obtained from natural products or inspired by natural compounds (Harvey, 2008). Furthermore, five natural-product-related drugs were approved as the first members of new classes of drugs, including the ziconotide and exenatide peptides and the small molecules ixabepilone, retapamulin, and trabectedin. Other products are composed of compounds from plant sources, e.g., elliptinium, galantamine and huperzine: from microbes, e.g., daptomycin, from animals e.g., exenatide and ziconotide: and from synthetic or semi-synthetic compounds processes based on natural products, e.g., tigecycline (**1**), everolimus (**2**), telithromycin (**3**), micafungin (**4**) and caspofungin (**5**). These drugs (Figure 1.1) cover a range of therapeutic activities, such as anti-infective, anti-diabetic, and anti-cancer activities, and have highly diverse chemical structures (Cragg and Newman , 2007; Lam, 2007; Harvery, 2008; Cragg and Newman, 2016).

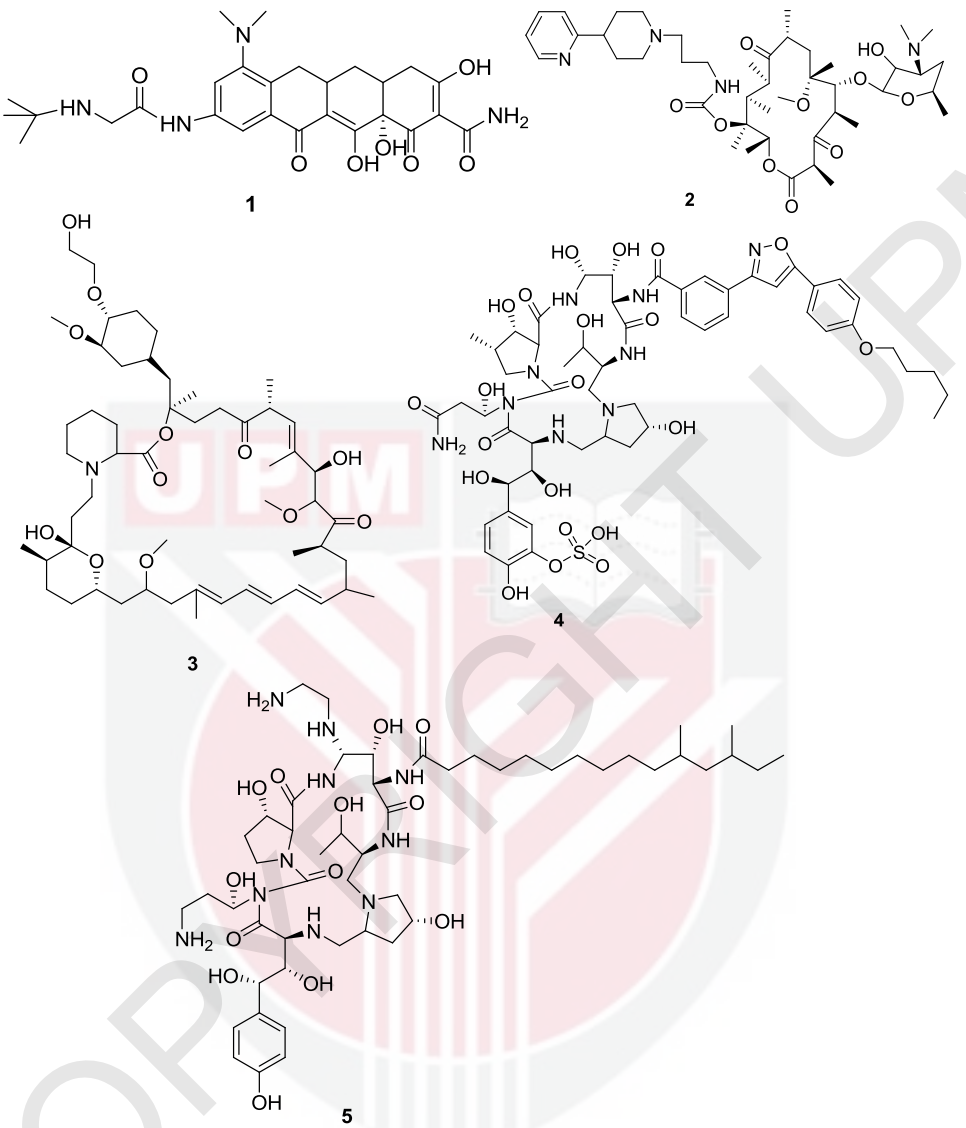

Figure 1.1 : Secondary Metabolites Contributing to New Medicinal Properties

1.3 Problem Statement and Justification

It is now known that radical oxygen species (ROS) such as hydroxyl radical ($OH^{\cdot}$), superoxide anion radical ($O^{\cdot-}_2$), and nitric oxide (NO), produced in the body are capable of destroying other cellular constituents and they act as secondary messenger for many chronic diseases (Vina et al., 2004; Stuchbury and Munch, 2005; Ferreira *et al*., 2006). This is generally considered to be linked to many chronic health problems such as cancers, cardiovascular problems, inflammation, diabetes and Alzheimer`s disease (Mata *et al*., 2007; Biglari *et al*., 2007).

Treatment of such diseases (Alzheimer, diabetes, and skin diseases) involves the inhibitions of enzymes known to be acetylcholinesterase for a person with Alzheimer`s disease, α-glucosidase in diabetes conditions and tyrosinase for skin diseases. The licensed medicines for inhibiting these enzymes presently in use for the treatment of these disorders such as donepezil, tacrine, galantamine, acarbose, miglitol, and hydroquinone have been reported to exhibit several severe side effects (Li *et al*., 2005; Choi et al., 2010; Wszelaki *et al*., 2010). Some of the frequent reported adverse effects include peripheral and central side effects such as fatigue or depression, gastrointestinal disturbances, insomnia, intestinal pain, flatulence and diarrhea, among other (Germanas *et al*., 2007; Bolen *et al*., 2007; Yilmazer-Musa, 2012).

In view of the fact that most of the inhibitors used for the treatments of such diseases acquired some toxic effects and caused another serious ailment, chemical and biological studies on medicinal and edible plants have been increased in order to find bioactive compounds from natural sources. Therefore, the present study is focused on screening, profiling and isolation of the phytochemicals from the leaves of *Cynometra cauliflora* and *Callicarpa maingayi*. Although, these species have been used broadly in traditional medicine for the treatment of many ailments, biological testing and purification of the bioactive compounds has been lacking. As part of intensive studies on Malaysian medicinal plant in the development of the source of useful pharmaceutical agents, this research was conducted based on the following objectives:

1. To screen the methanol extract and different polarity fractions (hexane, dichloromethane, ethyl acetate, n-butanol and aqueous) of *C cauliflora* and *C. maingayi* leaves for their antioxidant, acetylcholinesterase, tyrosinase and α-glucosidase inhibitory activities.
2. To profile the bioactive fraction(s) using liquid chromatography–diode array detection–electrospray ionization–tandem mass spectrometry (LC-DAD-ESIMS/MS).
3. To isolate the chemical constituents from the active fractions of *C. cauliflora* and *C. maingayi* using several chromatographic techniques.
4. To elucidate and characterize the structure of the isolated compound(s) using various spectroscopic techniques.
5. To evaluate the antioxidant, cholinesterase and α glucosidase inhibitory activities of the isolated compounds.

BIBLIOGRAPHY

Abas, F., Kalsom, Y.U., and Lajis, N.H. 2003. Antioxidative and radical scavenging properties of the constituents isolated from *Cosmos caudatus* Kunth. *Natural Product Sciences* 4: 245–248.

Abd Aziz, A.F., and Iqbal, M. 2013. Antioxidant activity and phytochemical composition of *Cynometra cauliflora*. *Journal of Experimental and Integrative Medicine* 4: 337–341.

Abdillahi, H.S., Finnie, J.F., and Van Staden, J. 2011. Anti-inflammatory, antioxidant, anti-tyrosinase and phenolic contents of four *Podocarpus* species used in traditional medicine in South Africa. *Journal of Ethnopharmacology* 136: 496–503.

Ado, M.A., Abas, F., Ismail, I.S., Ghazali, H.M., Shaari, K. 2014. Chemical profile and antiacetylcholinesterase, antityrosinase, antioxidant and α-glucosidase inhibitory activity of *Cynometra cauliflora* L. leaves. *Journal of the Science of Food and Agriculture* 95: 635–642.

Ado, M.A., Abas, F., Mohammed, A.S., and Ghazali, H.M. 2013. Anti-and pro-lipase activity of selected medicinal, herbal and aquatic plants, and structure elucidation of an anti-lipase compound. *Molecules* 18: 14651–14669.

Afjalus, S.M., Malik, S., Emrul, H., Sanjana, S., and Farjana, Y. 2013. Evaluation of neuropharmacological, antibacterial and antinociceptive activity of methanolic extract of the bark of *Cynometra ramiflora* Linn. (Leguminosae). *International Journal of Research in Ayurveda and Pharmacy* 4: 192–197.

Agra, M.D.F., Freitas, P.F.D., and Barbosa-Filho, J.M. 2007. Synopsis of the plants known as medicinal and poisonous in Northeast of Brazil. *Revista Brasileira de Farmacognosia* 17: 114–140.

Alam, M.S., Chopra, N., Ali, M., and Niwa, M. 1995. Oleanen and stigmasterol derivatives from *Ambroma augusta*. *Phytochemistry* 41: 1197–1200.

Alam, N., Yoon, K.N., Lee, K.R., Shin, P.G., Cheong, J.C., Yoo, Y.B., and Lee, T.S. 2010. Antioxidant activities and tyrosinase inhibitory effects of different extracts from *Pleurotus ostreatus* fruiting bodies. *Mycobiology* 38: 295–301.

Ali, M.S., Ibrahim, S.A., Jalil, S., and Choudhary, M.I. 2007. Ursolic acid: a potent inhibitor of superoxides produced in the cellular system. *Phytotherapy Research* 21: 558–561.

Asiri, S.M., Shaari, K., Abas, F., Al-Mekhlafi, N.A., and Lajis, N.H. 2012. Two new naphthoquinone derivatives from the stem bark of *Callicarpa maingayi*. *Natural Product Communications* 7: 1333–1336.

Ayyanar, M., and Ignacimuthu, S. 2011. Ethnobotanical survey of medicinal plants commonly used by Kani tribals in Tirunelveli hills of Western Ghats, India. *Journal of Ethnopharmacology* 134: 851–864.

Baker, D.D., Chu, M., Oza, V., and Rajgarhia, D. 2007. The value of natural products to future pharmaceutical discovery. *Natural Product Reports* 24: 1225–1244.

Barros, L., Dueñas, M., Alves, C.T., Silva, S., Henriques, M., Santos-Buelga, C., and Ferreira, I.C. 2013. Antifungal activity and detailed chemical characterization of *Cistus ladanifer* phenolic extracts. *Industrial Crops and Products* 41: 41–45.

Biglari, F., Abbas F.M., AlKarkhi, and Azhar, M.E. 2008. Antioxidant activity and phenolic content of various date palm (Phoenix dactylifera) fruits from Iran. *Food Chemistry* 107: 1636–1641.

Bolen, S., Feldman, L., Vassy, J., Wilson, L., and Yeh, H.C. 2007. Marinopoulos S, Systematic review: comparative effectiveness and safety of oral medications for type 2 diabetes mellitus. *Annals of Internal Medicine* 147: 386–399.

Burits, M., Asres, K., and Bucar, F. 2001. The antioxidant activity of the essential oils of *Artemisia afra*, *Artemisia abyssinica* and *Juniperus procera*. *Phytotheraphy Research* 15: 103–311.

Burkill, I. H. 1966. A dictionary of the economic products of the Malay Peninsula (2nd ed.). Ministry of agriculture and cooperatives (Vol. 1–2). Malaysia: Kuala Lumpur.

Cai, H., Xie, Z., Liu, G., Sun, X., Peng, G., Lin, B., and Liao, Q. 2014. Isolation, Identification and activities of natural antioxidants from *Callicarpa kwangtungensis* Chun. *Plant Chemicals and Antioxidant Activity* 9 (3).

Cantino, P.D. 1992. Evidence for a polyphyletic origin of the Lamiaceae. *Annals of the Missouri Botanical Garden* 79: 361–379.

Cetin, H., Cinbilgel, I., Yanikoglu, A., and Gokceoglu, M. 2006. Larvicidal activity of some Labiatae (Lamiaceae) plant extracts from Turkey. *Phytotherapy Research* 12: 1088–1090.

Chan, E.W.C., Lim, Y.Y., Wong, S.K., Lim, K.K., Tan, S.P., Lianto, F.S., and Yong, M. Y. 2009. Effects of different drying methods on the antioxidant properties of leaves and tea of ginger species. *Food Chemistry* 113: 166–172.

Chen, J.J., Wu, H.M., Peng, C.F., Chen, I.S., and Chu, S.D. 2009. Seco-abietane diterpenoids, a phenylethanoid derivative, and antitubercular constituents from *Callicarpa pilosissima*. *Journal of Natural Product* 72: 223–228.

Chen, R.S., Lai, J.S., and Wu, T.S. 1986. Studies on the constituents of *Callicarpa formosana* Rolfe. *Journal of the Chinese Chemical Society* 33: 329–334.

Chew, Y.L., Chan, E.W.L., Tan, P.L., Lim, Y.Y., Stanslas, J., Joo Kheng and Goh, J.K. 2011. Assessment of phytochemical content, polyphenolic composition, antioxidant and antibacterial activities of Leguminosae medicinal plants in Peninsular Malaysia. *BMC Complementary and Alternative Medicine* 11:12.

Choi, CW., Choi, Y.H., Cha, M.R., Yoo, D.S., Kim, Y.S., and Yon, G.H. 2010. Yeast α-glucosidase inhibition by Isoflavones from plants of Leguminosae as an in vitro alternative to acarbose. *Journal of Agriculture and Food Chemistry* 58: 9988–9993.

Choi, J.S., Islam, M.N., Ali, M.Y., Kim, E.J., Kim, Y.M., and Jung, H.A. 2014. Effects of C-glycosylation on anti-diabetic, anti-Alzheimer's disease and anti-inflammatory potential of apigenin. *Food and Chemical Toxicology* 64: 27–33.

Choudhury, S., A., Mukherjee, S.C., Pattnaik, P., and Bapuji, M. 2005. *In vitro* antibacterial activity of extracts of selected marine algae and mangroves against fish pathogens. *Asian Fisheries Science* 18: 285–294.

Chung, P. Y., Chung, L. Y., and Navaratnam, P. 2014. Potential targets by pentacyclic triterpenoids from *Callicarpa farinose* against methicillin-resistant and sensitive *Staphylococcus aureus*. *Fitoterapia*, 94: 48-54.

Clement, B.A., Goff, C.M., and Forbes, T.D.A. 1998. Toxic amines and alkaloids from *Acacia rigidula*. *Phytochemistry* 49: 1377–1380.

Colin, W.G.F., and Foster, R.J. 1996. A short dipolar cycloaddition approach to δ-lactam alkaloida from *Cynometra hankei*. Tetrahedron Letters 37: 3915–3918.

Cos, P., Vlietinck, A.J., Berghe, D.V., and Maes, L. 2006. Anti-infective potential of natural products: how to develop a stronger in vitro 'proof-of-concept'. *Journal of Ethnopharmacology* 106: 290–302.

Cragg, G.M., and Newman, D.J. 2013. Natural products: a continuing source of novel drug leads. *Biochimica et Biophysica Acta (BBA)-General Subjects* 6: 3670–3695.

Desai, M.N., and Chavan, N.S. 2010. Antibacterial activity and phytochemical screening of *Cynometra iripa* Kostel. *International Journal of Pharma and Bio Sciences* 1: 1–4.

Du, J., Cullen, J.J., and Buettner, G.R. 2012. Ascorbic acid: chemistry, biology and the treatment of cancer. *Biochimica et Biophysica Acta (BBA)-Reviews on Cancer* 1826: 443–457.

Duraipandiyan, V., Ayyanar, M., and Ignacimuthu, S. 2006. Antimicrobial activity of some ethnomedicinal plants used by Paliyar tribe from Tamil Nadu, India. *BMC Complementary and Alternative Medicine* 6: 35.

Edeoga, H.O., Okwu, D.E., and Mbaebie, B.O. 2005. Phytochemical constituents of some Nigerian medicinal plants. *African Journal of Biotechnology* 4: 685–688.

Ellman, G.L., Courtney, K.D., Andres, V., and Featherstone, R.M. 1961. A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochemical Pharmacology* 7: 88–95.

Fabricant, D.S., and Farnsworth, N.R. 2001. The value of plants used in traditional medicine for drug discovery. *Environmental Health Perspectives* 109: 69.

Fang, Z., Jeong, S.Y., Jung, H.A., Choi, J.S., Min, B. S., and Woo, M.H. 2010. Anticholinesterase and antioxidant constituents from *Gloiopeltis furcata*. *Chemical and Pharmaceutical Bulletin* 58: 1236–1239.

Farnsworth, N.R., Akerele, O., Bingel, A.S., Soejarto, D.D., and Guo, Z. 1985. Medicinal plants in therapy. *Bulletin of the world health organization* 63: 965–981.

Feng, J., Yang, X.W., and Wang, R.F. 2011. Bio-assay guided isolation and identification of α-glucosidase inhibitors from the leaves of *Aquilaria sinensis*. *Phytochemistry* 72: 242–247.

Feng, J., Yang, X.W., Wang, R.F. 2011. Bio-assay guided isolation and identification of α-glucosidase inhibitors from the leaves of *Aquilaria sinensis*. *Phytochemistry* 72: 242–247.

Ferreira, A., Proença, C., Serralheiro, M.L.M., and Araújo M.E.M. 2006. The in vitro screening for acetylcholinesterase inhibition and antioxidant activity of medicinal plants from Portugal. *Journal of Ethnopharmacology* 108**:** 31–37.

Ganesan, P., Kumar, C.S., and Bhaskar, N. 2008. Antioxidant properties of methanol extract and its solvent fractions obtained from selected Indian red seaweeds. *Bioresource Technology* 99: 2717–2723.

Gao, J., Xu, P., Wang, Y., Wang, Y., and Hochstetter, D. 2013. Combined effects of green tea extracts, green tea polyphenols or epigallocatechin gallate with acarbose on inhibition against α-amylase and α-glucosidase in vitro. *Molecules* 18: 11614–11623.

Gao, T., Sun, Z., Yao, H., Song, J., Zhu, Y., Ma, X., and Chen, S. 2011. Identification of Fabaceae plants using the DNA barcode matK. *Planta Medica* 77: 92–94.

Gao, T., Yao, H., Song, J., Liu, C., Zhu, Y., Ma, X., and Chen, S. 2010. Identification of medicinal plants in the family Fabaceae using a potential DNA barcode ITS2. *Journal of Ethnopharmacology* 1:116–121.

Georgiev, M., Alipieva, K., Orhan, K., Abrashev, R., Denev, P., and Angelova, M., 2011. Antioxidant and cholinesterases inhibitory activities of *Verbascum xanthophoeniceum* Griseb. and its phenylethanoid glycosides. *Food Chemistry* 128: 100–105.

Germanas, J.P., Wang, S., Andrew Miner, A., Ha, W., and Ready, J.M. 2007. Discovery of small-molecule inhibitors of tyrosinase. *Bioorganic and Medicinal Chemistry Letters* 17: 6871–6875.

Gnoatto, S.C., Dassonville-Klimpt, A., Da Nascimento, S., Galéra, P., Boumediene, K., Gosmann, G., and Moslemi, S. 2008. Evaluation of ursolic acid isolated from *Ilex paraguariensis* and derivatives on aromatase inhibition. *European Journal of Medicinal Chemistry* 43: 1865–1877.

Gulpinar, A.R., Orhan, I.E., Kan, A., Senol, F.S., Celik, S.A., and Kartal, M. 2012. Estimation of in vitro neuroprotective properties and quantification of rutin and fatty acids in buckwheat (*Fagopyrum esculentum* Moench) cultivated in Turkey. *Food Research International* 46: 536–543.

Gürbüz, P., Demirezer, L.Ö., Güvenalp, Z., Kuruüzüm-Uz, A., Kazaz, C., Chen, G., and Chalermglin, R. 2015. Isolation and Structure Elucidation of Uncommon Secondary Metabolites from *Cistus salviifolius* L. *Records of Natural Products* 9: 175–183.

Harvey, A.L. 2008. Natural products in drug discovery. *Drug discovery today*, 13: 894–901.

Hedrick, U.P. 1919. Sturtevant's Notes on Edible Plants. Albany, NY: J.B. Lyon Company.

HMRC. 2002. Compendium of medicinal plants used in Malaysia, Volume I and II. *Herbal Medicine Research Centre Publications.*

Hoareau, L., and DaSilva, E.J. 1999. Medicinal plants: a re-emerging health aid. *Electronic Journal of biotechnology* 2: 3–4.

Hosozawa, S., Kato, N., and Munakata, K. 1972. 5,6,7-Trimethoxy flavone from *Callicarpa japonica*. *Phytochemistry* 11: 2362.

Hudson, J.B., Lee, M.K., and Rasoanaivo, P. 2000. Antiviral activities in plants endemic to Madagascar. *Pharmaceutical Biology* 38: 36-39.

Ikram, E.H.K., Eng, K.H., Jalil, A.M.M., Ismail, A., Idris, S., Azlan, A., and Mokhtar, R.A.M. 2009. Antioxidant capacity and total phenolic content of Malaysian underutilized fruits. *Journal of Food Composition and Analysis* 22: 388–393.

Jachak, S. M. and Jain, R. 2006. Current status of target-based antimy-cobacterial natural products. Anti-Infect. *Agents in Medicinal Chemistry* 5: 123–133.

Jaiswal, R., Jayasinghe, L., and Kuhnert, N. 2012. Identification and characterization of proanthocyanidins of 16 members of the *Rhododendron* genus (Ericaceae) by tandem LC–MS. *Journal of Mass Spectrometry* 47: 502–515.

Jamzad, Z., Ingrouille, M., and Simmonds, M.S.J. 2003. Three new species of *Nepeta* (Lamiaceae) from Iran. *Taxon* 52: 93–98.

Jayasinghe, L., Amarasinghe, N.R., Arundathie, B.S., Rupasinghe, G.K., Jayatilake, N. A.N., and Fujimoto, Y. 2012. Antioxidant flavonol glycosides from *Elaeocarpus serratus* and *Filicium decipiens*. *Natural Product Research* 26: 717–721.

Jia, A., Yang, Y., and Kong, D. 2012. Isolation and structural identification of C-Glycosylflavones from *Callicarpa Kwangtungensis* Chun. and their preliminary anti-inflammatory activities. *Chinese Journal of Pharmaceuticals* 43: 263–267.

John, J., Ragi, P.R., Sujana, K.A., and Kumar, A. 2012. Analysis of Phytochemical Contents and Antibacterial Activity of an Endangered Tree (*Cynometra travancorica* Bedd.) of Western Ghats, India. *Advances in Biological Research* 6: 1–5.

Jones, W.P., and Kinghorn, A.D. 2008. Biologically active natural products of the genus *Callicarpa*. *Current Bioactive Compounds* 4: 15–32.

Jones, W.P., Lobo-Echeverri, T., Mi, Q., Chai, H.B., Soejarto, D.D., Cordell, G.A., and Kinghorn, A.D. 2007. Cytotoxic constituents from the fruiting branches of *Callicarpa americana* collected in Southern Florida, 1. *Journal of Natural Products* 3: 372–377.

Kamatou, G.P.P., Makunga, N.P., Ramogola, W.P.N., and Viljoen, A.M. 2008. South African *Salvia* species: A review of biological activities and phytochemistry. *Journal of Ethnopharmacology* 3: 664–672.

Katalinić, M., Rusak, G., Barović JD. 2010. Structural aspects of flavonoids as inhibitors of human butyrylcholinesterase. *European Journal of Medicinal Chemistry* 1: 186–192.

Kawazu, K., and Mitsui, T. 1966. Callicarpone, a fish-killing component of *Callicarpa candicans*. *Tetrahedron Letters* 30: 3519–3524.

Khan, M.A., Ali, M., and Alam, P. 2010. Phytochemical investigation of the fruit peels of Citrus Reticulata Blanco. *Natural Product Research* 24: 610-620.

Kim, I.S., Yang, M., Lee, O.H., and Kang, S.N. 2011. The antioxidant activity and the bioactive compound content of *Stevia rebaudiana* water extracts. *LWT-Food Science and Technology* 44: 1328–1332.

Kim, J.S., Kwon, C.S., and Son, K.H. 2000. Inhibition of alpha-glucosidase and amylase by luteolin, a flavonoid. *Bioscience Biotechnology and Biochemistry* 64: 2458–2461.

Kim, K.Y., Nam, K.A., Kurihara, H., and Kim, S.M. 2008. Potent α-glucosidase inhibitors purified from the red alga *Grateloupia elliptica*. *Phytochemistry* 69: 2820–2825.

Kim, Y.S., and Shin, D.H. 2004. Volatile constituents from the leaves of *Callicarpa japonica* Thunb. and their antibacterial activities. *Journal of Agricultural and Food Chemistry* 4: 781–787.

Kobaisy, M., Tellez, M.R., Dayan, F.E., and Duke, S.O. 2002. Phytotoxicity and volatile constituents from leaves of *Callicarpa japonica* Thunb. *Phytochemistry* 1: 37–40.

Koo, K.A., Sung, S.H., Park, J.H., Kim, S.H., Lee, K.Y., and Kim, Y.C. 2005. In vitro neuroprotective activities of phenylethanoid glycosides from *Callicarpa dichotoma*. *Planta Medica* 71: 778–780.

Kumar, V., Rani, A., Dixit, A.K., Pratap, D., and Bhatnagar, D. 2010. A comparative assessment of total phenolic content, ferric reducing-anti-oxidative power, free radical-scavenging activity, vitamin C and isoflavones content in soybean with varying seed coat colour. *Food Research International* 43: 323–328.

Lam, K.S. 2007. New aspects of natural products in drug discovery. *Trends in Microbiology* 15: 279–289.

Leeratiwong, C., Chantaranothai, P., and Paton, A. 2007. Notes on the genus *Callicarpa* L. (Lamiaceae) in Thailand. *Thai Forest Bulletin (Botany)* 35: 73–79.

Li, X., Kim, M.K, Lee, U., Kim, S.K., Kang, J.S., and Choi, H.D. 2005. Myrothenones A and B, cyclopentenone derivatives with tyrosinase inhibitory activity from the marine-derived fungus *Myrothecium* sp. *Chemical and Pharmaceutical Bulletin* 53: 453–455.

Li, Y.L., Li, J., Wang, N.L., and Yao, X.S. 2008. Flavonoids and a new polyacetylene from *Bidens parviflora* Willd. *Molecules* 13: 1931–1941.

Liu, Y.W., Cheng, Y.B., Liaw, C.C., Chen, C.H., Guh, J.H., Hwang, T.L., and Shen, Y. C. 2012. Bioactive diterpenes from *Callicarpa longissima*. *Journal of Natural Products* 4: 689–693.

López, V., Akerreta, S., Casanova, E., García-Mina, J. M., Cavero, R. Y., and Calvo, M. I. 2007. In vitro antioxidant and anti-rhizopus activities of Lamiaceae herbal extracts. *Plant Foods for Human Nutrition* 4: 151–155.

Lou, S.N., Yu, M.W., and Ho, C.T. 2012. Tyrosinase inhibitory components of immature calamondin peel. *Food Chemistry* 135: 1091–1096.

Lu, Y., Foo, L.Y. 2001. Salvianolic acid L, a potent phenolic antioxidant from *Salvia officinalis*. *Tetrahedron Letters* 42: 8223–8225.

Luo, Y. H., Zhou, Z. Q., Ma, S. C., and Fu, H. Z. 2014. Three new antioxidant furofuran lignans from *Callicarpa nudiflora*. *Phytochemistry Letters* 7: 194–197.

Mackinder, B.A., and Wieringa, J. 2013. Ann ea gen. nov.(Detarieae, Caesalpinioideae, Leguminosae): a home for two species long misplaced in *Hymenostegia sensu* lato. *Phytotaxa* 142: 1–14.

Mata, A., Proença, C., Ferreira, A., Serralheiro, M., Nogueira, J., and Araújo, M. 2007. Antioxidant and antiacetylcholinesterase activities of five plants used as Portuguese food spices. *Food Chemistry* 103: 778–86.

Mayachiew, P., and Devahastin, S. 2008. Antimicrobial and antioxidant activities of Indian gooseberry and galangal extracts. *LWT-Food Science Technology* 41: 1153–1159.

Mazlan, N.A., Mediani, A., Abas, F., Ahmad, S., Shaari, K., Khamis, S., and Lajis, N. H. 2013. Antioxidant, antityrosinase, anticholinesterase, and nitric oxide inhibition activities of three Malaysian *Macaranga* species. *The Scientific World Journal 2013*.

McChesney, J.D., Venkataraman, S.K., Henri, J.T. 2007. Plant natural product: back to the future or into extinction? *Phytochemistry* 68: 201–2022.

Mei, W.L., Han, Z., Cui, H.B., Zhao, Y.X., Deng, Y.Y., and Dai, H.F. 2010. A new cytotoxic iridoid from *Callicarpa nudiflora*. *Natural Product Research* 10: 899–904.

Mossa, A.H., and Nawwar, G.A. 2011. Free radical scavenging and antiacetylcholinesterase activities of *Origanum majorana* L. essential oil. *Human and Experimental Toxicology* 30: 1501–1513.

Munir, A.A. 1982. A taxonomic revision of the genus *Callicarpa* L. (Verbenaceae) in Australia. *Journal of the Adelaide Botanic Gardens* 1: 5–39.

Naghibi, F., Mosaddegh, M., Motamed, S.M., and Ghobani, A. 2005. Labiatae family in folk medicine in Iran: from ethnobotany to pharmacology Iranian. *Journal of Pharmaceutical Research* 2: 63–79.

Newman, D.J., and Cragg, G. M. 2007. Natural products as sources of new drugs over the last 25 years. *Journal of Natural Products* 70: 461–477.

Newman, D.J., and Cragg, G. M. 2012. Natural products as sources of new drugs over the 30 years from 1981 to 2010. *Journal of Natural Products* 75: 311–335.

Newman, D.J., and Cragg, G. M. 2016. Natural products as sources of new drugs from1981 to 2014. *Journal of Natural Products* 79: 629–661.

Nishino, C., Kawazu, K., and Mitsui, T. 1971. Isolation and structure of maingayic acid, a piscicidal constituent of *Callicarpa maingayi*. *Agricultural and Biological Chemistry* 35: 1921–1930.

Ogunbinu, A.O., Okeniyi, S., Flamini, G., Cioni, P.L., and Ogunwande, I.A. 2013. Monoterpenoid constituents of the volatile oils of *Cynometra megalophylla* Harms., *Caesalpinia pulcherrima* L. Swartz and *Pachylobus edulis* G. Don., growing in Nigeria. *Journal of Essential Oil Research* 22: 536–539.

Okamoto, T. 2000. The protective effect of glycyrrhizinonanti-Fas antibody-induced hepatitis in mice. *European Journal of Pharmacology* 387: 229–232.

Oleszek, W., Stochmal, A., Karolewski, P., Simonet, A.M., Macias, F.A., and Tava, A. 2002. Flavonoids from *Pinus sylvestris* needles and their variation in trees of different origin grown for nearly a century at the same area. *Biochemical Systematics and Ecology* 30: 1011–1022.

Ono, K., Hiradate, S., Morita, S., Ohse, K., and Hirai, K. 2011. Humification processes of needle litters on forest floors in Japanese cedar (*Cryptomeria japonica*) and *Hinoki cypress* (*Chamaecyparis obtusa*) plantations in Japan. *Plant and Soil* 338: 171–181.

Orhan, I., Şenol, F.S., Gülpinar, A.R., Kartal, M., Şekeroglu, N., Deveci, M., and Şener, B. 2009. Acetylcholinesterase inhibitory and antioxidant properties of *Cyclotrichium niveum*, *Thymus praecox* subsp. *caucasicus* var. *caucasicus*, *Echinacea purpurea* and *E. pallida*. *Food and Chemical Toxicology* 47: 1304–1310.

Paguigan ND, Castillo DHB, Chichioco-Hernandez CL. 2014. Anti-ulcer activity of Leguminosae plants. *Arquivos de Gastroenterologia*, 51: 64–67.

Rabeta, M.S., and Nur Faraniza, R. 2013. Total phenolic content and ferric reducing antioxidant power of the leaves and fruits of *Garcinia atrovirdis* and *Cynometra cauliflora*. *International Food Research Journal* 4: 1691–1696.

Robya, M.H.H., Sarhan, M.A., Selim, K.A.H., and Khalel, K.I. 2013. Evaluation of antioxidant activity, total phenols and phenolic compounds in thyme (*Thymus vulgaris* L.), sage (*Salvia officinalis* L.), and marjoram (*Origanum majorana* L.) extracts. *Industrial Crops and Products* 43: 827–831.

Sasidharan, S., Chen, Y., Saravanan, D., Sundram, K.M., and Latha, L.Y. 2011. Extraction, isolation and characterization of bioactive compounds from plants' extracts. *African Journal of Traditional Complementary and Alternative Medicines* 8: 1–10.

Sedgley, M., and Gardner, J.A. 1989. International survey of under exploited tropical and subtropical perennials. Wageningen, ISHS. *Acta Horticulturae* p. 206.

Sen, M., and Pal, B.C. 1974. Chemical investigation of the bark of *Callicarpa arborea* (Verbenaceae). *Journal of the Indian Chemical Society* 51: 903.

Senol, F.S., Woźniak, K.S., Khan, M.T.H., Orhan, I.E., Sener, B., and Głowniak, K. 2011. An in vitro and in silico approach to cholinesterase inhibitory and antioxidant effects of the methanol extract, furanocoumarin fraction, and major coumarins of *Angelica officinalis* L. fruits. *Phytochemistry Letters* 4: 462–467.

Shai, L.J., Masoko, P., Mokgotho, M.P., Magano, S.R., Mogale, A.M., Boaduo, N., and Eloff, J.N. 2010. Yeast alpha glucosidase inhibitory and antioxidant activities of six medicinal plants collected in Phalaborwa, South Africa. *South African Journal of Botany* 76: 465–470.

Shao, Y., Hu, L.H., Sim, K.Y., and Goh, S.H. 2006. Lignanoids and diterpenoids from *Callicarpa furfuraceae*. *Helvetica Chimica Acta* 1: 64–72.

Shi, Y., Wu, C., Chen, Y., Liu, W., Feng, F., and Xie, N. 2013. Comparative analysis of three *Callicarpa* herbs using high performance liquid chromatography with diode array detector and electrospray ionization-trap mass spectrometry method. *Journal of Pharmaceutical and Biomedical Analysis* 75: 239–247.

Siciliano, T., De Tommasi, N., Morelli, I., and Braca, A. 2004. Study of flavonoids of *Sechium edule* (Jacq) Swartz (Cucurbitaceae) different edible organs by liquid chromatography photodiode array mass spectrometry. *Journal of Agricultural and Food Chemistry* 52: 6510–6515.

Simirgiotis, M.J., and Schmeda-Hirschmann, G. 2010. Determination of phenolic composition and antioxidant activity in fruits, rhizomes and leaves of the white strawberry (*Fragaria chiloensis* spp. chiloensis form chiloensis) using HPLC-DAD–ESI-MS and free radical quenching techniques. *Journal of Food Composition and Analysis* 23: 545–553.

Singh, A.K., Chanotiya, C.S., Yadav, A., and Kalra, A. 2010. Volatiles of *Callicarpa macrophylla*: a rich source of selinene isomers. *Natural Product Communications* 2: 269–272.

Singh, D., Gawande, D.Y., Singh, T., Poroikov, V. 2014. Revealing pharmacodynamics of medicinal plants using in silico approach: A case study with wet lab validation. *Computer in Biology and Medicine* 47: 1–6.

Sreelatha, S., and Padma, P.R. 2009. Antioxidant activity and total phenolic content of *Moringa oleifera* leaves in two stages of maturity. *Plant Foods for Human Nutrition* 64: 303–311.

Stintzing, F.C., Kammerer, D., Schieber, A., Adama, H., Nacoulma, O.G., and Carle, R. 2004. Betacyanins and phenolic compounds from *Amaranthus spinosus* L. and *Boerhavia erecta* L. *Zeitschrift fur Naturforschung C* 59: 1–8.

Stuchbury, G., and Munch, G. 2005. Alzheimer's associated inflammation, potential drug targets and future therapies. *Journal of Neural Transmission* 112: 429–453.

Tajudin, T.J.S.A., Johari, S. A., Mat, N., Siti-Aishah, A.B., Yusran, A.A.M., Alwi, A., and Ali, A. M. 2012. Cytotoxicity, antiproliferative effects, and apoptosis induction of methanolic extract of *Cynometra cauliflora* Linn. whole fruit on human promyelocytic leukemia HL-60 cells. *Evidence-Based Complementary and Alternative Medicine* 2012: 1–6.

Talapatra, S.K., Polley, M., and Talapatra, B. 1994. Terpenoids and related compounds. Part 32 Calliphyllin, a new diterpene from the leaves of *Callicarpa macrophylla*. *Journal of the Indian Chemical Society* 71: 527–532.

Tiwari, P., Rahuja, N., and Kumar, R. 2008. Search for antihyperglycemic activity in few marine flora and fauna. *Indian Journal of Sciences and Technology* 1: 1–5.

Topçu, G., and Gören, A. C. 2007. Biological activity of diterpenoids isolated from Anatolian Lamiaceae plants. *Records of Natural Products* 1: 1–16.

Tosun, F., and Akyuz, Ç. 1997. Flavonoids of Gonocytisus angulatus. *Pharmacy and Pharmacology Communications*, 3: 377–378.

Tu, Y., Sun, L., Guo, M., and Chen, W. 2013. The medicinal uses of *Callicarpa* L. in traditional Chinese medicine: An ethnopharmacological, phytochemical and pharmacological review. *Journal of Ethnopharmacology* 2: 465–481.

Uddin, S.J., Grice, I.D., and Tiralongo, E. 2009. Cytotoxic effects of Bangladeshi medicinal plant extracts. *Evidence-Based Complementary and Alternative Medicine* 2011: 1–7.

Ulubelen, A., Topcu, G., Chai, H.B., and Pezzuto, J.M. 1999. Cytotoxic activity of diterpenoids isolated from *Salvia hypargeia*. *Pharmaceutical Biology* 37: 148–151.

Vats, V., Grover, J.K., and Rathi, S.S. 2002. Evaluation of anti-hyperglycemic and hypoglycemic effect of *Trigonella foenum-graecum* Linn, *Ocimum sanctum* Linn and *Pterocarpus marsupium* Linn in normal and alloxanized diabetic rats. *Journal of Ethnopharmacology* 79: 95–100.

Velioglu, Y.S., Mazza, G., Gao, L., and Oomah, B.D. 1998. Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products. *Journal of Agricultural and Food Chemistry* 46: 4113–4117.

Vina, J., Lloret, L.A., Orti, R., and Alonso, D. 2004. Molecular bases of the treatment of Alzheimer's disease with antioxidants: prevention of oxidative stress. *Molecular Aspects of Medicine* 25: 117–123.

Wang, K.H., Lin, R.D., Hsu, F.L., Huang, Y.H., Chang, H.C., Huang, C.Y., and Lee, M. H. 2006. Cosmetic applications of selected traditional Chinese herbal medicines. *Journal of Ethnopharmacology* 106: 353–359.

Wang, Y.M., Xiao, H., Liu, J.K., and Wang, F. 2010. New iridoid glycosides from the twigs and leaves of *Callicarpa formosana* var. formosana. *Journal of Asian natural products research* 3: 220–226.

Watanabe, H., Miyaji, C., Makino, M., and Abo, T. 1996. Therapeutic effects of glycyrrhizin in mice infected with LP-BM5 murine retrovirus and mechanisms involved in the prevention of disease progression. *Biotherapy* 9: 209–220.

Waterman, P.G., and Faulkner, D.F. 1981. Imidazole alkaloids from *Cynometra hankei*. *Phytochemistry* 12: 2765–2767.

Williams, M.C., and Molyneux, R.J. 1987. Occurrence, concentration, and toxicity of pyrrolizidine alkaloids in *Crotalaria* seeds. *Weed Science* 35: 476–481.

Wojakowska, A., Piasecka, A., García-López, P.M., Zamora-Natera, F., Krajewski, P., Marczak, Ł., and Stobieck, M., 2013. Structural analysis and profiling of phenolic secondary metabolites of Mexican lupine species using LC–MS techniques. *Phytochemistry* 92: 71–86.

Woo, K.W., Han, J.Y., Choi, S., Kim, K.H., and Lee, K.R. 2014. Triterpenes from *Perilla frutescens* var. *acuta* and their cytotoxic activity. *Natural Product Science* 20: 71–75.

Wolfender, J.L., Verotta, L., Belvisi, L., Fuzzati, N., and Hostettmann, K. 2003. Structural investigations of isomeric oxidised forms of hyperforin by HPLC-NMR and HPLC-MSn. *Phytochemical Analysis* 14: 290–297.

Wszelaki, N., Kuciun, A., and Kiss, A. 2010. Screening of traditional European herbal medicines foracetylcholinesterase and butyrylcholinesterase inhibitory activity. *Acta Pharmaceutical* 60: 119–128.

Xu, J., Harrison, L.J., Vittal, J.J., Xu, Y.J., and Goh, S.H. 2000. Four new clerodane diterpenoids from *Callicarpa pentandra*. *Journal of Natural Products* 8: 1062–1065.

Yazaki, K. 2006. ABC transporters involved in the transport of plant secondary metabolites. *FEBS letters* 580: 1183–1191.

Yi, L.G., Gray, A.I., and Waterman, P.G. 1989. Pentacyclic triterpenes from the fruits of *Rosa sterilis*. *Journal of Natural Products* 52: 162–166.

Yilmazer-Musa, M., Griffith, A.M., Michels, A.J., Schneider, E., and Frei, B. 2012. Grape Seed and Tea Extracts and Catechin 3-Gallates are Potent Inhibitors of α-Amylase and α-Glucosidase Activity. *Journal of Agriculture and Food Chemistry* 60: 8924–8929.

Zaidan, M.R.S., Noor Rain, A., Badrul, A.R., Adlin, A., Norazah, A., and Zakiah, I. 2005. *In vitro* screening of five local medicinal plants for antibacterial activity using disc diffusion method. *Tropical Biomedicine* 2: 165–170.

Zareen, S., Choudhary, M.I., Akhtar, M.N., and Khan, S.N. 2008. α-Glucosidase inhibitory activity of triterpenoids from *Cichorium intybus*. *Journal of Natural Products 71*: 910–913.

Zhang, B.B., Yuan, D.A.I., and Zhi-Xin, L. 2011. Chemical constituents of *Saussurea eopygmaea*. *Chinese Journal of Natural Medicines* 9: 33–37.

Zhang, J., Li, B., Feng, F., Tang, Y., and Liu, W. 2010. Chemical constituents from *Callicarpa nudiflora* and their hemostatic activity. *China Journal of Chinese Material Medica* 24: 3297–3301.

Zheng, Z.P., Cheng, K.W., Chao, J., Wu, J., and Wang, M. 2008. Tyrosinase inhibitors from paper mulberry (*Broussonetia papyrifera*). *Food Chemistry* 106: 529–535.

Zhou, C., Sun, C., Chen, K., and Li, X. 2011. Flavonoids, phenolics, and antioxidant capacity in the flower of *Eriobotrya japonica* Lindl. *International Journal of Molecular Sciences* 12: 2935–2945.

Zhu, C.C., Gao, L., Zhao, Z.X., and Lin, C.Z. 2012. Triterpenes from *Callicarpa integerrima* Champ. *Acta Pharmaceutica Sinica* 1: 77–83.