CHEMICAL CONSTITUENTS AND BIOACTIVITY STUDIES OF *Bauhinia rufescens* Lam. (FABACEAE) AND *Ficus platyphylla* Del. (MORACEAE)

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

Faculty of Science
Universiti Teknologi Malaysia

JUNE 2014

To my beloved Father Late Alhaji Muhammad Sarki and my beloved mother Hajiya Hauwa Muhammad Sarki

ACKNOWLEDGEMENT

Praise belongs to almighty Allah, the lord of all being. May his peace be upon his messenger Muhammad (PBUH). My appreciation goes to my Supervisor Prof. Dr. Hasnah Mohd Sirat for her guidance, patience, motivation, support, and advices during my research.

A special thanks to Assoc. Prof. Dr. Farediah Ahmad for her kindness and her precious help. I would like to thank Mr. Rasydi and Mr. Azmi for their assistance in obtaining the NMR spectra at Faculty of Science, UTM.

My appreciation to all academic and technical staffs of Chemistry Department, UTM, as well as my fellow postgraduate colleagues; Mr. Salam, Mr. Nuzul, Mr. Shamsul, Mr. Nazifi, Mrs. Athira, Mrs. Shariha, Syafiqah, Ernie, Iman, Edeline, for their advice and encouragement. Special thanks to my parents, family, siblings and my friends for their constant support and encouragement.

Last but not the least, I would also acknowledge BUK-MacArthur Grant, Bayero University Kano, Nigeria for my doctoral fellowship. International Doctoral fellowship, UTM for partial financial support, and to the Faculty of Science Universiti Teknologi Malaysia for facilities. Thank you.

ABSTRACT

The chemical constituents and bioactivities of the stem barks and leaves of *Bauhinia rufescens* and the stem barks of *Ficus platyphylla* have been studied. Extraction of the plant materials using Soxhlet method with petroleum ether, ethyl acetate, and methanol afforded nine crude extracts. Fractionation and purification of the ethyl acetate and methanol extracts of stem bark of *B. rufescens* yielded two new compounds identified as 4-(2′-hydroxyphenethyl)-5-methoxy-2-methylphenol and bauhiniside. Compounds identified as 6-methoxy-7-methyl-8-hydroxydibenz [*b,f*]oxepin, *α*-amyrin acetate, *β*-sitosterol 3-*O*-*β*-D-xylopyranoside, menisdaurin, sequoyitol, 6-hydroxy-7,7a-dihydrobenzofuran-2(*6H*)-one, glyceryl trilinoleate, linoleic acid, stigmasterol and *β*-sitosterol were also isolated from the stem barks. The leaves extract yielded glyceryl trilinoleate, sequoyitol and bauhiniside. *F. platyphylla* yielded two new phenolic compounds from its methanolic extract which was identified as ficuside A and ficuside B. In addition, hordenine was isolated *via* alkaloid extraction, and also 3,4-dihydroxybenzoic acid anhydride, epicatechin, lupeol, lupeol acetate and *α*-amyrin acetate were identified from *F. platyphylla*. Bioactivities including antioxidant, antimicrobial, inhibitory studies on the brine shrimp larvae, tyrosinase, acetylcholinesterase and cyclooxygenase-2 were investigated. Among the nine crude extracts from the two plants, the methanol extract from the stem barks of *B. rufescens* showed a strong DPPH radical scavenging activity (14.31 µg/mL), and similar extract from *F. platyphylla* showed higher total phenolic content (719.58 µg GAE/mg), while the methanol extract from the leaves of *B. rufescens* showed the highest antioxidant value based on *β*-carotene bleaching assay (78.75%). The antimicrobial activity revealed the methanol extract from *F. platyphylla* was active against Gram-positive bacteria, *Staphylococcus aureus* (113 µg/mL) and a fungus, *Aspergillus niger* (56 µg/mL). Meanwhile, toxicity study indicated that, ethyl acetate from the leaves of *B. rufescens* was toxic against brine shrimp larvae (LC_{50} 0.06 mg/mL). The methanol extract from *F. platyphylla* was found active against tyrosinase enzyme (49.2%). Evaluation of acetylcholinesterase inhibition using the microplate and bioautographic assays showed the methanol extract from *F. platyphylla* inhibited the enzyme activity with percent inhibition of 44.7% (L_D 30.0 µg). In addition, among the isolated compounds, oxepin from the stem barks of *B. rufescens* was found to be active against *Staphylococcus aureus* (113 µg/mL) and lupeol acetate from *F. platyphylla* was found active against *Candida glabrata* (56 µg/mL). It was also found that, hordenine inhibited acetylcholinesterase activity at 58.6% (L_D 33.0 µg). Lupeol and 3,4-dihydroxybenzoic acid anhydride from *F. platyphylla* showed the highest inhibitory activity against tyrosinase (67.7%) and COX-2 (IC_{50} 0.15 µM) enzymes, respectively.

ABSTRAK

Komponen kimia dan bioaktiviti terhadap kulit batang dan daun *Bauhinia rufescens* serta kulit batang *Ficus platyphylla* telah dikaji. Pengekstrakan tumbuhan menggunakan Soxhlet dengan petroleum eter, etil asetat dan metanol telah menghasilkan sembilan ekstrak mentah. Pengasingan dan penulenan ekstrak mentah etil asetat dan metanol daripada kulit batang *B. rufescens* berjaya menghasilkan dua sebatian baru yang dikenalpasti sebagai 4-(2′-hidroksifenetil)-5-metoksi-2-metilfenol dan bauhinisida. Sebatian dikenalpasti sebagai 6-metoksi-7-metil-8-hidroksidibenz[b,f]oksepin, α-amirin asetat, *β*-sitosterol 3-*O*-*β*-D-xilopiranosida, menisdaurin, sekuoyitol, 6-hidroksi-7,7a-dihidrobenzofuran-2(6H)-on, gliseril trilinoleat, asid linoleik, stigmasterol dan *β*-sitosterol turut diasingkan daripada kulit batang. Ekstrak daun menghasilkan gliseril trilinoleat, sekuoyitol dan bauhinisida. *F. platyphylla* menghasilkan dua sebatian fenolik baru daripada ekstrak metanol dan dikenalpasti sebagai fikusida A dan fikusida B. Di samping itu, hordenin diasingkan melalui pengekstrakan alkaloid dan 3,4-dihidroksibenzoik asid anhidrida, epikatekin, lupeol, lupeol asetat dan α-amyrin asetat juga dikenalpasti daripada *F. platyphylla*. Bioaktiviti termasuk antioksidan, antimikrob, kajian perencatan ke atas larva udang air masin, tirosinase, asetilkolinesterase dan siklooksigenase-2 turut dikaji. Antara sembilan ekstrak mentah daripada dua tumbuhan, ekstrak metanol daripada kulit batang *B. rufescens* menunjukkan aktiviti perencatan radikal yang tinggi (14.31 μg/mL) dan ekstrak yang sama daripada *F. platyphylla* menunjukkan kandungan fenolik keseluruhan yang lebih tinggi (719.58 μg GAE/mg). Ekstrak metanol daripada daun *B. rufescens* menunjukkan nilai antioksidan tertinggi berdasarkan aktiviti pelunturan *β*-karotena (78.75%). Aktiviti antimikrob menunjukkan ekstrak metanol daripada *F. platyphylla* adalah aktif terhadap bakteria Gram-positif, *Staphylococcus aureus* (113 μg/mL) dan kulat *Aspergillus niger* (56 μg/mL). Sementara itu, kajian ketoksikan menunjukkan etil asetat daripada daun *B. rufescens* adalah toksik terhadap larva udang air masin (LC_{50} 0.06 mg/mL). Ekstrak metanol daripada *F. platyphylla* didapati aktif terhadap enzim tirosinase (49.2%). Penilaian perencatan asetilkolinesterase menggunakan plat mikro dan bioautografi menunjukkan ekstrak metanol daripada *F. platyphylla* merencat aktiviti enzim dengan peratus perencatan 44.7% (L_D 30.0 μg). Selain itu, antara sebatian yang diasingkan, oksepin daripada kulit batang *B. rufescens* didapati aktif terhadap *Staphylococcus aureus* (113 μg/mL) dan lupeol asetat daripada *F. platyphylla* didapati aktif terhadap *Candida glabrata* (56 μg/mL). Hordenin turut didapati menrencat aktiviti asetilkolinesterase sebanyak 58.6% (L_D 33.0 μg). Lupeol dan 3,4-dihidroksibenzoik asid anhidrida daripada *F. platyphylla* menunjukkan penrencatan aktiviti tertinggi masing-masing terhadap enzim tirosinase (67.7%) and COX-2 (IC_{50} 0.15 μM).

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LIST OF ABBREVIATIONS

α	-	Alpha
AA	-	Ascorbic Acid
Abs	-	Absorbance
Ac	-	Acetone
β	-	Beta
br	-	broad
BHT	-	Butylated hydroxytoluene
BCB	-	*β*-carotene bleaching
$BaCl_2$	-	Barium chloride
BSLT	-	Brine Shrimps Lethality Test
c	-	concentration
^{13}C	-	Carbon-13
CC	-	Column Chromatography
COSY	-	Correlation Spectroscopy
$CDCl_3$	-	Deuterated chloroform
CD_3COCD_3	-	Deuterated acetone
$CHCl_3$	-	Chloroform
cm	-	Centimeter
cm^{-1}	-	Per centimeter
1D	-	1 Dimension
2D	-	2 Dimension
δ	-	Chemical shift
d	-	doublet
dd	-	doublet of doublets
ddd	-	doublet of doublets of doublets
dq	-	doublet of quartet
dt	-	doublet of triplet

DCM	-	Dichloromethane
DEPT	-	Distortionless Enhancement by Polarization Transfer
DMSO	-	Dimethyl sulfoxide
DPPH	-	2,2-Diphenyl-1-picrylhydrazyl
EtOAc	-	Ethyl acetate
EIMS	-	Electron Impact Mass Spectrometry
ESIMS	-	Electrospray Ionization Mass Spectrometry
Et_2O	-	Diethyl ether
EtOH	-	Ethanol
FT-IR	-	Fourier Transform Infrared
γ	-	Gamma
GC	-	Gas Chromatography
GC-MS	-	Gas Chromatography-Mass Spectrometry
^1H	-	Proton
HRESIMS	-	High Resolution Electrospray Ionization Mass Spectrometry
HMBC	-	Heteronuclear Multiple Bond Correlation
HMQC	-	Heteronuclear Multiple Quantum Coherence
H_2O	-	Water
H_2SO_4	-	Sulfuric acid
Hz	-	Hertz
HCl	-	Hydrochloric acid
IR	-	Infrared
IC_{50}	-	Inhibition Concentration at 50%
J	-	coupling constant
KBr	-	Potassium bromide
K_2HPO_4	-	Potassium phosphate dibasic anhydrous
λ	-	Lamda
L	-	Liter
L_D	-	Detection Limit
lit.	-	Literature
$MgSO_4.7H_2O$	-	Magnesium sulfate heptahydrate
MIC	-	Minimum Inhibition Concentration
MMC	-	Minimum Microbicidal Concentration
MS	-	Mass Spectrometry

M^+	-	Molecular ion
m/z	-	mass to charge ion
MeOH	-	Methanol
m.p	-	melting point
$MgSO_4$	-	Magnesium sulphate
MHz	-	Megahertz
mg	-	Miligram
m	-	multiplet
min.	-	Minute(s)
mm	-	milimeter
mL	-	mililiter
NA	-	Nutrient agar
NB	-	Nutrient broth
NMR	-	Nuclear Magnetic Resonance
nm	-	nanometer
NaOH	-	Sodium hydroxide
NaCl	-	Sodium chloride
O_2	-	Oxygen
PE	-	Petroleum ether
ppm	-	parts per million
q	-	quartet
R_f	-	retention factor
rpm	-	Revolutions per minute
SD	-	Standard Deviation
s	-	singlet
t	-	triplet
td	-	triplet of doublets
t_r	-	Retention time
tr	-	trace
TLC	-	Thin Layer Chromatography
µM	-	Micro molar
UV	-	Ultraviolet
VLC	-	Vacuum Liquid Chromatography

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CHAPTER 1

INTRODUCTION

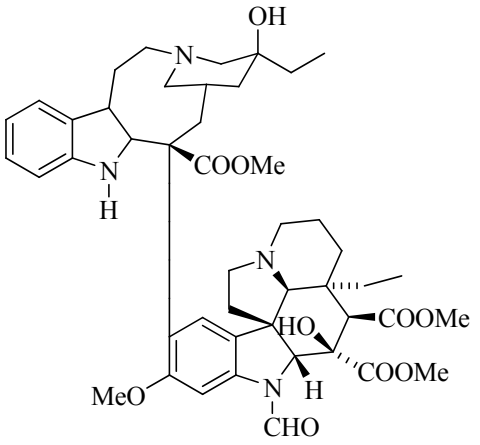
1.1 Background of Study

Over the centuries human being has relied on plants for basic needs such as food, clothing, and shelter, which are produced from matrices or storage parts of a plant i.e. leaves, woods, fibers, fruits, tubers etc. Plants have also been utilized for some other purposes, such as, poisons used for hunting, hallucinogens used for ritual purposes, stimulants for endurance, and hunger suppression, as well as medicines. The plant chemicals used for these latter purposes are largely the secondary metabolites, which are derived biosynthetically from plant primary metabolites, carbohydrates, amino acids, and lipids. The substances are also not directly involved in the growth, development, or reproduction of plants [1, 2]. These secondary metabolites can be classified into several groups according to their chemical classes, such as flavonoids, alkaloids, terpenoids and coumarins.

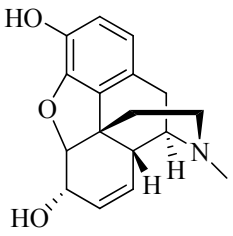
The plant medicines initially took the form of crude drugs such as tinctures, teas, poultices, powders, and other herbal formulations. The specific plants to be used and the methods of application for particular ailments were passed down through oral history. Eventually information regarding medicinal plants was recorded in herbals [3]. The first written record on the medicinal uses of plants appeared in about 2600 BC from the Sumerians and Akkaidians [4]. The “Ebers Papyrus”, the best known Egyptian pharmaceutical record, which documented over 700 drugs, represents the history of Egyptian medicine dated from 1500 BC. The Chinese “Materia Medica”, describes more than 600 medicinal plants, well documented dating from about 1100 BC [5]. Documentation of the Ayurvedic system recorded in Susruta and Charaka

date from about 1000 BC [6]. The Greeks also contributed substantially to the rational development of the herbal drugs. Dioscorides, the Greek physician (100 A.D.), described more than 600 medicinal plants in his work “De Materia Medica” [4].

Drug discovery from medicinal plants include numerous fields of inquiry and various methods of analysis. The process typically begins with a botanist who collects and identifies the plants of interest. Collection may involve species with known biological activity for which active compounds have not been isolated or may involve taxa collected randomly for a large screening program [3]. Consequently, a number of drugs have been developed from natural products, for examples, anticancer drug vincristine (**1**) from *Vinca rosea*, narcotic analgesic morphine (**2**) from *Papaver somniferum*, antimalarial drug artemisinin (**3**) from *Artemisia annua* and antibiotic penicillin G (**4**) from *Penicillium sp* [7].



(**1**)



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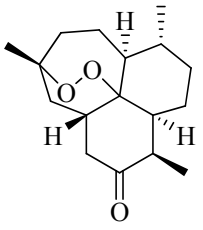
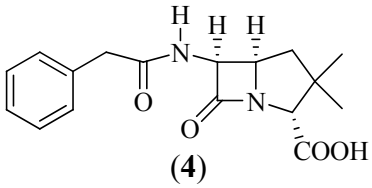


(**3**)



(**4**)

Natural products are used directly in the ‘natural’ pharmaceutical industry, consequently traditional medicine programmes are incorporated into the primary health care systems in some parts of the world, such as Mexico, China, Nigeria and Malaysia [8]. Conventional methods of drug discovery from natural products could be viewed based on two approaches. Bioassay-guided isolation, where a drug targets

are exposed to crude extracts, any evidence of activity in an extract will then go through fractionation and isolation of the active compound. Secondly, is the isolation of a number of natural compounds (compounds library) suitable for undergoing any bioactivity screening [7].

Modern drug discovery approach involving High-Throughput Screening (HTS), where, applying full automation and robotics in which hundreds of molecules can be screened using several assays within a short time, and with very little amounts of compounds. In order to incorporate natural products in the modern HTS programmes, a natural product library (a collection of dereplicated natural products) is required. This will allow dereplication; a process by which one can eliminate re-isolation of similar compounds from various extracts. In an attempt to improve the dereplication, a number of techniques are to be developed, including liquid chromatography–photo-diode-array detector (LC-PDA), liquid chromatography–mass spectrometry (LC-MS) and liquid chromatography–nuclear magnetic resonance spectroscopy (LC-NMR) [9].

1.2 Medicinal Plants

Medicinal plants have had a sort of continuous and critically controlled clinical trial, represent a primary source for the discovery of new drugs. It is, therefore, amazing that many medicinal plants from the Mediterranean (Greek–Latin–Arabic) tradition were over looked for so long by Modern medicine.

The birth of drug discovery is closely connected to the study of plant natural products and was shaped by two seminal events, the isolation of morphine (**2**) from *opium* by the pharmacist Serturner in 1817 and the introduction in the clinics of Antipyrin (phenazone) 70 years later, in 1887. The isolation of a pure compound responsible for the medicinal properties of a crude drug marked the beginning of medicinal chemistry, triggering the transition from botanical extracts to pure molecules and eventually leading to the isolation of the active substance of most drugs. The importance of medicinal plants can be ascertained from the fact that

according to the World Health Organization (WHO) estimates, 80% of the World's population fulfill their healthcare needs from phytomedicinal sources [10].

1.3 Statement of the Problem

The plants from Fabaceae and Moraceae families have been claimed to have medicinal applications which include antidiabetic, antitumor, antihypertensive, antimicrobial infections. The bioactivities exhibited are associated to the presence of various classes of secondary metabolites in the plants, such as flavonoids, alkaloids, terpenoids, phenyl propanoids and glycosides. Thus, there are need to investigate the bioactive compounds present in the *Bauhinia rufescens* (Fabaceae) and *Ficus platyphylla* (Moraceae).

1.4 Objectives of Research

The objectives of this research are to isolate the chemical constituents of *Bauhinia rufescens* (Fabaceae) and *Ficus platyphylla* (Moraceae) and to evaluate bioactivity of the crude extracts and the isolated compounds for antioxidant, antimicrobial, antityrosinase, brine shrimp lethality test, antiacetylcholinesterase and antiinflammatory inhibitors. The research is divided into two parts. The first part is to isolate and identify the phytochemicals from the dried samples followed by the study of bioactivity of crude extracts and pure compounds.

1.5 Significance of the Research

This study is to ascertain the medicinal values of *Bauhinia rufescens* (Fabaceae) and *Ficus platyphylla* (Moraceae) through phytochemicals and bioactivity studies and make necessary recommendations that could lead to a scientific evidence of their medicinal applications.

1.6 Scope of Work

This research is focused on the *Bauhinia rufescens* (Fabaceae; leaves and stem bark) and *Ficus platyphylla* (Moraceae; stem bark) for the studies on their chemical constituents and bioactivities. The dried samples will be extracted using soxhlet extraction with different organic solvents in an increasing polarity gradient. The crude extracts will be fractionated using vacuum liquid chromatography (VLC).

Purification of the fractions will be carried out by gravity column chromatography (CC) to obtain the pure compounds. An elucidation of chemical structure for the isolated compounds in pure forms will be conducted using spectroscopic techniques, which includes; UV, IR, ^{1}H NMR, ^{13}C NMR, 2D NMR (COSY, HMQC, HMBC) and mass spectrometry.

Finally, the bioactivity studies, including DPPH radical scavenging assay, total phenolic content assay, β-carotene/linoleic acid bleaching assay, brine shrimp lethality test, antibacterial, antifungal, antityrosinase, acetylcholinesterase, anti-inflammatory assays will be carried out on the crude extracts and pure compounds.

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