PHYTOCHEMICALS AND BIOLOGICAL ACTIVITIES OF *ARTOCARPUS FULVICORTEX* JARRETT

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Dedicated to..

My parents, Othman bin Taib and Rahimah bte Mohamed

My husband, Mohd Nazrul bin Mohd Amin

My sisters and brothers

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PREFACE

This thesis is the result of my work carried out in the Department of Chemistry, Universiti Teknologi Malaysia and Kuliyyah of Pharmacy, International Islamic University Malaysia between December 2007 and December 2009 under the supervision of Dr. Shajarahtunnur bte Jamil and Dr. Muhamed Taher. Parts of my work described in this thesis have been reported in the following publications:

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ABSTRACT

Phytochemical studies of the leaves and barks of *Artocarpus fulvicortex* Jarrett have been carried out. Cold extraction method was applied to obtain the crude extracts. Twelve compounds, comprising of chalcones, flavones, and triterpenes were successfully isolated from this plant. The structures of these pure compounds were determined on the basis of spectral studies including nuclear magnetic resonance, infrared, ultraviolet spectroscopies, mass spectrometry as well as by comparison with literature data. Multiple purification of the leaves crude extracts yielded a new flavonoid, identified as 5-hydroxy-2",2"-dimethylpyrano-[5",6",11",12":6,7,3',4'] flavone together with known compounds i.e. carpachromene, cycloartocarpesin, 2′-hydroxy-4,4′,6′-trimethoxychalcone, norartocarpetin, friedelin, lupeol, and β-sitosterol. Five known compounds, characterized as artobiloxanthone, artonin E, catechin, lupeol 3-acetate, and friedelin were isolated from the barks crude extracts. Evaluations on biological activities were performed by using antibacterial, antioxidant, and cytotoxic assays on the pure compounds. Artobiloxanthone and artonin E were found to have antibacterial activity against *Pseudomonas aeruginosa* and *Bacillus subtilis* with MIC values in the range of 62.5-250 µg/mL, while MBC values were at 125-500 µg/mL. Catechin and artonin E showed high ability to act as free radical scavenger with SC_{50} values of 19.4 µg/mL and 38.2 µg/mL respectively. The cytotoxic activity was done by using 3-(4,5-dimethylthiazol-2-yl)-2-5-diphenyltetrazolium bromide (MTT) assay on esophagus cancer (TE-1), lung cancer (A549), breast cancer (MCF-7), cervix cancer (C33A), and colon cancer ($CaCO_2$) cell lines. Artonin E displayed significant effect against esophagus cancer (TE-1) and colon cancer ($CaCO_2$) cell lines with the percentage of cell survival of 24.72% and 47.29% at 11 µg/mL. Carpachromene, cycloartocarpesin, 2′-hydroxy-4,4′,6′-trimethoxychalcone, friedelin, and lupeol showed moderate activity against colon cancer ($CaCO_2$) cell line with percentage of cell survival of 52 to 70%.

ABSTRAK

Kajian fitokimia ke atas daun dan kulit batang *Artocarpus fulvicortex* Jarrett telah dilakukan. Pengekstrakan secara sejuk telah dijalankan bagi mendapatkan ekstrak mentah. Dua belas sebatian yang terdiri daripada kalkon, flavon, dan triterpena telah berjaya diasingkan daripada tumbuhan ini. Struktur bagi sebatian tulen dikenalpasti berdasarkan kajian spektroskopi yang terdiri daripada spektroskopi resonans magnetik nukleus, infra merah, ultra lembayung, spektrometri jisim, dan juga perbandingan dengan data dari kajian terdahulu. Penulenan yang berulangkali terhadap ekstrak mentah daun telah memberikan flavonoid baru, dikenalpasti sebagai 5-hidroksi-2",2"-dimetilpirano-[5",6",11",12":6,7,3',4']flavon bersama dengan sebatian yang diketahui, iaitu karpakromen, sikloartokarpesin, 2′-hidroksi-4,4′,6′-trimetoksikalkon, norartokarpetin, friedelin, lupeol, dan β-sitosterol. Lima sebatian yang diketahui dan dikenalpasti sebagai artobiloxanton, artonin E, katekin, lupeol 3-asetat, dan friedelin telah diasingkan daripada ekstrak mentah kulit batang. Kajian bioaktiviti telah dilakukan ke atas sebatian tulen dengan menggunakan cerakinan antibakteria, antioksidan, dan sitotoksik. Artobiloxanton dan artonin E didapati mempunyai aktiviti antibakteria terhadap *Pseudomonas aeruginosa* and *Bacillus subtilis* dengan nilai MIC pada anggaran 62.5-250 μg/mL, sementara nilai MBC pada 125-500 μg/mL. Katekin dan artonin E menunjukkan keupayaan yang tinggi sebagai perencat radikal bebas dengan nilai 19.4 μg/mL and 38.2 μg/mL. Kajian sitotoksik telah dilakukan dengan menggunakan cerakinan 3-(4,5-dimetiltiazol-2-il)-2-5-difeniltetrazolium bromid (MTT) ke atas sel kanser esofagus (TE-1), kanser paru-paru (A549), kanser payudara (MCF-7), kanser serviks (C33A), dan kanser kolon ($CaCO_2$). Artonin E memperlihatkan kesan yang signifikan terhadap sel kanser esofagus (TE-1) dan kanser kolon ($CaCO_2$) dengan peratusan sel kanser hidup 24.72% dan 47.29% pada 11 μg/mL. Karpakromen, sikloartokarpesin, 2′-hidroksi-4,4′,6′-trimetoksikalkon, friedelin, dan lupeol menunjukkan aktiviti sederhana terhadap sel kanser kolon ($CaCO_2$) dengan peratusan sel kanser hidup antara 52 hingga 70%.

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LIST OF ABBREVIATIONS

Abs	Absorbance
$AlCl_3$	Aluminium trichloride
br	broad
CC	Column Chromatography
COSY	Correlation Spectroscopy
^{13}C	Carbon-13
$CDCl_3$	Deuterated chloroform
CD_3COCD_3	Deuterated acetone
DPPH	2,2-Diphenyl-1-picrylhydrazyl
d	doublet
dd	doublet of doublet
ddd	doublet of doublet of doublet
dt	doublet of triplet
DEPT	Distortionless Enhancement by Polarization Transfer
DMSO	Dimethylsulfoxide
EtOAc	Ethyl acetate
EIMS	Electron Impact Mass Spectrometry
Et_2O	Diethyl ether
EtOH	Ethanol
FABMS	Fast Atom Bombardment Mass Spectrometry
GC	Gas Chromatography
GC-MS	Gas Chromatography-Mass Spectrometry
^1H	Proton
HMBC	Heteronuclear Multiple Bond Correlation
HMQC	Heteronuclear Multiple Quantum Coherence
Hz	Hertz

HCl	Hydrochloric acid
IR	Infrared
IC_{50}	Inhibition Concentration at 50%
J	coupling constant
KBr	Potassium bromide
KI	Kovats Index
lit.	Literature
LWT	Lebensm.-Wiss. u.-Technol / Food Science and Technology
MIC	Minimum Inhibition Concentration
MBC	Minimum Bactericidal Concentration
MS	Mass Spectrometry
mM	milimolar
m/z	mass to charge ion
MeOH	Methanol
mp	melting point
$MgSO_4$	Magnesium sulphate
MHz	Megahertz
m	multiplet
NMR	Nuclear Magnetic Resonance
nm	nanometer
NaOH	Sodium hydroxide
NaCl	Sodium chloride
Ph	Phenyl
PE	Petroleum ether
ppm	parts per million
q	quartet
RDA	Retro Diels-Alder rearrangement
R_f	retention factor
SD	Standard Deviation
SFE	Supercritical Fluid Extraction
s	singlet
sh	shoulder
t	triplet
tr	trace

TLC	Thin Layer Chromatography
UV	Ultraviolet
VLC	Vacuum Liquid Chromatography
μM	micromolar
δ	chemical shift
$[\alpha]_D$	specific rotation
c	concentration

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CHAPTER 1

INTRODUCTION

1.1. General Introduction

Medicine and natural products have been closely linked through the use of traditional medicines for thousands of years. Fossil records date human use of plants as medicines at least to the Middle Paleolithic age some 60,000 years ago [1]. Plants have formed the basis of sophisticated traditional medicine systems which have been used in countries such as China and India [2]. These plant-based systems continue to play an essential role in health care, and it were estimated by the World Health Organization that approximately 80% of the world's inhabitants rely mainly on traditional medicines for their primary health care [3].

Traditional medicine is widespread throughout the world and Malaysia is no exception. Traditional medicine plays an important role in the Malaysian society and is expected to play more important role in the future. Malaysia with its tropical rain forest is richly endowed with flora abounds with plants of diverse nature and uses. Moreover, it has a good potential to be developed into various useful natural products. The past ethnomedical surveys suggest that at least about 20% of the estimated total of higher plants of 15,000 species comprise plants which have been reported to possess medicinal and other therapeutic properties [4]. It offers unlimited opportunity for scientists' who are interested in the search of biologically active compounds from plants. There are many reports of these plants which exhibit interesting biological properties including cytotoxic, antimicrobial [5], anti-inflammatory, antioxidant [6], antiplatelet [7], and anti HIV activities [8].

Clinical, pharmacological, and chemical studies of these natural products, which were derived predominantly from plants, were the basis of most early medicines such as aspirin, digitoxin, morphine, quinine, and pilocarpine [9]. An analysis of the origin of the drugs developed between 1981 and 2002 showed that natural products comprised 28% of all new chemical drugs launched into the market. In addition, 24% of these new chemical drugs were synthetic or natural mimic compounds, based on the study of pharmacophores related to natural products. This phenomenon suggests that natural products are important sources for new drugs and also good lead compounds suitable for further modification during drug development [10].

To date, pharmacologists, microbiologists, botanists, and natural-products chemists are combing for phytochemicals and lead molecule that could be developed for the treatment of various diseases.

1.2. Family Moraceae

Moraceae is a family of flowering plants in the order Urticales. Moraceae is often called the mulberry family or fig family. They are usually woody plants with alternate leaves and presence of milky latex. Most species have multiple fruits due to combination of different flowers [11]. This plant family which is distributed in the tropical and subtropical regions of Asia, comprises of 1,400 species divided among 60 genera. Only 9 genera and 137 species could be found in Malaysia, distributed from lowlands to mountain forests [12]. A few of these genera such as *Morus*, *Ficus*, and *Artocarpus* have received the majority of phytochemical investigations and in some cases directed towards the isolation of biologically active constituents.

1.3 Genus *Artocarpus*

Artocarpus is the most commonly encountered genus, representatives of the Moraceae. The genus *Artocarpus* consists of approximately 50 species, which are

native to the region of Southeast Asia. However, the greatest diversity is in Indonesia, Malaysia, and the Philippines [13]. There are 47 species of *Artocarpus* that could be found in Malaysia including the cultivated plants as showed in **Table 1.1**. *Artocarpus* are evergreen or deciduous small plants in which only 20 species are large monoecious trees, with all parts containing white latex. Economically, the genus of *Artocarpus* is appreciable importance as a source of edible fruits, such as *A. heterophyllus* (jackfruit), *A. integer* (cempedak), and *A. communis* (breadfruit). Many species of *Artocarpus* also yield fairly good timber. Some members of this genus have been used medicinally to treat various diseases such as malaria, fever, dysentery and tuberculosis [14].

Table 1.1: *Artocarpus* Species Found in Malaysia [12]

Species	**Local Name**
A. anisophyllus	Keledang babi
A. bracteata	Ipoh
A. communis	Sukun
A. dadah	Tampang bulu
A. denisonian	Nangka pipit
A. elasticus	Terap nasi
A. fulvicortex	Tampang gajah
A. glaucus	-
A. gomezianus	Tampang hitam
A. heterophyllus	Nangka
A. hispidus	Temponek
A. integer	Cempedak
A. integer var. silvestris	Bangkok
A. kemando	Pudu
A. lakoocha	Keledang beruk
A. lanceifolius	Keledang
A. lowii	Miku
A. maingayi	Pudu
A. nitidus	Tampang
A. odoratissima	-
A. penduncularis	Miku
A. rigidus	Temponek
A. scortechinni	Terap hitam
A. teysmanii	-

1.3.1 *Artocarpus fulvicortex* Jarrett

Artocarpus fulvicortex Jarrett is one of the Malaysian rare *Artocarpus* species. *A. fulvicortex* Jarrett is locally known as 'keledang tampang gajah'. It is a medium-sized tree with orange brown or reddish brown bark. *A. fulvicortex* can be found in the lowland forests at Perak, Pahang, Negeri Sembilan, Melaka, and Terengganu [12]. However, there is no information on the traditional and medicinal usage of this plant.

1.4 Problem Statement

The *Artocarpus* species is noted as an abundant source of isoprenylated flavonoids, a class of compounds which has the chemical and biological properties attraction. Thus, during the last couple of years, many researches on *Artocarpus* species have been carried out especially by researchers from Japan, Taiwan, and Indonesia. Many phenolic constituents have been discovered and characterized. These metabolites were also significantly bioactive. This information throws a vivid light on *Artocarpus* as medicinal plants. However, there are only few phytochemical and biological activity studies on Malaysian *Artocarpus* species. Within the scope of continuation in search for bioactive compounds from natural plants, this research will focus on the phytochemical and biological activity studies of *Artocarpus fulvicortex* Jarrett.

1.5 Research Objectives

The objectives of this research are to determine the chemical constituents of *A. fulvicortex* which involves isolation and characterization of phytochemicals as well as bioactivity screening since there is no report on the phytochemical study of *A. fulvicortex* Jarrett.

1.6 Scope of Study

This research will focus on two parts of *A. fulvicortex* Jarrett plant which are the leaves and the barks. Several approaches will be endeavoured in order to determine the chemical constituents of *A. fulvicortex* Jarrett. Basically, the dried samples of leaves and barks of *A. fulvicortex* Jarrett will be extracted by using different polarity of organic solvents at room temperature. The crude extracts obtained after removal of solvents will be fractionated into several fractions based on the polarity using vacuum column chromatographic technique. The chemical constituents of each fraction will be analyzed using thin layer chromatography and will then be purified by using gravity column chromatography and recrystallisation to obtain the pure compounds. The structures of the pure compounds will be characterized on the basis of spectroscopic methods including MS, IR, ^{1}H NMR, ^{13}C NMR, 2D NMR (COSY, HMQC, HMBC) and UV.

The evaluation of the biological activities of the crude extracts and pure compounds will be carried out by using several bioassays. The crude extracts and the pure compounds will be screened for antibacterial, antioxidant, and cytotoxic activities. The antibacterial activity will be tested using disc diffusion method with strains of Gram +ve; *Bacillus subtilis*, *Staphylococcus aureus* and Gram –ve bacteria; *Pseudomonas aeruginosa* and *Escherichia coli*. The antioxidant activity will be performed by using 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay and measured by UV spectrophotometer. While, 3-(4, 5-dimethylthiazol-2-yl)-2-5-diphenyltetrazolium bromide (MTT) assay was used to evaluate the cytotoxic activity.

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