UNIVERSITI PUTRA MALAYSIA

CHEMICAL CONSTITUENTS OF Syzygium aquem (MYRTACEAE) AND Dysoxylum acuntangulum (MELIACEAE)

SITI NOOR KAMILAH BINTI HAJI MOHAMAD

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CHEMICAL CONSTITUENTS OF *Syzygium aquem* (MYRTACEAE) AND *Dysoxylum acuntangulum* (MELIACEAE)

By

SITI NOOR KAMILAH BINTI HAJI MOHAMAD

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science.

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of requirements for the Master of Science

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SITI NOOR KAMILAH BINTI HAJI MOHAMAD

February 2014

Chairman : Professor Mawardi Rahmani, PhD

Faculty : Science

Two plant species, *Syzygium aquem* and *Dysoxylum acutangulum* were phytochemically studied. The chemical studies on the leaves and twigs of *Syzygium aquem* involves extraction using three organic solvents of different polarity and isolation of compounds by using several chromatographic techniques including gravity column chromatography. The structures of the compounds were elucidated by various spectroscopic techniques including UV, IR, EIMS and NMR.

Detail study on the chloroform crude extract twig and leaves of *Syzygium aquem* afforded three compounds where one of the compounds was identified as a chalcone, 2’,4’-dihyroxy-3’,5’-dimethyl-6’-methoxychalcone **(1)**, obtained as orange needle-shaped crystals. The other two compounds are urs-12-en-3β-ol **(30)** and lup-20(29)-en-3β-ol **(31)**.

Chemical investigation on the hexane crude extract leaves and twigs of the plant have resulted a flavanone, 5-hydroxy-7-methoxy-6,8-dimethylflavanone **(26)**, friedelin **(28)**, and two phytosterols, stigmasterol **(30)** and β-sitosterol **(32).** A chrotacumine C **(33)** were isolated from root bark of *Dysoxylum acutangulum.*

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

KANDUNGAN KIMIA DARIPADA *Syzygium aquem* DAN *Dysoxylum acutangulum*

Oleh

SITI NOOR KAMILAH BINTI HAJI MOHAMAD

Februari 2014

Pengerusi: **Profesor Mawardi Rahmani, PhD**

Fakulti: **Sains**

Dua jenis spesis tumbuhan, *Syzygium aquem* dan *Dysoxylum acutangulum* telah dikaji secara fitokimia. Kajian kimia ke atas daun dan ranting *Syzygium aquem* melibatkan pengestrakan yang menggunakan tiga pelarut organik yang berbeza kekutubannya dan pemencilan sebatian dengan menggunakan beberapa teknik kromatografi termasuklah kromatografi turus graviti. Struktur-struktur sebatian ini telah dikenalpasti dengan menggunakan pelbagai kaedah spektroskopi termasuk UV, IR, MS dan NMR.

Kerja pemencilan terhadap ekstrak bahan mentah kloroform daun dan rating tumbuhan itu telah menghasilkan tiga sebatian sebatian dimana salah satunya dikenalpasti sebagai kalkon, 2’,4’-dihidrosi-3’,5’-dimetil-6’-metoksikalkon **(1)**, didapati dalam bentuk kristal oren. Dua lagi sebatian tersebut ialah urs-12-en-3β-ol **(30)** dan lup-20(29)-en-3β-ol **(31)**. Kajian terperinci ke atas ekstrak bahan mentah hexane daun dan ranting telah menghasilkan 5-hidroksi-7-metoksi-6,8-dimetilflavanon **(26)**, friedelin **(28)**, dan dua fitosterol, stigmasterol **(27)** dan β-sitosterol **(29).** Satu krotakumine C (**32**) telah dipencilkan daripada kulit akar *Dysoxylum acutangulum.*

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

Mawardi Rahmani, PhD
Professor
Faculty of Science
Universiti Putra Malaysia
(Chairman)

Intan Safinar Ismail, PhD
Associate Professor
Faculty of Science
Universiti Putra Malaysia
(Member)

Amin Ismail, PhD
Professor
Faculty of Medical and Health Science
Universiti Putra Malaysia
(Member)

BUJANG BIN KIM HUAT, PhD
Professor and Dean
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Universiti Putra Malaysia

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Signature: ________________
Name of Member of Supervisory Committee: ________________

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

α	Alpha
β	Beta
δ	Chemical shift in ppm
γ	Gamma
µg	Micro gram
brs	Broad singlet
^{13}C	Carbon-13
$CHCl_3$	Chloroform
$CDCl_3$	Deuterated chloroform
CD_3OD	Deuterated methanol
COSY	Correlated Spectorscopy
d	Doublet
dd	Doublet of doublet
DEPT	Distortionless Enhancement by Polarization Transfer
DMSO	Dimethylsulfoxide
dt	Doublet of triplet
EtOAc	Ethyl acetate
EtOH	Ethanol
EI-MS	Electron Ionization Mass Spectroscopy
g	Gram
GC	Gas Chromatography
GC-MS	Gas Chromatography- Mass Spectroscopy
^{1}H	Proton
HMBC	Heteronuclear Multiple Bond Connectivity by 2D Multiple Quantum
HPLC	High Performance Liquid Chromatography
HR-MS	High Resolution Mass Spectroscopy

HSQC	Heteronuclear Single Quantum Coherence
Hz	Hertz
IR	Infra Red
J	Coupling constants in Hz
L	Litre
m	Multiplet
$(CD_3)_2CO$	Deuterated Acetone
MeOH	Methanol
m.p	Melting point
MS	Mass Spectrum/Spectra/Spectrometer
NMR	Nuclear Magnetic Resonance
ppm	Parts per million
s	Singlet
t	Triplet
TLC	Thin Layer Chromatography
UV	Ultra Violet
WHO	World Health Organization

CHAPTER I

INTRODUCTION

1.1 General Introduction

Organic compounds are found in various fungi, micro-organism, plants or animals. Some of these compounds can be found in many different organisms. Sometimes these compounds can only be found in specific species only. A natural product is a study on organic compounds and considered as a major driving force in development of organic chemistry and medicinal chemistry. Plants that are used in traditional medicine provide some of the first prototype drugs used clinically in the treatment of wide variety of diseases. The need to purify natural products from complex mixtures and to determine their structures have led to the development of more sophisticated methods for separations of compounds and structural analysis by chemical, and spectroscopic procedures. Some of the organic compounds are useful for biological activity and perform various functions in nature.

According to the World Health Organization (WHO), as much as 80 % of the world's population depends on traditional medicine for their primary health care needs (Cordell, 1995). The major part of traditional therapy involves the use of plants extracts. Today, traditional medicine still remains a popular method of treatment. Plants are almost exclusive source of medicine for the majority of the world populations, especially in the developing countries. No accurate data are available to assess the value and extent of the use of plants or active principles derived from them in the health care systems throughout the world.

Over half of all deaths in the United States are caused by diseases of the heart and malignant neoplasm as stated by the Centers of Disease Control and Prevention (Minino et al., 2006). A diet which is high in vegetables and fruits suggested by epidemiological evidences are linked to a reduced incidence of heart disease, cancer, and some neurodegenerative disorders (Arts and Hollman, 2005).

Mammalian system naturally produced reactive oxygen species (ROS) as a result of oxidative metabolism. Reactive oxygen species damage cell membranes and DNA, cancerous mutation, and the oxidation of low-density lipoprotein are major factors in the promotion of heart disease. In chronic inflammatory disease, as well as the etiology of cancers and heart disease, inflammation plays a major factor (Hu and Willett, 2002). Endogenous antioxidants can balance oxidative damage. Nutritive and non-nutritive elements from food provided are one of the additional protections for disease chemoprevention.

According to the (Art and Hollman, 2005) in the prevention of oxidative and inflammatory disease, colourful fruits are a potentially rich source of many dietary phenolic antioxidants and are believed to play an important role. Anthocyanins pigments contain in the fruit colour plants are responsible for many of the bright fruits and flowers colours. It acts as a strong antioxidants and anti-inflammatories, with antimutagenic and cancer chemopreventative activities (Reynertson et al., 2006).

1.2 The Family of Myrtaceae

The plant family Myrtaceae is a pan-tropical plant and widely found in South America, Southeast Asia, and Australia. The plants in the family can generate economic returns for these countries in food production, agricultural crops, and ornamentals, such as the Mediteranean genus *Myrtus* (myrtle), spices such as clove (*Syzygium aromaticum*) and bay rum (*Pimenta racemosa*). Another example of fruit plants under this family are *Psidium* (guavas), *Myrciaria, Eugenia, Syzygium, Plinia* and *Luma*.

Syzygium species are known for their medicinal properties. *Syzygium aqueum* is the watery rose apple or water apple. It is originated from south of India. It is still grown wild in India and parts of Malaysia. The fruits have an uneven shape, being wider at the apex than base. The colour varies from white to bright pink. It is, crispy and watery flesh makes a good thirst quencher. Although they have a high water content, their skins are full of fruit sugars and vitamin A. Other names include: '*jambu air'* (Malaysia/Indonesia) and '*tambis*' (Philippines). The samples of *Syzygium aqueum* for this research were collected from Bandar Baru Bangi, Selangor.

In this study, twigs and leaves of *syzygium aqueum* was investigated in detail. The purposes of this research are to extract and identify the chemical constituents of twigs and leaves *syzygium* aquem species using chromatographic and modern spectroscopic methods. Some members of this genus have been investigated or studied in detail, however, *syzygium aquem* have not been well studied especially twigs and leaves parts. Thus, there is a need to identify bioactive compounds from this genus for effective drug development (Chattopadhayay et al., 1998).

Figure 1.1: The Fruits of *Syzygium aquem*

Figure 1.2: The Buds of *Syzygium aquem*

Figure 1.3: The Tree of *Syzygium aquem*

1.3 The Family of Meliaceae

This family comprises of 51 genera and about 575 species of trees and shrubs, native to tropical and subtropical regions also known as mahogany family of flowering plants. Most members of the family have fruit which are fleshy and coloured or leathery and also have large compound leaves. The leaflets arranged in the form of a feather, and branch flower clusters.

The bark of *dysoxylum acutangulum* was chosen as the second plant for this research. The plant was collected in Terengganu, Malaysia. In Sumatera, Indonesia seeds of *Dysoxylum acutangulum* have been traditionally used as fish-poison. The active principles of this plant have been investigated by monitoring the toxicity against a species of fish, *Oryzias latipes,* with the isolation of a phenolic sesquiterpene as a major toxic constituent. The compound showed a significant fish- toxicity against *Oryzias latipes* at 5 ppm concentration and moderate antibacterial activity against gram-positive bacteria, such as *Staphylococcus aureus*, *Candida albicans* and *Trichophyton mentagrophytes* at 5-20 ppm (MIC), but it is ineffective against Gram-negative bacteria, such as *Esherichia coli* or *Pseudomonas aeruginosa* (Nishizawa et al., 1983).

Figure 1.4: The Tree of *Dysoxylum acutangulum*

Figure 1.5: The Leaves of *Dysoxylum acutangulum*

Figure 1.6: The Barks of *Dysoxylum acutangulum*

1.4 Objectives of study

1. To extract and isolate the chemical constituents of the twigs and leaves of *Syzygium aquem* and bark of *Dysoxylum acutangulum.*
2. To identify and elucidate the structure of the compounds by using spectroscopic methods.

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