



UNIVERSITI PUTRA MALAYSIA

***PHYTOCHEMISTRY OF *Garcinia rostrata* Hassk. ex Hook.f. AND
Garcinia nervosa Miq.***

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PHYTOCHEMISTRY OF *Garcinia rostrata* Hassk. ex Hook.f. AND *Garcinia nervosa* Miq.

By

WONG KA WOONG

**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 the thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of requirement for the degree Master of Science

PHYTOCHEMISTRY OF *Garcinia rostrata* Hassk. ex Hook.f. AND *Garcinia nervosa* Miq.

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July 2017

Chairman: Professor Gwendoline Ee Cheng Lian, PhD
Faculty: Science

Garcinia is a plant genus from the family Clusiaceae. The genus *Garcinia* is known to be a rich source of phenolic compounds like xanthenes, benzophenones and flavonoids. These compounds were reported to have good biological activities and they have potential to be drug candidates. *Garcinia* plants are available in our country especially in Sarawak, and many have still yet to be studied. Literature search indicated no previous reports on chemical compounds from *Garcinia rostrata*. Large scale extractions of the stem bark of *G.rostrata* and *G.nervosa* were conducted using conventional solvent extraction method at room temperature for three days. The isolation and purification of the extracts obtained were carried out by a combination of various chromatographic techniques such as vacuum column chromatography, gravity column chromatography and Thin-layer Chromatography (TLC). The structural elucidations of the pure compounds isolated were assisted by numerous spectroscopic methods including Nuclear Magnetic Resonance (NMR), Gas Chromatography-Mass Spectrometry (GC-MS), Infrared Spectroscopy (IR), Ultra Violet Spectroscopy (UV) and also by comparison with literature data. The crude extracts obtained were tested for their anti-bacterial activities.

Phytochemical studies on *G.rostrata* afford six known xanthenes: ananixanthone (**42**), 6-deoxyjacareubin (**43**), 6-deoxyisojacareubin (**44**), 8-deoxygartanin (**45**), 1,7-dihydroxyxanthone (**21**) and 1,3,7-trihydroxyxanthone (**46**) along with two common triterpenoids stigmasterol (**24**) and β -sitosterol (**23**).

Detail chemical studies on *Garcinia nervosa* led to isolation of one new xanthone, garnerxanthone (**47**), three known xanthenes: 6-deoxyisojacareubin (**44**), 1,5-dihydroxyxanthone (**20**) and 12b-hydroxy-des-D-garcigerrin A (**32**) as well as two common triterpenoids stigmasterol (**24**) and β -sitosterol (**23**).

The ethyl acetate extracts of both plants and the acetone extract of *G.nervosa* showed very significant activities against *Bacillus subtilis*, *Bacillus cereus*, *Bacillus megaterium* and *Bacillus pumilus*. On the other hand, the acetone extract of *G.rostrata*

showed very significant activities against *B.subtilis* and *B.megaterium*. The other extracts showed results comparable to the positive control used against the bacteria tested. Extracts that showed good activities can be used in anti-bacterial formulations.



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

FITOKIMIA *Garcinia rostrata* Hassk. ex Hook.f. DAN *Garcinia nervosa* Miq.

Oleh

WONG KA WOONG

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Garcinia merupakan genus tumbuhan daripada keluarga Clusiaceae. Genus *Garcinia* dikenali sebagai sumber yang kaya dengan sebatian fenolik seperti xanton, benzofenon dan flavonoid. Sebatian tersebut dilaporkan mempunyai aktiviti biologi yang baik dan mereka berpotensi menjadi calon dadah. Tumbuhan *Garcinia* boleh didapati di negara kita terutamanya di Sarawak, dan kebanyakannya masih belum dipelajari. Kajian lepas tidak menunjukkan sebarang laporan mengenai sebatian kimia daripada *Garcinia rostrata*. Pengekstrakan skala besar-besaran kulit batang *G.rostrata* dan *G.nervosa* telah dijalankan dengan menggunakan pengekstrakan pelarut pada suhu bilik selama tiga hari. Kajian literatur menunjukkan bahawa tidak ada laporan mengenai bahan kimia diasingkan daripada *G.rostrata* sebelum ini. Pengasingan dan penulenan daripada ekstrak yang diperolehi dilakukan oleh gabungan pelbagai teknik kromatografi seperti kromatografi turus vakum, kromatografi turus graviti dan kromatografi lapisan nipis (TLC). Penentuan struktur sebatian yang diperolehi dibantu oleh pelbagai kaedah spektroskopi termasuk Resonans Magnetik Nuklear (NMR), Kromatografi Gas-Spektrometer Jisim (GC-MS), Spektroskopi Inframerah (IR), Spektroskopi Ultraungu-nampak (UV) dan juga oleh perbandingan dengan data literatur. Ekstrak mentah yang diperolehi telah diuji untuk mengetahui aktiviti anti-bakteria mereka.

Kajian fitokimia pada *G.rostrata* menghasilkan enam xanton yang telah dikenali: ananixanton (42), 6-deoksijacareubin (43), 6-deoksiisojacareubin (44), 8-deoksigartanin (45), 1,7-dihidroksixanton (21) and 1,3,7-trihidroksixanton (46) bersama-sama dengan dua triterpenoid biasa: stigmasterol (24) and β -sitosterol (23). Kajian terperinci kimia pada *G.nervosa* memperoleh satu xanton baru, garnerxanton (47), tiga xanton yang telah dikenali 6-deoksiisojacareubin (44), 1,5-dihidroksixanton (20) and 12b-hidroksi-des-D-garcigerrin A (32) bersama dengan dua triterpenoid biasa: stigmasterol (24) and β -sitosterol (23).

Ekstrak etil asetat kedua-dua tumbuhan dan ekstrak aseton *G.nervosa* menunjukkan aktiviti yang sangat ketara terhadap *Bacillus subtilis*, *Bacillus cereus*, *Bacillus*

megaterium dan *Bacillus pumilus*. Sebaliknya, ekstrak aseton *G.rostrata* menunjukkan aktiviti yang sangat ketara terhadap *B.subtilis* dan *B.megaterium*. Ekstrak lain menunjukkan keputusan yang setanding dengan kawalan positif digunakan terhadap bakteria yang telah diuji. Ekstrak yang menunjukkan aktiviti yang bagus boleh digunakan dalam formulasi anti-bakteria.



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I certify that a Thesis Examination Committee has met on 14 July 2017 to conduct the final examination of Wong Ka Woong on his thesis entitled "Phytochemistry of *Garcinia rostrata* Hassk. ex Hook.f. and *Garcinia nervosa* Miq." 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 Master of Science.

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LIST OF ABBREVIATIONS

α	alpha
β	beta
γ	gamma
<i>s</i>	singlet
<i>d</i>	doublet
<i>t</i>	triplet
<i>q</i>	quartet
<i>m</i>	multiplet
<i>dd</i>	doublet of doublet
<i>dt</i>	doublet of triplet
^1H	proton
^{13}C	carbon-13
λ_{max}	maximum wavelength in nm
δ	chemical shift in ppm
ppm	part per million
<i>J</i>	coupling constant in Hz
Hz	hertz
R_f	retention factor
M^+	molecular ion
mp	melting point
g	gram
kg	kilogram
mg	milligram
$^{\circ}\text{C}$	degree in Celsius
m	meter
cm	centimeter
nm	nanometer
L	liter
mL	milliliter
μL	microliter
<i>m/z</i>	mass per charge
IR	Infrared
FTIR	Fourier Transform Infra-Red
MS	Mass Spectrum
GC-MS	Gas Chromatography-Mass Spectrometry
EIMS	Electron Ionization Mass Spectroscopy
LCMS-qToF	Liquid chromatography mass spectrometry - quadrupole-Time of Flight
TLC	Thin Layer Chromatography
UV-Vis	Ultraviolet Visible
NMR	Nuclear Magnetic Resonance
DEPT	Distortionless Enhancement by Polarization Transfer
HMQC	Heteronuclear Quantum Coherence
HMBC	Heteronuclear Multiple Bond Correlation
COSY	Correlated Spectroscopy
CHCl_3	chloroform
CDCl_3	deuterated chloroform
EA	ethyl acetate

ETOAc
MeOH
EtOH

ethyl acetate
methanol
ethanol



CHAPTER 1

INTRODUCTION

1.1 General Introduction

Plants are rich in chemical constituents which are very useful in drug discovery. Natural products are organic and inorganic compounds found in plants (leaves, needles, bark, roots, flowers and seeds) and microbial organisms in highly diverse and sometimes extreme conditions. A majority of natural products are secondary metabolites, produced by microorganisms and plants for the purpose of protection, procreation and survival against herbivores and other interspecies defense mechanisms (Sarker and Nahar, 2012). Therefore, it is not surprising that some natural products will contain toxic compounds harmful to human and animals.

The search for fine natural products has been carried out since prehistoric times and new compounds are still being discovered till now. This is because there are many plants that have not been studied yet. Malaysia is one of the 12 mega diversity countries that are known to be rich in phanerogamic and cryptogamic flora. The biodiversity of Malaysia's plant resources contain more than 15000 species of higher plants. From this, less than 10% of them have medicinal values and many of them have not been evaluated for their potentials yet (Goh, 1988).

Although natural products have been widely used for thousands of years, their modern and systematic studies did not begin until the late 18th century. The development of modern isolation methods, such as various analytical techniques and preparative chromatographic methods made it possible to separate compounds present in extremely small quantities, while the development of spectroscopic techniques such as UV, NMR, MS, IR, etc. have led to rapid structural elucidation even with trace quantity (Kumar and Chopra, 2005).

Nowadays, natural products are applied in many fields to enhance the products and their efficiencies in the agro-chemical and pharmaceuticals field. They are used as herbicides, anti-parasitic agents and bio-insecticides, anti-bacterial (Zapf *et al.*, 2005), anti-inflammatory (Kwok *et al.*, 2001), anti-oxidant agents (Velioglu *et al.*, 1998) and many other uses to treat diseases. The pharmaceutical industry isolates natural products as lead compounds that may potentially, after chemical modification, give rise to new compounds with better bioactivity.

1.2 Botanical Aspect of the Plants

1.2.1 The Family Clusiaceae

The family Clusiaceae comprises about 43 genera and 1610 species of tropical tree and shrubs. There are four most common genera which include *Garcinia*, *Calophyllum*,

Mesua and *Mammea*. Members of the Clusiaceae family usually have broad-ended, oblong leaves; these may be leathery and have a strong, central vein from which branch many delicate, horizontal veins. The plants have resinous, sticky sap, flowers with numerous stamens often united in bundles, and separate petals and sepals. Male and female organs often occur in separate flowers. It has many species of economic importance, ranging from large trees grown for their timber, to those grown for drugs, dyes, resins, and essential oils used in cosmetics. Some of the Clusiaceae plants produce edible fruits, including *Garcinia mangostana*, (Mangosteen) and *Mammea americana* (Mamme Apple).

1.2.2 The Genus *Garcinia*

Genus *Garcinia* is native to Asia, Australia, tropical and southern Africa, and Polynesia. The species of *Garcinia* genus are evergreen trees and shrubs, dioecious and in several cases apomictic. It consists of 240 species of trees and shrubs found throughout the tropics, but especially in the Paleotropics. The tree is large, having elliptic, oblong with deep-green glossy leaves up to 5–8 cm long and 2–3 cm broad. The flowers are fleshy, dark pink, solitary or in spreading cluster. The fruit is brownish or purple about the size of an orange, marbled with yellow, and is crowned by the 4-parted, stalkless stigma. The fruit pulp is juicy, white, and delicious in taste and odor, and consists of 6–8 seeds (Hemshekhar *et al.*, 2011).



Figure 1.1: Sketch diagram of *Garcinia rostrata*.

1.2.3 The species *Garcinia rostrata*

Garcinia rostrata is a medium-sized glabrous tree which can grow up to 90 feet high and 3 feet girth. The bark surface is smooth, grey-brown, becoming shallowly cracked and flaked. In addition, the exudate is pale yellow in colour, opaque and sticky. The leaf blade of *G.rostrata* is elliptic to frequently obovate, thin coriaceous and drying pale tawny. Besides, it has flowers with four sepals and four petals. The trees of *G.rostrata* are distributed in West Java, Sumatra, Malaya, Tennasserim, and Borneo. They are frequently found in Sarawak on leached yellow sandy clay soils and on skeletal soils to at least 1400 m altitude. They are occasionally found on podzols in Health forest in Brunei (Ridley, 1922; Whitmore and Ng, 1989).

1.2.4 The species *Garcinia nervosa*

Garcinia nervosa is a medium-sized unbuttressed tree. It can grow up to 100 feet high and 5 feet girth. The tree bark surface is smooth, hoop bark and pale brown colour. Moreover, the exudate is pale yellow, opaque and sticky. The twig and leaf undersurface are sometimes caducous puberulent. The leaf blade of *G.nervosa* is very large but variable in size and shape. It is thickly coriaceous and pale yellowish brown. Besides, the flowers have 4-6 sepals and 5 petals. It has fruits with ripening yellow colour and 1-5 brown seeds imbedded in pale orange pulp. The trees of *G.nervosa* are distributed in Sumatra, Malaya and Borneo. They are scattered in primary Mixed Dipterocarp forest, especially near streams, and in hill forest around 2000 m altitude (Corner, 1952; Ridley, 1922; Whitmore and Ng, 1989).



Figure 1.2: Stem bark, leaves, fruits and flower of *Garcinia nervosa*.

1.3 Problem Statement

Borneo is frequently acknowledged for being an important centre of plant diversity in the world. It is conservatively estimated to harbor 10000-12000 species of flowering plants, representing about 5-6% of the world total (Mat-Salleh *et al.*, 1992). There are up to 80% of endemic species in Borneo occurring in Sabah and Sarawak. The presence of high species diversity in the natural forests of Sabah and Sarawak indicates that there are countless natural products waiting to be investigated. However, research on higher plant as a natural source of drugs is still undiscovered. It was identified that 2000 species in Sabah and Sarawak are being harvested for medicinal properties (Perry and Metzger, 1980). So, there is still plenty of work needed to be carried out by natural product chemists on our natural resources.

The genus *Garcinia* is often being studied because of its potential in various medicinal properties such as anti-oxidant, anti-inflammatory anti-immunosuppressive and anti-microbial activities (Aravind *et al.*, 2016; Ilyas *et al.*, 1994; Ilyas *et al.*, 2002; Jamila *et al.*, 2015; Parveen *et al.*, 2004; Tran *et al.*, 2016). Furthermore, the genus *Garcinia* has been reported mostly for its rich secondary metabolites such as xanthenes, flavonoids and benzophenones which are responsible for its medicinal properties. Although *Garcinia* plants have been studied by many researchers, new compounds are still being discovered until today. *Garcinia rostrata* is a new plant that has not been studied by other researchers, so it is a high potential to find new compounds from it. On the other hand, *G. nervosa* has been reported previously to have good anti-oxidant and anti-inflammatory activities. The plant also showed positive response towards HeLa, MCH-7, and HT-29 cell lines (Seruji *et al.*, 2013). Therefore, more studies should be carried out on both *G.rostrata* and *G.nervosa* so that more biological activities can be discovered.

1.4 Objectives

This research project was designed for the isolation, characterization, elucidation and evaluation of the phytochemical compounds from *Garcinia rostrata* and *Garcinia nervosa*. The discovery of new natural compounds and evaluation of the biological activities of the plant extracts are the ultimate goals of this project.

As such, the following specific objectives are to be met as the goals of this project:

1. To extract and isolate new compounds from the crude extracts obtained from the stem bark of *Garcinia rostrata* and *Garcinia nervosa*.
2. To elucidate the structures of compounds isolated with the aid of various spectroscopic techniques (IR, NMR, GC-MS and UV-Vis).
3. To screen and evaluate the anti-bacterial activities of the crude extracts obtained.

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