UNIVERSITI PUTRA MALAYSIA

SECONDARY METABOLITES FROM STEM BARK OF Garcinia beccarii PIERRE AND Garcinia cuneifolia PIERRE

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SECONDARY METABOLITES FROM STEM BARK OF *Garcinia beccarii* PIERRE AND *Garcinia cuneifolia* PIERRE

By

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Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment 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 fulfillment of the requirement for the Degree of Master of Science

SECONDARY METABOLITES FROM STEM BARK OF *Garcinia beccarii* PIERRE AND *Garcinia cuneifolia* PIERRE

By

A MUNIANDY A/L ARUMUGAM

March 2017

Chairman : Professor Gwendoline Ee Cheng Lian, PhD
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The genus *Garcinia* is from the family Guttiferae of evergreen trees and shrubs distributed in tropical Asia, Africa and Polynesia. The genus *Garcinia* is known to be a rich source of polyisoprenylated benzophenones and xanthones. Medium scale extractions of the grounded stem bark of *Garcinia beccarii* Pierre and *Garcinia cuneifolia* Pierre were conducted by using the solvent extraction method at room temperature for three days. The extracts afforded some xanthones and triterpenoids. These compounds were isolated by using various chromatographic techniques and their structures were elucidated by using spectroscopy techniques such as NMR, GC-MS and FTIR.

Bioassays such as cytotoxic activities and antimicrobial screenings were investigated by using on the crude extracts of the two plants. Four xanthones namely rubraxanthone, trapezifolixanthone, α-mangostin and β-mangostin were isolated from the chloroform and ethyl acetate extracts of the stem bark of *Garcinia beccarii* Pierre. Two xanthones, dulxanthone C and osajaxanthone were obtained from the chloroform extract of the stem bark of *Garcinia cuneifolia* Pierre. Two common terpenoids were also obtained from both plants. These are stigmasterol from both plants, while beta sitosterol acetate was only obtained from the hexane crude extract of the stem bark of *Garcinia cuneifolia* Pierre. The hexane extract of *Garcinia beccarii* Pierre gave low IC_{50} values against the MCF 7 and HL 60 cancer lines.

The antimicrobial screening was also carried out using six bacteria, namely *Bacillus Subtilis B29, Bacillus Subtilis B125, Staphylococcus aureus, Staphylococcus epidermidis, Methicillin Resistant Staphylococcus aureus* and *Streptococcus sp*. Only low to moderate inhibition towards these bacteria was observed.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Sarjana Sains

METABOLIT SEKUNDER DARI BATANG KULIT *Garcinia Beccarii* PIERRE DAN *Garcina Cuneifolia* PIERRE

Oleh

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Pengerusi : Profesor Gwendoline Ee Cheng Lian, PhD
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Genus *Garcinia* ialah sejenis tumbuhan dari keluarga Guttiferae yang merupakan tumbuhan hijau dan pokok berkayu kecil terlentang luas di kawasan tropika Asia, Afrika dan Polinesia. Genus ini termasyhur sebagai tumbuhan yang kaya dengan sumber polisoprenyl benzofenon dan xanton. Pengekstrakan terhadap batang kulit *Garcinia beccarii* Pierre dan *Garcinia cuneifolia* Pierre telah dijalankan dengan menggunakan pelarut ekstrak di suhu bilik selama tiga hari. Pengekstrakan ini menghasilkan beberapa sebatian xanton dan terpena. Kesemua sebatian ini dikenalpasti dengan analisis teknik spektroskopi seperti NMR,GC-MS dan FTIR. Kajian biologi turut dilakukan terhadap semua ekstrak mentah setiap tumbuhan dalam kajian ini. Empat sebatian xanton iaitu rubraxanthone, trapezifolixanthone, α-mangostin dan β-mangostin berjaya diasingkan dengan menggunakan pelarut chlorofom dan ethyl acetat dari batang kulit *Garcinia beccarii* Pierre. Manakala dua sebatian xanton diasingkan iaitu dulxanthone C dan osajaxanthone dengan menggunakan pelarut chlorofom dari batang kulit *Garcinia cuneifolia* Pierre. Dua sebatian terpena juga dapat dihasilkan dari ekstrak kedua-dua tumbuhan ini, dimana stigmasterol ditemui dalam kedua-dua tumbuhan kajian dan beta-sitosterol acetate hanya diperolehi dari ekstrak heksana tumbuhan *Garcinia cuneifolia* Pierre.

Ujian sitotoksik telah dijalankan dengan menggunakan sel kanser MCF-7 dan HL-60 ke atas ekstrak mentah *Garcinia beccarii* Pierre dan *Garcinia cuneifolia* Pierre. Ekstrak heksana *Garcinia beccarii* Pierre didapati menunjukkan sitotoksik dengan IC_{50} nilai yang rendah terhadap kedua-dua sel kanser tersebut.

Ujian aktiviti antimikrob juga dijalankan dengan menggunakan bakteria *Bacillus Subtilis B29, Bacillus Subtilis B125, Staphylococcus aureus, Staphylococcus epidermidis, Methicililin Resistant Staphylococcus aureus and Stretococcus sp.*Kajian

mempamerkan aktiviti perencatan dari tahap rendah ke tahap sederhana sahaja oleh kesemua ekstrak.

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Thanks also goes to my beloved family members. A word of appreciation and deepest affection is inadequate to my wife, mother, and siblings. Their love and understanding inspired me and gave me hope whenever I feel disappointed and stressed.

I certify that a Thesis Examination Committee has met on 14 March 2017 to conduct the final examination of A Muniandy a/l Arumugam on his thesis entitled "Secondary Metabolites from Stem Bark of *Garcinia beccarii* Pierre and *Garcinia cuneifolia* Pierre" 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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The thesis was submitted to the Senate of the Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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LIST OF ABBREVIATIONS

^{13}C-NMR	Carbon Nuclear Magnetic Resonance
$CHCl_3$	chloroform
cm	centimeter
δ	chemical shift in ppm
J	coupling constant in Hz
˚C	degree in Celsius
$CDCl_3$	deuterated chloroform
d	doublet
dd	doublet of doublet
ETOAc	ethyl acetate
g	gram
GC-MS	Gas Chromatography-Mass Spectrometry
Hz	hertz
IR	Infrared
FTIR	Fourier Transform Infra-Red
Kg	kilogram
mp	melting point
MeOH	methanol
MS	Mass Spectrum
mL	milliliter
mg	milligram
^{1}H	proton
ppm	part per million
s	singlet

q	quartet
t	triplet
m	multiplet
α	alpha
β	beta
γ	gamma
HMBC	Heteronuclear Multiple Bond Correlation
HMQC	Heteronuclear Quantum Coherence
DEPT	Distortionless Enhancement by Polarization Transfer
TLC	Thin Layer Chromatography
UV-Vis	ultraviolet visible
Lit.	Literature
m/z	mass per charge
R_f	retention factor
M^+	molecular ion

CHAPTER 1

INTRODUCTION

1.1 The Value of Natural Products

Plant science, has become one of the favorable field of study by researchers today. The scope of research has widen from household products to natural products for medicinal uses. Today scientific research in natural product chemistry on the other hand takes on social and commercial values. From ancient to modern times, herbs and plants have been used for their medicinal properties. The difference is medicine among the primitive people included application of heat and cold, bloodletting, massage and the use of herbs while the modern approach for drugs in medicine was developed based on scientific basis. For example Karuvagai with scientific name *Albizia odoratissima* is used in Ayurveda and Siddha herbal medicine around the world especially in India as a traditional medicine. The bark of this plant is used for external application to treat bacteria and infections and as an astringent. The application of the above medicine is found in Indian literature as long as 1000 years ago. (Quattrocchi, 2012)

Natural products of plant origin offer a wide variety of bioactive compounds that could meet the demand for base compounds of drugs development. (Kumar *et al*., 2013) The perennial herb, *Galega officinalis* was used in folk medicine to treat diabetes. It gave rise to metformin, one of the most popular diabetes medications in the world. Metformin is biguanide, synthesized by fusing two guanidine together molecules, forming a biguanide.

Part of the drug used in modern medicine were either directly isolated from plants or synthetically modified from a lead compound of natural origin. Thus natural products play an important role in drug discovery and development programs in the pharmaceutical industry.

Malaysia is well known to be one of the 12 mega diversity among countries in the world that are rich in phanerogamic and cryptogamic flora. The biodiversity of Malaysia's plant resources is estimated to have 15000 species of higher plants. Among these, approximately 1300 are plants with medicinal values. However only a few hundred have been investigated for their chemical properties and biological activities up to date. There are huge opportunities for researchers to explore these Malaysian flora and fauna to find their properties and they are keen to investigate them. (Goh, 1988).

Phytochemistry is the study of chemicals derived from plants. Extraction, isolation and structural elucidation techniques are used in the field of phytochemistry. In short this field of study describes the large number of secondary metabolic compounds that were found in plants. Such compounds naturally provide protection against insect

attacks and plant diseases. Nevertheless, clinically proven secondary metabolites exhibit a number of protective functions for human consumers as well.

As per exhibit natural products towards human anatomy, there are diverse biological profiles which can be considered including antihypertensive, antioxidative, antithrombotic and anticancer activities. These issues have attracted many scientists to isolate or synthesize natural product compounds as novel drug candidates. One example compound with biological target is gambogic acid for apoptosis and KDR/Flk-1 phosphorylation. (Na, 2009)

Furthermore, combination therapies based on natural products and approved drugs have become a new approach in fighting against certain diseases especially cancer. Over 30 natural active principles are recently studied to treat malignant melanoma, the most aggressive form of skin cancer. Drug candidates which are able to diminish the production of cancer cells together with combination therapies based on natural products and approved drugs, such as dacarbazine and quercetin and sulforaphane are being investigated. (Al Qathama *et al*., 2015)

1.2 Botany of plants studied

1.2.1 The Family Clusiaceae (Guttiferae)

A total of 40 genera of Guttiferaeous plant contain at least 1000 species on earth. This **family** is approximately 0.4 percent of all the plant species on earth. In Malaysia alone 4 genera and 121 species are found and approximately 12.1 percent species are scattered around the country. In Guttiferae also known as Clusiaceae, family members are *Garcinia*, *Calophyllum* and *Mesua.* Among these three common genera the *Garcinia* are fruit trees. The genus *Garcinia* is the major group in the Clusiaceae family and it is also called Angiosperms (Flowering plants). Statistics taken in 2010 with the collaboration between the Royal Botanic Gardens, Kew and Missouri Botanical Garden showed that there are 610 species for the genus *Garcinia*. However only 418 are accepted for the species name. The remaining scientific names are under review.(http://www.theplantlist.org/)

Plants belonging to *Clusiaceae* are evergreen shrubs or trees. Some of the species grow on another plant for mechanical support but are unlikely parasites (epiphytic). These species are characteristic ally glabrous with uni or multicellular hairs and leaves are opposite.

Concerning the vegetative morphology of *Clusiaceae*, almost all the species are woody plants. The trunks are buttressed with prop roots. The plants that grow in drier areas appear a swelling at the base of the stem or underground stems or roots (lignotuber). Although these three genera grow in different habitats they appear strong and thick (stout).

Furthermore, the *Clusiaceae* species comprises all genera growing upward from a single point. They add leaves to the apex each year and the stem grows longer accordingly (monopodial). Most *Garcinia* species are comparable with the other two genera, branches out from orthotropic to plagiotropic.

The buds of almost all taxa lack scales and frequently *Garcinia* may have one or more pairs of leaves. The branches on the other hand, develop from the axils of the uppermost pair of leaves.

The *Clusiaceae* species are often glabrous. *Calophyllum* has irregular multicellular hairs. Whereas in *Garcinia* the terminal buds are often enclosed in the petiole bases. In *Mesua*, axillary buds are immersed in stem tissue. (Stevens, 2007)

1.2.2 The Genus *Garcinia*

The genus *Garcinia* is a member of the Clusiacea family. The genus *Garcinia* was named after Laurent Garcin (1683-1751) a French botanist and who authored several botanical works and carried out research in India (Glen, 2004). *Garcinia* is a major genus with over 400 plant species found worldwide (Perry and Metzger, 1980). The *Garcinia* plant species is small to medium tree and it could be found from the seashore to the lowland and up to the upper mountain forest. It is a tropical evergreen tree distributed mainly in Southeast Asia and South America (Obolskiy, 2009). In peninsular Malaysia, there are 49 species found in all types of lowland forests (Nazre, *et al*, 2009). In Borneo Island inclusive of Sabah and Sarawak this species is found abundantly.

The taxonomic hierarchy of the genus *Garcinia* is shown in the table below.

KINGDOM	Plantae
DIVISION	Magnoliophyta
CLASS	Eudicotyledoneae
ORDER	Malpighiales
FAMILY	Clusiaceae
TRIBE	Garcinieae
GENUS	*Garcinia*
SPECIES	(*Ca* 600 species worldwide)

Garcinia species have attracted natural product chemists for many years due to their phytochemicals and pharmacological effects. It was reported compounds isolated are mostly xanthones (39%), flavonoids (27%), triterpenoids (10%), benzophenones (8%) and other classes of compounds (16%).

In recent years *Garcinia* plants have become familiar in the field of ethnomedicine. The plant extracts are used as a decoction, meaning to say as a juice. Such decoctions are applied as a remedy for certain diseases. For example, toothache, inflammations, wound healing, jaundice, ulcers, dysentery and many respiratory related diseases (Gulumian, 1984).

A recent report on the importance of natural products talks about the natural active principles being used in the treatment of advanced or terminal stage cancer. It is reported at over 30 natural active compounds shows good pharmacological effects such as antimigratory compounds for metastasis of melanoma cells. (Al Qathama *et al*., 2015)

Although the studies of *Garcinia* species are important to be worthy significantly, there are challenges waiting for the researchers. This concerns both the safety and toxicity aspects of some pure compounds. Therefore further investigations currently undergone in clinical studies are cytotoxicity as well as screening tests in order to develop drugs from this species around the world.

1.3 The species *Garcinia beccarii* Pierre

General description

Garcinia beccarii Pierre is named after an Italian botanist O. Beccarii. The tree is sub canopy growing up to 26 meter tall and 35 centimeter of diameter at breast height. The stem is appeared with yellow latex and has a sticky inner bark. The leaves grow opposite and are characterized by a simple penni vein and glabrous. The flowers of *Garcinia beccarii* Pierre are green and yellowish and are located in bundles in leaf axils. The fruits are yellow-orange-red in colour with fleshy berry and seeds with aril.

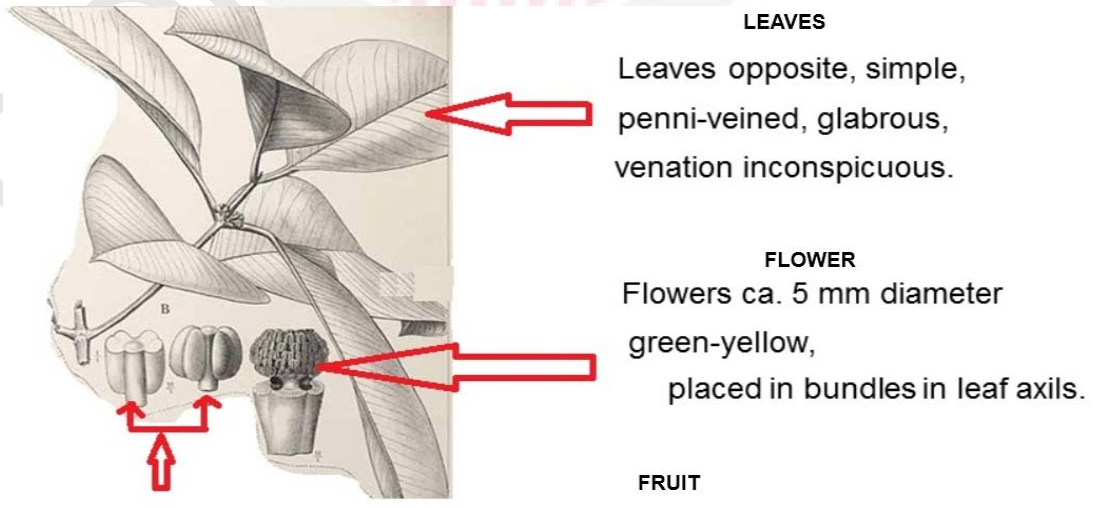

Figure 1 : Leaves, flower and fruit of *Garcinia beccarii* Pierre.

Morphology of Plant

Garcinia beccarii Pierre are woody plants. They grow in undisturbed mixed dipterocarp and are found in sub mountain forests up to 1000 meter altitude. However this species abundantly grow well on hillsides and ridges with sandy soils. It is actually present as a pre-disturbance remnant tree in secondary forests. (John Proctor *et al*, 1983).

This evergreen tree is distributed widely in Borneo which consists of 3 contingents Sarawak, Brunei and East Kalimantan. The local name in Borneo is *'Kandis'*. (Asthon, 1988).

1.4 The species *Garcinia cuneifolia* Pierre

General description

Garcinia cuneifolia Pierre is a small and unbuttressed tree and grows up 20 meters tall. The leaf is blade type, pale orange –brown at the bottom and pale grey above. The flowers are long, dense cymes and pedicels with 4 sepals and petals. The stamens groups in 4 and anthers have 2 cells. The fruits size around 6 millimeter with sub globose and when ripe become bright red.

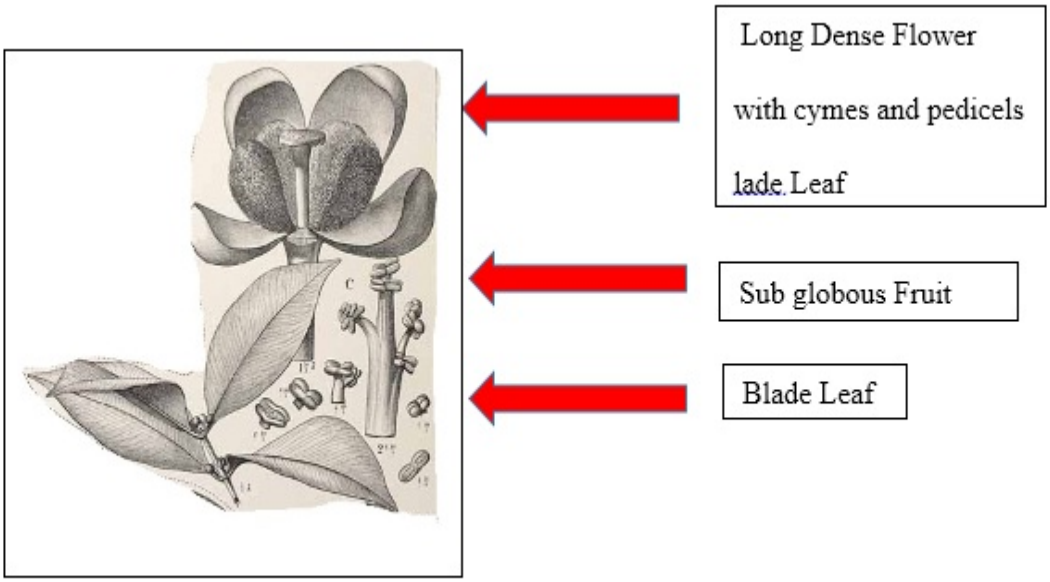

Figure 2: Leaves, flower and fruit of *Garcinia cunefolia.*

Morphology of Plant

Garcinia cuneifolia Pierre species are widely distributed in Sarawak until west Sabah. These species grow very commonly everywhere especially in the open area somewhere in Padang Keruntum of Sarawak vegetation Centre of the Baram peat

swamps. This species frequently grows as a tree in mixed and Alan Peat Swamp forests area.

1.5 Problem Statement

An emerging disease 'cancer' has appeared in the world population and is rapidly increasing currently and is the futuristic killer disease of human kind. Up to date, there are no drug remedies completely for it. Therefore new compounds from new medicinal plants such as *Garcinia Beccarii* Pierre and *Garcinia Cuneifolia* Pierre will necessitate in the effort to search for novel and effective drugs from this research to complement the existing synthetic drugs.

1.6 Objective of Study

This research is specifically designed to meet an outbreak of the above mention problem statement. Thus, the project covers isolation, characterization, elucidation and evaluation of compounds from both *Garcinia beccarii* Pierre and G*arcinia cuneifolia* Pierre. Eventually the discovery of bioactive from the natural products under study is the ultimate gift to the medicinal world.

Thus, the specific objectives of this research project are:

1. To extract and isolate chemical constituents from the stem bark of *Garcinia beccarii* Pierre and *Garcinia cuneifolia* Pierre.

2. To identify and elucidate the structures of the pure compounds using spectroscopic methods such as NMR, MS and IR.

3. To determine the biological activities of the crude extracts through cytotoxic and antimicrobial bioassays to search for anti-cancer and anti-microbial lead compounds

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