UNIVERSITI PUTRA MALAYSIA

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**MASTER OF SCIENCE**
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# CHEMICAL CONSTITUENTS AND BIOLOGICAL ACTIVITIES OF *GARCINIA MANGOSTANA* L. AND *PIPER BETLE*

**By**

**YEAP SOO FONG**

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

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**October 2009**

**Chairman : Mawardi bin Rahmani, PhD**

**Faculty : Science**

Young fruits of *Garcinia mangostana* L. from Guttiferae family and leaves of *Piper betle* from Piperaceae family were phytochemically studied and screened for their biological activities. The young fruits of *Garcinia mangostana* L. were collected from Negeri Sembilan while the leaves of *Piper betle* were collected from Sabah. The phytochemical works involved extraction of the plant materials with organic solvents of different polarity and chromatographic separation of the extracts with several techniques to obtain pure compounds. The structures of the compounds were determined by using spectroscopic techniques such as IR, MS, NMR and UV. The crude extracts from both plants were screened for antimicrobial (against four pathogenic bacteria and 3 pathogenic fungi), cytotoxic activities and antioxidant using disc diffusion method, Tetrazolium Salt (MTT) assays and 1,2-Diphenyl-2-picrylhydrayl (DPPH) respectively. Three isolated compounds, epicatechin (**39**), 4-hydroxybenzoic acid (**114**) (both from *mangostana* L.) and 2-allyl-3,4-dihydroxybenzaldehyde (**115**) (from *P. betle*) were tested for antioxidant by using DPPH.

Separation of the extracts of young fruits of *Garcinia mangostana* L. afforded seven chemical compounds identified as methylparaben (**110**), methyl 3,4,5-trihydroxybenzoate (**111**), parvifoliol A1 (**112**), methyl 2,3-dihydroxybenzoate (**113**), 4-hydroxybenzoic acid (**114**), epicatechin (**39**) and a xanthone, mangostanin (**20**) after extensive various chromatographic techniques. Two compounds, methylparaben (**110**) and methyl 3,4,5-trihydroxybenzoate (**111**) have not been previously reported to occur in *Garcinia mangostana*. This is the first report on the occurrence of these compounds in *Garcinia mangostana* and the proper technical name for methylparaben (**110**) is methyl 4-hydroxybenzoate. On isolation and purification of the leaves extracts of *Piper betle* led to the isolation of four compounds chavibetol (**77**), 2-hydroxychavicol (**80**), β-sitosterol (**47**) and 2-allyl-3,4-dihydroxybenzaldehyde (**115**).

The antimicrobial activity test for both plant extracts was carried out using seven microbes namely, *methicilin resistant Staphylococcus aureus* (MRSA), *Bacillus substili, Salmonella typhimurium, Pseudomonas aeruginosa, Candida albicans, Aspergillus ochraceaus* and *Saccharomyces cerevisiae*. However, no activity was observed in the crude extracts of both *Garcinia mangostana* L. and *Piper betle*. The same results were obtained for the cytotoxic activity using Tetrazolium Salt (MTT) assay. When tested for antioxidant by using 1,2-Diphenyl-2-picrylhydrayl (DPPH), all the crude extracts failed to exhibit any activity. However two of the isolated compounds, epicatechin (**39**) and 2-allyl-3,4-dihydroxybenzaldehyde (**115**) showed strong activity with $IC_{50}$ < 7.81 μg/mL in comparison with the standard, ascorbic acid ($IC_{50}$ < 11.70 μg/mL).

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

**KANDUNGAN KIMIA DAN AKTIVITI BIOLOGI DARIPADA *GARCINIA MANGOSTANA* L. DAN *PIPER BETLE***

Oleh

**YEAP SOO FONG**

**Oktober 2009**

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Buah muda *Garcinia mangostana* L. daripada famili Guttiferae dan daun *Piper betle* daripada famili Piperaceae telah dikaji secara fitokimia dan diuji aktiviti biologi. Buah muda *Garcinia mangostana* L. diperoleh dari Negeri Sembilan manakala daun *Piper betle* pula dibawa dari negeri Sabah. Kajian fitokimia ini melibatkan pengekstrakan sebatian daripada tumbuhan dengan menggunakan pelarut organik yang berbeza kekutubannya serta pemisahan dengan pelbagai teknik kromatografi ke atas ekstrak untuk memperolehi sebatian tulen. Struktur sebatian kemudiannya dikenalpasti melalui teknik spektroskopi seperti IR, MS, NMR and UV. Ekstrak mentah daripada kedua-dua tumbuhan diuji aktiviti antimikrob (terhadap empat bakteria patogen dan tiga fungi patogen), sitotoksik, dan antioksidan masing-masing dengan menggunakan kaedah peresapan cakera, garam Mikrokultur Tetrazolium (MTT) dan 1,2-difenil-2-pikrilhidrazil (DPPH). Tiga sebatian tulen yang diperolehi, epikatekin (**39**), asid hidroksi benzoik (**114**) (kedua-duanya daripada *G. mangostana* L.) dan 2-alil-3,4-dihidroksibenzaldehid (**115**) (daripada *P. betle*) telah diuji aktiviti antioksidan.

Pemisahan berterusan dengan pelbagai teknik kromatografi ke atas ekstrak buah muda *Garcinia mangostana* L. telah menghasilkan tujuh sebatian kimia yang dikenalpasti sebagai metilparaben (**110**), metil 3,4,5-trihidroksibenzoat (**111**), parvifoliol A1 (**112**), metil 2,3-dihidroksibenzoat (**113**), asid hidroksi benzoik (**114**), epikatekin (**39**) dan juga xanthone iaitu mangostanin (**20**). Dua sebatian, metilparaben (**110**) dan metil 3,4,5-trihidroksibenzoat (**111**) belum pernah dilaporkan berlaku dalam *Garcinia mangostana*. Ini merupakan laporan pertama di mana dua sebatian ini dipisahkan daripada *Garcinia mangostana* dan nama teknikal yang betul untuk metilparaben (**110**) ialah metil 4-hydroxybenzoat. Pemisahan dan penulenan ekstrak daun *Piper betle* telah mendorong kepada pemisahan dan pengenalpastian empat sebatian iaitu kavibetol (**77**), 2-hidroksikavikol (**80**), β-sitosterol (**47**) dan 2-alil-3,4-dihidroksibenzaldehid (**115**).

Ujian aktiviti antimikrob ke atas ekstrak mentah kedua-dua tumbuhan dijalankan dengan menggunakan tujuh mikrob iaitu *methicilin resistant Staphylococcus aureus* (MRSA), *Bacillus substili, Salmonella typhimurium, Pseudomonas aeruginosa*, *Candida albicans, Aspergillus ochraceaus* and *Saccharomyces cerevisiae*. Walau bagaimanapun, tiada aktiviti diperhatikan ke atas ekstrak mentah kedua-dua spesies kajian. Keputusan yang sama juga diperoleh untuk ujian aktiviti sitotoksik dengan garam Mikrokultur Tetrazolium (MTT). Apabila diuji aktiviti antioksidan dengan 1,2-difenil-2-pikrilhidrazil (DPPH), didapati semua ekstrak mentah gagal menunjukkan sebarang aktiviti manakala dua sebatian tulen yang diuji, epikatekin (**39**) dan 2-alil-3,4-dihidroksibenzaldehid (**115**) menghasilkan aktiviti yang kuat dengan $IC_{50}$ < 7.81 μg/mL berbanding dengan standard, asid askorbik ($IC_{50}$ < 11.70 μg/mL).

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I certify that an Examination Committee has met on 21 October 2009 to conduct the final examination of Yeap Soo Fong on her Master of Science thesis entitiled "Chemical Constituents and Biological Activities Of *Garcinia Mangostana* L. and *Piper Betle*" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the Candidate be awarded the relevant degree.

Members of the Examination Committee are as follows:

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This thesis was submitted to the Senate of Universities 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:

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Date: 14 January 2010

## DECLARATION

I declare that the thesis is based on my original work except for quatations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or other institutions.

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**YEAP SOO FONG**

Date: 1 February 2010

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| | |
|---|---|
| α | alpha |
| β | beta |
| δ | delta |
| δ | chemical shift in ppm |
| $\lambda_{max}$ | maximum wavelength in nm |
| μ | microgram |
| μL | microliter |
| $^{13}C$ | carbon-13 |
| $^{o}C$ | degree celcius |
| $CDCl_3$ | deuterated chloroform |
| $CHCl_3$ | chloroform |
| $cm^{-1}$ | per centimeter |
| COSY | Correlated Spectroscopy |
| *d* | doublet |
| *dd* | doublet of doublet |
| DEPT | Distortionless Enhancement by Polarization Transfer |
| DMSO | dimethyl sulphoxide |
| EtOAC | ethyl acetate |
| EIMS | Electron Impact Mass Spectrometry |
| g | gram |
| GC-MS | Gas Chromatography-mass spectroscopy |
| $^{1}H$ | proton |

| | |
|---|---|
| hex | hexane |
| HMBC | Heteronuclear Multiple Bond Connectivity by 2D Multiple |
| HMQC | Heteronuclear Multiple Quantum Coherence |
| $IC_{50}$ | Inhibition Concentration at 50 percent |
| Id | Inhibition diameter |
| *br* | broad |
| *t* | triplet |
| *s* | singlet |
| *m* | multiplet |
| MeOH | methanol |
| m.p | melting point |
| MS | mass spectrum |
| *m/z* | mass per charge |
| NMR | Nuclear Magnetic Resonance |
| TLC | Thin Layer Chromatography |
| IR | Infrared |
| UV | Ultraviolet |
| ε | molar absorptivity |

# CHAPTER I

# INTRODUCTION

## 1.1 General Introduction

Focus on plant research has recently increased all over the world. It is well known that plants synthesize poisonous chemicals to defend themselves against hostile environment and various predators. Some of these chemical are very dangerous to human, but some may be very useful and can be used to treat diseases. Research on medicinal plants, especially in tropical areas of the Ancient World, is of special importance from a therapeutic point of view. Nature, both flora and fauna give us some interesting model compounds, providing chemists with lead compounds for the design and synthesis of more pharmacologically viable derivatives.

Natural product research plays a significant role in drug discovery especially in nutraceuticals, agrochemicals and traditional medicines research. Two of the challenges in natural products research are the unknown nature and complexity of natural products extracts and the detection of minor active compounds in biological assay.

Nature probably provides unlimited sources of valuable secondary metabolites, which might be of high biological importance for several kinds of applications. The World Health Organization has listed over 21,000 plant species used around the world for medicinal purposes. Asian countries are enormously rich in still widely unexplored medicinal plants and natural products of unknown biological activities. Malaysia is identified as one of 12 mega-diversity countries in the world. It is estimated that 1,200

plants species in Peninsular Malaysia and 2000 species in Sabah and Sarawak have been harvested for medicinal or herbal purposes (Perry, 1980). The rainforest of Malaysia is rich with many species of herbal and medicinal plants and offers great opportunities for chemical investigation. However, the research on higher plant as a natural source of drugs is still largely unexplored. According to Perry (1980), there are 12000 species of flowering plants found in Malaysia. Unfortunately, only about 100 of 1300 that are said to be medicinal have been investigated. Hence, there is huge potential in research on medicinal plants.

*Garcinia mangostana* L. is well studied plant of its different parts like leaves, heartwood, ripe fruits and especially fruit hull (pericarp or rind) which was reported to be a source of mangostin, xanthone, tannin, isoflavone and other bioactive substances (Deachathai *et al*., 2005). However, phytochemical investigation of whole young fruit of Garcinia *mangostana* L. has never been reported. Hence, it was chosen to be studied further for its chemical constituent and biological activity. While *Piper betle* leaves were studied in order to identify the bioactive substance as it is widely use in traditional medicine in Malaysia.

## 1.2 Objectives of Study

The objectives of this study are:

1. To extract and isolate the chemical constituents of the young fruits of *Garcinia mangostana* L. and *Piper betle* leaves using chromatographic techniques.

2. To identify and elucidate the structure of the isolated pure compounds using various spectroscopic techniques (IR, UV, MS and NMR).

3. To test the bioactivity of the crude extracts and isolated compounds.

# CHAPTER 2

# LITERATURE REVIEW

## 2.1 Botanical Aspects Of The Plants

### 2.1.1 The Family Guttiferae

The Guttiferae is widely distributed family of evergreen tropical trees with milky or colored sap, comprising of about 40 genera and 1000 species. It is also known as Clusiaceae and mainly found in humid and hot regions (Babu *et al*., 1988). There are 4 genera and 121 species found spreading in all kind of habitats in Malaysia with the most common genera of *Garcinia*, *Calophyllum*, *Mesua* and *Mammea*. *Garcinia* and *Mesua* can be found in dry land forests; *Calophyllum* in swampy forests while *Mesua* in lowlands (Morton, 1987).

Guttiferae plants grow as trees, shrubs and herbs with yellow or brightly coloured resinous juice which can be used as timber and as a source of resins, gums, pigments, dyes, edible oil and fruits (Mabberly, 1987). The leaves are simple, entire, opposite and stipulate with the present of resin or oil gland. The flowers are regular and often bright in colour (Dale and Greenway, 1961).

### 2.1.2 *Garcinia*

*Garcinia* is the most numerous genus of the Guttiferae family with about 400 species widely distributed in tropical Asia, Africa, New Caledonia and Polynesia (Morton,

1987). Plants of this genus normally reach up to 20 metres in height; have green leaves, edible fruits and produce yellow latex or resins.

*Garcinia* is well known as a genus of fruit trees in Malaysia. The fruits of many species are edible. Mangosteen (*Garcinia mangostana* L.*)* in which the flesh is encased within an outer harden shell (rind) is eaten fresh; the acidic fruits like *Garcinia atroviridis, Garcinia cambogia and Garcinia planconi* serve as a substitute for tamarind in curries. Furthermore, the fruits can be preserved in a dry state, with or without the aid of salt (Burkill, 1993).

*Garcinia* is often used for traditional medicines to threat abdominal pain, dysentery, diarrhoea, infected wound and gonorrhoea (Jayaprakasha *et al*., 2006). The fruits of *Garcinia xanthochymus* have been widely used for bilious condition, diarrhoea and dysentery in Thailand (Perry, 1980). Meanwhile the fruit hull of *Garcinia mangostana* L. used for healing skin infections and wound (Mahabusarakum *et al*., 1987). In Indonesia, the leaves and seeds of *Garcinia dulcis* have been used for the treatment of lymphatitis, parotitis and struma (Kosela *et al*., 2000).

### 2.1.3 *Garcinia mangostana* L.

The origin of *Garcinia mangostana* L. is in Southeast Asia and distributed in Northern Australia, Brazil, Central America, Hawaii, Southern India, Indonesia, Malaysia, Thailand and other tropical countries (Morton, 1987).