CHEMICAL CONSTITUENTS FROM THE LEAVES OF *GARCINIA PARVIFOLIA*

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Dedicated to my beloved family…

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ABSTRACT

The chemical constituents of the leaves of *G. parvifolia* (known as kandis) have been investigated. Extraction of dried leaves was successfully done by cold extraction method using ethanol as solvent, followed by partitioning using *n*-hexane, chloroform and ethyl acetate. Fractionation and purification on the *n*-hexane crude extract by using Vacuum Liquid Chromatography (VLC) and Column Chromatography (CC) have resulted four compounds. The elucidation of the structures were carried out by spectroscopic techniques using IR, ^{1}H, ^{13}C, DEPT and GC-MS. Analysis by spectroscopic data showed the isolated compounds are β-sitosterol, squalene, friedelin-3β-ol, and mixture of friedelin and friedelin-3β-ol. The antibacterial activity of crude extracts was carried out using disc diffusion methods with eight strains of bacteria, *Enterococcus faecalis, Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus cereus, Escherichia coli, Pseudomonas aeruginosa*, *Klebsiella pneumonia* and *Pseudomonas putida.* Antibacterial screening showed that the chloroform crude extract gave a strong towards *Klebsiella pneumonia*, *Pseudomonas aeruginosa* and *Pseudomonas putida* with minimum inhibition concentration of 450 μg/mL.

ABSTRAK

Sebatian kimia daripada daun *G. parvifolia* (dikenali sebagai kandis) telah dikaji. Pengekstrakan daun kering telah berjaya dilakukan menggunakan kaedah pengekstrakan sejuk yang menggunakan pelarut etanol, diikuti dengan pembahagian menggunakan *n*-heksana, kloroform and etil asetat. Pemeringkatan dan penulenan esktrak mentah menggunakan Kromatografi Cecair Vakum dan Kromatografi Turus telah menghasilkan empat sebatian. Pencirian struktur sebatian telah dilakukan dengan teknik spektroskopi menggunakan IM, ^{1}H, ^{13}C, DEPT RMN dan KG-SJ. Analisa terhadap data spektroskopi menunjukkan sebatian yang telah diasingkan ialah β-sitosterol, skualena, friedelin-3β-ol dan campuran friedelin dan friedelan-3β-ol. Ujian bakteria ke atas ekstrak mentah dan β-sitosterol telah dilakukan dengan menggunakan teknik pembauran cakera terhadap lapan jenis bakteria, *Enterococcus faecalis, Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus cereus, Escherichia coli, Pseudomonas aeruginosa*, *Klebsiella pneumonia* and *Pseudomonas putida.* Penyaringan antibakteria menunjukkan ekstrak mentah kloroform memberikan perencatan yang kuat terhadap bakteria *Klebsiella pneumonia, Pseudomonas aeruginosa* dan *Pseudomonas putida* dengan kepekatan perencatan minimum 450 μg/mL.

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LIST OF ABBREVIATIONS

B	-	Beta
Δ	-	Chemical shift
^{13}C NMR	-	Carbon- 13 Nuclear Magnetic Resonance
^{1}H NMR	-	Proton Nuclear Magnetic Resonance
CC	-	Column Chromatography
$(CD_3)_2CO$	-	Deuterated acetone
$CDCl_3$	-	Deuterated Chloroform
$CHCI_3$	-	Chloroform
D	-	Doublet
Dd	-	doublet of doublet
DDM	-	Disc Diffusion Method
DEPT	-	Distortionless Enhancement by Polarization Transfer
EtOAc	-	Ethyl acetate
Et_2O	-	Diethyl ether
GC	-	Gas Chromatography
GC-MS	-	Gas Chromatography-Mass Spectrometry
HMBC	-	Heteronuclear multiple bond correlation
HMQC	-	Heteronuclear multiple quantum correlation
IR	-	Infrared
J	-	Coupling constant
Lit.	-	Literature
M	-	Multiplet
MeOH	-	Methanol
Mg	-	Milligram
mL	-	Mililiter
MBC	-	Minimum Bactericidal Concentration

MIC	-	Minimum Inhibitory Concentration
NA	-	Nutrient Agar
NB	-	Nutrient Broth
µg/mL	-	Microgram per mililitre
Ppm	-	part per million
R_f	-	Retention factor
T	-	Triplet
TLC	-	Thin layer chromatography
VLC	-	Vacuum liquid chromatography

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CHAPTER 1

INTRODUCTION

1.1 General Introduction

Natural products are derived from natural sources such as plants, animals or microorganisms. There are 250,000 to 500,000 species of plants were estimated on earth. Unfortunately, among the species, there is only 1-10% that used as food for human and animals and more species of plant more used for medicinal purposes [1]. Plant is always important role in traditional systems of medicine to prevent and treat the disease worldwide. The traditional medicines in all countries were employed naturally occurring plant medicines for thousands of years. People preferred in natural medicines rather than synthetic medicines because of its safe and effective [2].

Plants can be used as medicine because of the richest bio-resources of drugs of traditional medicinal systems, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceuticals, intermediate and chemical entitled for synthetic drugs. Plants have their intrinsic ability to resist pathogenic microorganisms [3]. Some plant can be used in traditional medicine because they contain a vast array of substances and active constituents that can be treat chronic and infectious diseases. Medicinal plants are considerably useful and economically essential [4].

According to World Health Organization, medicinal plants would be the best source to variety of drugs. Approximately 20 % of the plants found in the world have

been submitted to pharmaceutical and biological test. Nowadays, many researchers were screened of antibacterial plant extracts to find new compounds with the potential to act against multi resistant bacteria. The development of medicine was started with the Ayurveda in India. Ayurveda system of medicine has its long history of therapeutic potential. After Ayurveda, current advancements in drug discovery technology and search for novel chemical diversity have intensified the efforts for exploring leads. For a long period of time, plants have been valuable source of natural products for maintaining human health [5].

Plants can be divided into their main classes of bioactive compounds based on their similar characteristics. The main classes included flavonoids, terpenes, alkaloids, saponins, coumarins, steroid, lactones, phenols, cyclic ketones, stilbenes and acidamides [6, 7]. The drugs are derived from the whole plant or from different organs like leaves, stem, bark, root, flower, seeds and twig. Some drugs also can be prepared from excretory plant product such as gum, resins and latex [8]. The different parts of plant have different potential as medicinal properties.

Over the last two decades, intensive effort has been made to discover chemically useful antibacterial or antifungal drugs of plant origin. Medicinal plant based antimicrobials represent a vast unstapped source of pharmaceuticals. Further exploration of plant antimicrobials need to occur for treatment of infectious diseases both in plants and humans while simultaneously for mitigating many of the side effects that are often associated with synthetic antimicrobials. However, only a small portion of the several hundred thousand medicinal plant species has been investigated both phytochemically and pharmacologically [3].

1.2 The Guttiferae Family

Guttiferae (or known as Clusiaceae) is one of the higher plant families of the plant kingdom. Guttiferae is comprised into six subfamilies, Kielmeyeroideae, Calophylloideae, Clusiodeae, Moronoboideae, Lorostemonodeae, Hypericodeae.

Every subfamily is divided into tribes. Subfamily of Clusiodeae is divided into two tribes: Clusieae and Garcinieae. Garcinieae is further divided into four genera: *Allanblackia*, *Garcinia*, *Pentaphalangium* and *Rheedia* while the tribe Clusieae consists of two genera (*Clustu* and *Tovomita*) [9].

Guttiferae family contains more than 1000 species and widely distributed in tropical region but majority of *Hypercium* genus occurs widely in temperature region. Oxygenated xanthones and benzophenones have been found widely in all genera of the Guttiferae [9]. Whitmore, T.C. reported there are 4 genera and 121 species in Malaysia. The genera that can be found in Malaysia are *Garcini*a, *Calophyllum*, *Mesua* and *Mammea*. *Mammea* can be found only on lowlands whereas *Garcinia*, *Calophyllum* and *Mesua* are found from sea level to the tops of the highest mountain. Meanwhile *Garcinia* and *Mesua* are found in dry land forests and only *Calophyllum* is common in swampy forests [10].

1.3 Genus *Garcinia*

Garcinia belongs to the family Guttiferae (Clusiaceae), which is large genus of polygamus trees in the world tropics. It is widely distributed in tropical Africa, Asia, New Caledonia and Polynesia. In Indonesia, these plants were reported to be rich in natural chemical substances. Mostly, *garcinia* was grown and distributed in all over Indonesia [11].

Garcinia contains 35 genera and divided into 800 species and approximately 28 species that can be found in Malaysia. Genus *Garcinia* in Malaysia can be also called as fruit trees because it can provide palatable fresh fruit pulp and fruit peel. The best known fruit in Malaysia is the mangosteen (*Garcinia mangostana*). Asam gelugor (*Garcinia atroviridis*) used as tamarind slices in local dishes. *Garcinia* is very popular as high nutritional values but still underutilized due to lack of popularity among the local citizen. Most of these species have too acidic fruit pulp,

lack information on nutritional compositions and physical qualities and lack of promotional activities for these species [11, 12].

1.4 *Garcinia parvifolia*

Garcinia parvifolia is one of the species in genus *Garcinia.* It is synonyms with *G. dioica* and *G. globulosa* and also locally known as *'kandis'* or cherry mangosteen. It is widely distributed in Thailand, Peninsular Malaysia, Sumatra, Java, Borneo, Celebes, Moluccas and New Guinea. It is a tropical species which can be found wild in various area like peat swamp forests, primary or secondary undisturbed mixed dipterocarp forests in the low-lands and humid submontane and up to an attitude of 1000 m. It also can be lived on hillsides, ridges, on well-drained, alluvial sites and along rivers [13, 14].

The species of *G. parvifolia* can be described as an evergreen, small to medium-sized, sub-canopy, perennial tree, 5-25 m high with a bole of 23 cm and with black widely patent branches and exuding yellow latex when bruised. While their leaves are opposite, shortly petioliate, oblong or lanceolate-oblong, entire, simple, penniveined, glabrous, 3.5-17 cm long, 2-7 cm wide, petiole 0.5-1.5 cm long with a shallow furrow on the anterior side. Besides that, the flowers are unisexual-monoecious, bisexual or polygamous, pale yellow or orange coloured, 0.5-1 cm across, pedicellate, solitary or in axillary fascicles of 2-12. Other part is fruit which can be described as depressed globose, pale-green turning yellow or orange-yellow when ripe 2.5-3.5 by 3.5-4 cm across, crowned by sunken remains of the small stigma with thin skin [14].

In Indonesia, the young leaf with sour taste can be eaten as a vegetable by their residences [13]. The ripe fruits can be used as flavouring in rice and curries since the arils have a pleasant and agreeable sub-acid taste [14].

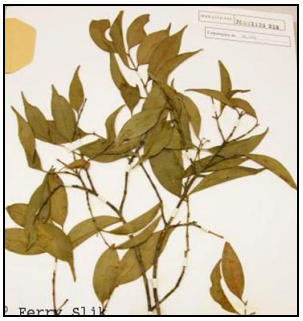

Figure 1.1: Leaves of *G. parvifolia*

1.5 Problem Statement

Garcinia is one of the genera in Guttiferae family. Many medicinal plants from genus of *Garcinia* used as herbal medicine. Phytochemical research of *Garcinia parvifolia* was greatly enhanced by the search for bioactive compound since there are not been many phytochemicals and bioactivity studies conducted on this species. The purpose of this study is to identify the chemical constituent from the leaves of *G. parvifolia*. The isolation and evaluation of chemical compounds in the *G. parvifolia* is essential to be carried out to determine the chemical compounds. It is also important to study the antimicrobial activity of the chemical constituents to determine the medicinal value of the plant.

1.6 Objectives

The objectives of this research are:-

1) To extract and isolate chemical compounds from the leaves of *G. parvifolia.*
2) To characterize the structure of the chemical compounds by spectroscopic method.
3) To screen the antibacterial activity of the crude extract and pure isolated compounds.

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