INVESTIGATION OF ESSENTIAL OIL, MAJOR CONSTITUENTS AND ANTIOXIDANT ACTIVITY FROM THE LEAVES AND STEM BARK OF *Garcinia urophylla* Scort. ex King (CLUSIACEAE)

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UNIVERSITI PENDIDIKAN SULTAN IDRIS

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ABSTRACT

The aim of this study was to investigate the essential oil, major constituents, and antioxidant activity of *Garcinia urophylla* leaf and stem bark. Essential oil was obtained from the leaves using hydrodistillation and its chemical composition was determined by gas chromatography (GC) and gas chromatography mass spectrometry (GC-MS). Phytochemicals were isolated using column chromatography techniques and their structures were elucidated via spectroscopic methods and comparisons with literature data. Antioxidant activity was assessed using the total phenolic content assay and the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay. Results showed that the leaf oil primarily consists of β-caryophyllene (56.2%), α-humulene (26.3%), and α-gurjunene (6.3%). Isolation and purification of the leaves and stem bark extracts yielded eight phytochemicals which were identified as α-mangostin, β-mangostin, β-amyrin, lupeol, lupeol acetate, ferulic acid, syringic acid, and caffeic acid. The hexane leaf extract exhibited the highest phenolic content with 425.9 mg GAE/g, while the isolated lupeol acetate showed the strongest DPPH free radical scavenging activity with an IC_{50} value of 33.0 µg/mL. In conclusion, the essential oil composition revealed sesquiterpene hydrocarbons as the principal components, whereas phytochemical studies of the leaves and stem barks extracts identified xanthones, phenolics, and terpenes as major compounds. These findings indicate that *G. urophylla* is a potential source of bioactive compounds with antioxidant properties for the prevention of free radical-related diseases such as atherosclerosis and diabetes.

KAJIAN MINYAK PATI, SEBATIAN UTAMA DAN AKTIVITI ANTIOKSIDAN DARIPADA DAUN DAN KULIT BATANG *Garcinia urophylla* Scort. ex King (CLUSIACEAE)

ABSTRAK

Tujuan kajian ini adalah untuk menyiasat minyak pati, komponen utama, dan aktiviti antioksidan oleh daun dan kulit batang *Garcinia urophylla*. Minyak pati diperolehi daripada daun menggunakan penyulingan hidro dan komposisi kimianya ditentukan oleh kromatografi gas (GC) dan kromatografi gas spektrometri jisim (GC-MS). Fitokimia diasingkan menggunakan teknik kromatografi turus dan struktur ditentukan melalui kaedah spektroskopi dan perbandingan dengan data literatur. Aktiviti antioksidan dinilai menggunakan jumlah kandungan fenolik total dan asai perencatan radikal bebas 2,2-difenil-1-pikrilhidrazil (DPPH). Keputusan menunjukkan minyak daun terutamanya terdiri oleh β-kariofilena (56.2%), α-humulena (26.3%), dan α-gurjunena (6.3%). Pengasingan dan penulenan ekstrak daun dan kulit batang menghasilkan lapan fitokimia dikenal pasti sebagai α-mangostin, β-mangostin, β-amirin, lupeol, lupeol asetat, asid ferulik, asid siringik, dan asid kafeik. Ekstrak heksana daun mempamerkan kandungan fenolik tertinggi dengan 425.9 mg GAE/g, manakala pemencilan lupeol asetat menunjukkan aktiviti perencatan radikal bebas DPPH yang paling kuat, dengan nilai IC_{50} sebanyak 33.0 µg/mL. Sebagai kesimpulan, komposisi minyak pati menunjukkan hidrokarbon seskuiterpena sebagai komponen utama, manakala kajian fitokimia oleh ekstrak daun dan kulit batang telah mengenal pasti xanton, fenolik, dan terpena sebagai sebatian utama. Penemuan ini menyatakan bahawa *G. urophylla* berpotensi sebagai sumber sebatian bioaktif dengan sifat antioksidan, untuk pencegahan penyakit berkaitan radikal bebas seperti aterosklerosis dan diabetes.

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LIST OF ABBREVIATIONS

α	Alpha
Abs	Absorbance
β	Beta
br	broad
^{13}C	Carbon-13
CC	Column Chromatography
$CDCl_3$	Deuterated chloroform
$CHCl_3$	Chloroform
cm^{-1}	Per centimeter
COSY	Correlation spectroscopy
1D	1 Dimension
2D	2 Dimension
δ	chemical shift
d	doublet
dd	doublet of doublets
DEPT	Distortionless Enhancement by Polarization Transfer
EIMS	Electron Impact Mass Spectrometry
Et_2O	Diethyl ether
GC	Gas Chromatography
GC-MS	Gas Chromatography-Mass Spectrometry
^{1}H	Proton
HMBC	Heteronuclear Multiple Bond Correlation

HMQC	Heteronuclear Multiple Quantum Coherence
Hz	Hertz
IR	Infrared
J	Coupling constant
KBr	Potassium bromide
KI	Kovats Index
L	Liter
m	multiplet
M^+	Molecular ion
MeOH	Methanol
MHz	Megahertz
min	Minute(s)
m/z	Mass to charge ion
mg	milligram
m.p	Melting point
$MgSO_4$	Magnesium sulphate
mL	milliliter
mm	millimeter
NMR	Nuclear Magnetic Resonance
nm	nanometer
s	singlet
SiO_2	Silica gel
t	triplet
TLC	Thin Layer Chromatography

CHAPTER 1

INTRODUCTION

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1.1 General Introduction

Plants play an essential part in the survival of life on Earth. The use of chemical analysis in medicine during the 19th century had a profound impact on the role of plants. Higher plants still hold historical significance today as significant sources of newly discovered compounds that can be used directly as medicinal agents, as biochemical or pharmacological probes, and as sources of inspiration for future generations of synthetic organic medicinal chemists (Auranwiwat et al., 2021). Numerous times, plants with helpful therapeutic qualities have been identified through folklore records from a wide range of civilisations. Over the last 200 years, a great deal of purified compounds that are essential to the practice of modern medicine have been produced by the chemical study and purification of plant extracts that were thought to have medicinal qualities (Dias et al., 2012).

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Medicinal plants have been identified and employed in traditional medicine practices since ancient times. Over the past three decades, there has been a significant increase in the usage of herbal medicines for some aspects of basic healthcare. As an example, pregnant women utilise herbal goods such as red raspberry leaf which is rich in iron, it helps to tone the uterus, increase milk production, decrease nausea, and ease labor pain (Abu Bakar et al., 2018). They use this herbal medicine to treat pregnancy-related ailments because they have past expertise on herbal treatment and the items are easily accessible and less expensive (Socha et al., 2023).

Malaysia is among the world's 12 megadiverse countries where endemism is highest (Abu Bakar et al., 2018). At least a quarter of our tree flora is not found elsewhere in the world, and many of our herbaceous flora and other groups of species are unique. In Malaysia, about 2000 medicinal plant species are reported to possess health benefit properties. The most common traditional Malaysian medicinal plants are *Aloe vera* (lidah buaya), *Andrographis paniculata* (hempedu bumi), *Eurycoma longifolia* (tongkat ali), *Labisia pumila* (kacip fatimah), *Ficus deltoidea* (mas cotek), *Cymbopogon nardus* (serai wangi), *Centella asiatica* (pegaga), *Melastoma malabathricum* (senduduk), *Morinda citrifolia* (mengkudu), *Phyllanthus niruri* (dukung anak), *Momordica charantia* (peria), *Orthosiphon stamineus* (misai kucing) and *Piper sarmentosum* (kaduk).

Plants have long been used as folk herbal medicines to treat various disorders, and their different natural products have inspired the design, discovery, and development of new drugs. Natural products will play a vital part in supplying this need via the continuous exploration of global biodiversity, the majority of which remains unexplored. Some of the useful plant drugs include taxol, demecolcine, caffeine, cocaine, quinidine, morphine, yohimbine, sparteine, galantamine, and atropine. The scientific study of traditional medicine, derivatization of drugs through bioprospecting, and systematic conservation of the concerned medicinal plants are thus of great importance (Hao & Xiao, 2020).

For the time being, experts are discovering new plants with therapeutic characteristics and possibilities for herbal treatments. Clusiaceae is one of the plant families that is thought to enhance therapeutic effects because several species of this family have been used for medicinal purposes worldwide.

1.2 Clusiaceae Family

Clusiaceae family, known as Guttiferae, is comprising about 14 genera and some 800 species of tropical trees and shrubs. It is mainly distributed in Asia, Central America, South America, West Indies, Africa, and Indian Ocean Islands. Several are important for their fruits, resins, or timbers, and a number of species are cultivated as ornamentals (Britannica, 2018).

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. Both fruits and seeds are highly variable. The seeds may be variously winged, have fleshy arils, or be borne in single-seeded berries or drupes. Although little is known about pollinators, there is evidence that in South America, at least, bees visit the family for the oils and resins the flowers contain. Only in a very few genera, such as *Symphonia*, do birds and other visitors take nectar from the flowers (Berry & Paul, 2023).

The most common genera are *Clusia*, *Garcinia*, *Calophyllum*, *Mammea*, *Kayea*, *Kielmeyera*, and *Chrysochlamys*. Genus *Clusia* (300-400 species) is restricted to the New World. Genus *Garcinia* (240 species, including former genera such as *Rheedia* and *Tripetalum*) as well as the genus *Calophyllum* (about 190 species) is found mostly in the Old World. Besides, the genus *Mammea* (about 75 species) is found throughout the tropics, with many species in Madagascar, while the genus *Kayea* (about 70 species) includes one species from Sri Lanka, with most of the rest from western Malesia. In addition, the genus *Kielmeyera* (50 species) is native to South America, especially Brazil, whereas the genus *Chrysochlamys* (55 species, including former genera such as *Tovomitopsis*) grows throughout the Neotropics.

The Clusiaceae are a rather economically important family. Many species, such as *Mesua ferrea* and *Garcinia paucinervis*, have hard wood. Numerous species of *Calophyllum*, *Clusia* Linnaeus, and *Garcinia* produce valuable commercial resin or gum. Gamboge is produced by *Garcinia morella*, Desrousseaux, and other species. *Garcinia mangostana* and *Mammea americana* Linnaeus produce well-known edible fruits. Other species, such as *Calophyllum inophyllum* and *Garcinia indica* Choisy, have oily seeds. Meanwhile, *Hypericum* is important in horticulture and medicine (Crocket, 2010).

1.3 Genus *Garcinia*

Genus *Garcinia* is mainly distributed throughout tropical Asia, Africa, New Caledonia, Polynesia, and Brazil. It comprises more than 400 species of trees and shrubs that are a common component of lowland tropical forests. In Africa, including Madagascar, and the forested areas in Cameroon, Equatorial Guinea, Gabon, Angola, and the Democratic Republic of the Congo, the genus *Garcinia* forms a significant constituent of the lower strata of dense lowland to submontane rain forests (Sosef et al., 2012). The common names for the plants in this genus are garciniasor, mangosteens, saptrees, kokum, and the ambiguously named 'monkey fruit' (Hemshekhar et al., 2011).

The plants of this family are very ornamental, with a dense canopy of green leaves and fragile, red-tinged developing leaves. The tree is big, with elliptic, oblong, glossy, deep-green leaves that can grow to be up to 5–8 cm long and 2-3 cm wide. The fleshy, dark pink blossoms can be found alone or in a spreading cluster. The fruit is orange-sized, brownish or purple, with yellow specks on the top, and a 4-parted, stalk-less stigma at the top. The fruit pulp has six to eight seeds and is juicy, white, and flavourful (Hemshekhar et al., 2011).

The genus *Garcinia* displays various features that are of general interest to taxonomists and ecologists. In many regions, the genus is distinguished by its occurrence of high numbers of sympatric species, and this diversity is primarily notable since species that are both dioecious and agamospermous may be common in the genus (Ngnitedem et al., 2023). Moreover, *Garcinia* species exhibit the highest diversity of floral forms, mostly in the androecium, as is found anywhere in angiosperms and as such, there exist many unresolved taxonomic problems around it (Sweeney, 2008). It was reported that *Garcinia* leaves contained 75% moisture, 2.3% protein, 0.5% fat, 1.24% fire, 17.2% carbohydrate, 14% iron, 25% calcium and 10% ascorbic acid, and 10% oxalic acid. The rind contains protein (1%), tannins (1.7%), total sugar (4.1%), pectins (0.9%), and fat (1.4%), moisture (80.0 g/100 g), all of which have important therapeutic properties (Noreen et al., 2023).

Among *Garcinia* species, *G. cambogia* has excellent properties beneficial to many health conditions. The fruit, which resembles a pumpkin in appearance, is presently most often used and heavily promoted as a supplement for weight loss. According to the study, hydroxycitric acid, the main organic acid present in the fruit rind, has antiobesity properties that include lowering appetite and reducing body fat gain. There are already several *G. cambogia* dietary supplements available for weight loss, however there are some safety concerns about the potential toxicity of continuous usage of these supplements (Noreen et al., 2023).

The genus *Garcinia* comprises a large collection of medicinal plants with therapeutic potential. Different plant parts including leaves, bark, stem, fruits, fruits rinds, and flowers have been used worldwide to treat several disorders. For instance, the pericarps of mangosteen have been used for centuries in the Asian region as a traditional medicine in treating skin infections, trauma, dysentery, abdominal pain, and wounds. In addition, the rind of this fruit has been used in Thailand for treating skin conditions, wounds, and diarrhoea; while the pulp has been used to treat fever (Abdallah et al., 2022). Table 1.1 lists the medicinal uses of several *Garcinia* species.

Table 1.1

Medicinal uses of several Garcinia species

Species	Part	Medicinal Uses
G. bancana	Leaves	Used to treat fever (Jabit et al., 2009)
G. cambogia	Fruits	Used for constipation, hemorrhoids, and intestinal parasites (Noreen et al., 2022)
G. celebica	Roots	Used to treat malaria and cancer (Pasaribu et al., 2021)
G. cowa	Barks	Used as an antipyretic and antimicrobial agent (Jabit et al., 2009)
	Latex	Used as antifever agent (Mahabusarakam et al., 2005)
G. gummigutta	Fruits	Treating gastrointestinal ailments, intestinal parasites, rheumatism, and digestive disturbances (Noreen et al., 2022)
G. kola	Fruits	Used as an antidiabetic agent (Nkono et al., 2022)
	Seeds	Used for liver disorders, bronchitis, throat infections, colic, head or chest colds (Nkono et al., 2022)
	Roots	Used for cleaning teeth by cutting into short chew sticks. (Farombi et al., 2011)
G. lanceifoila	Fruits	Used as a pain reliever and hypoglycaemic agent (Ghosh et al., 2018)
G. livingstonei	Fruits	Used to treat diabetes, parasitic infections, and microbial infections (Ramadwa & Taylor, 2023)
G. maingayi	Leaves	Used as antifever (Jabit et al., 2009)
G. opaca	Leaves	Used to improve blood circulation (Jabit et al., 2009)

Species	Part	Medicinal Uses
G. mangostana	Leaves	Used in traditional medicine in Southeast Asia as an anti-inflammatory and antiseptic food (Chaverri et al., 2008)
	Fruits	Treatment of abdominal pain, diarrhea, dysentery, infected wounds, suppuration, and chronic ulcer (Chaverri et al., 2008)
	Rinds	Used to reduce fever and to treat diarrhea, thrush, dysentery, aphthae, and various disorders of the urinary system (Mohamed et al., 2017)
G. merguensis	Leaves	Used in folk medicine for the treatment of edema (Jabit et al., 2009)
G. multiflora	Fruits	Used to treat acne, scabies, constipation, and inflammation (Nguyen et al., 2023)
G. nervosa	Leaves	Used to treat skin infections and wound healing (Jabit et al., 2009)
G. nigrolineata	Leaves	Used to treat eye diseases (Jabit et al., 2009)
G. oligantha	Stem bark	Used to treat inflammation, internal heat, toothache, and scald (Tan et al., 2023)
G. penangiana	Leaves	Used to treat skin diseases and fever (Jabit et al., 2009)
G. xanthochymus	Fruits	Used to treat bilious conditions, diarrhea, and dysentery. (Chen et al., 2017)
G. indica	Fruits	Effective in the treatment of dysentery, tumors, and heart complaints (Lakshmi et al., 2011)

Species	Part	Medicinal Uses
G. humilis	Fruits	Traditionally used as a hunger suppressant and for healing the skin (John et al., 2018)
G. pedunculata	Fruits	Used to treat gastrointestinal disorders (Bhattacharjee et al., 2021)
G. morella	Pulps	Used as a home remedy for stomach ailments, inflammatory disorders, and gastritis (Choudhury et al., 2018)
G. dulcis	Fruits	Treatment of ailments such as lymphatitis, parotitis, struma, scurvy, cough, and sore throat (Khamtong et al., 2017)
G. atroviridis	Leaves	The decoctions were used for ear-aches (Muchtaridi et al., 2022)
G. forbesii	Fruits	Used to provide relief from scurvy (Haris & Sani, 2021)
	Barks	Used to clean wounds (Haris & Sani, 2021)
	Leaves	Used to cure swellings (Haris & Sani, 2021)
G. schomburgkiana	Fruits	Used as a cough treatment, diabetes medication, and a laxative (Meechai et al., 2018)
G. lucida	Barks	Used to prevent food poisoning, cure stomach and gynecological pain, and snake bites (Sonfack et al., 2021)

Species	Part	Medicinal Uses
G. brasiliensis	Leaves	Used to treat tumors, inflammation of the urinary tract, and arthritis as well as to relieve pain (Cecelia et al., 2011)
G. madruno	Latex	Used to treat ulcers and oxidative-related diseases (Hormaza et al., 2023)
G. rubro-echinata	Barks	Used to treat fever, diarrhea, and dysentery (Espirito et al., 2020)
	Leaves	Used as a diuretic and for treating skin diseases (Espirito et al., 2020)
G. hanburyi	Barks	Drastic purgative, emetic, and a vermifuge for treating tape worm (John et al., 2018)
G. marophylla	Fruits	Treating dysentery, rheumatism, inflammation, and as an antiparasitic or antimicrobial medication (John et al., 2018)
G. cochinchinnensis	Barks	Used to cure allergy, itches and skin diseases (Farinazzi et al., 2016)
	Buds	Used for the treatment of threatened abortion in Vietnam (Farinazzi et al., 2016)
G. hombroniana	Fruits	Used to relieve itching and as a protective medicine against infections after childbirth (On, 2018)

In this study, *G. urophylla* (Figure 1.1) has been selected to investigate the essential composition, phytochemicals, and their biological activities. *G. urophylla* is locally known as “kandis hutan’ in Peninsular Malaysia. This plant is a small fruiting tree (3-10 m high), usually scattered throughout the hills. The native range of this species is Peninsula Malaysia to Sumatera. The fruits are used to treat stomachache and the leaves are used to treat fever (Jabit et al., 2009).

Figure 1.1

Garcinia urophylla

1.4 Problem Statement

The Clusiaceae family is a rich source of secondary metabolites, in which four major classes of compounds are found: xanthones, coumarins, biflavonoids, and benzophenones, produced by the plants mainly as a defense mechanism (Melo et al., 2014). The genus *Garcinia*, which belongs to the family Clusiaceae, is one of the largest genera of tropical evergreen trees that is endemic in Malaysia, however some of them remain to be underexplored and unresolved. A great diversity of interesting bioactive secondary metabolites was isolated from many species in the genus, which consists of mainly phenolic compounds in the classes of xanthones, coumarins, and flavonoids (Ullah et al., 2020). Therefore, this research aimed to investigate the phytochemicals from the leaves and stem bark of *G. urophylla*, as well as to assess the potential bioactivity of the crude extracts and isolated phytochemicals towards DPPH free radical scavenging assay and phenolic content.

To the best of our knowledge, there is only one study pertaining to phytochemical investigations conducted on the leaf part of *G. urophylla* (Khalid et al., 2007). However, no report was found on the isolation of phytochemicals from the stem bark. In addition, this is the first report on the chemical composition of *G. urophylla* leaves essential oil. Hence, there is a necessity to address the need of filling up the gap of knowledge on the phytochemicals that were present in these species to supply the demand of a prospective phytochemical database for future research use. As a result, the study's findings might benefit the pharmaceutical sector in the future.

1.5 Objectives of Study

The objectives of the study are:

1. To determine the chemical compositions of the essential oil of the leaves of *G. urophylla.*
2. To isolate the phytochemicals from the leaves and stem bark extracts of *G. urophylla* and characterised spectroscopically.
3. To determine the antioxidant activity of crude extracts and phytochemicals of *G. urophylla.*

1.6 Scopes of Study

The study was separated into three parts. The first part was the extraction of the essential oil by hydrodistillation method from the leaf part of *G. urophylla.* The chemical composition of the essential oils was analysed using GC-FID, GC-MS, and Kovats Indices. The second part was the isolation of phytochemicals from the leaves and stem bark extracts of *G. urophylla* using various chromatographic methods such as column chromatography. The chemical structures of the isolated phytochemicals were analysed spectroscopically using FT-IR, NMR (1D/2D), and MS (GC-MS/HR-EIMS). Finally, the antioxidant activities of the phytochemicals and crude extracts were carried out using DPPH free radical scavenging assay and total phenolic content.