



Faculty of Resource Science and Technology

**Secondary Metabolites from *Garcinia dryobalanoides* and
their Anti-bacterial Activity**

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Secondary Metabolites from *Garcinia dryobalanoides* and their
Anti-bacterial Activity

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DECLARATION

I declare that the work in this thesis was carried out in accordance with the regulations of Universiti Malaysia Sarawak. Except where due acknowledgements have been made, the work is that of the author alone. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree.

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ABSTRACT

Garcinia was discovered to have excellent sources of bioactive compounds with therapeutic potential and hence received significant attention due to its potential for medicinal applications. This study investigates *Garcinia* species' secondary metabolites and their anti-bacterial activity, specifically *Garcinia dryobalanoides*. Different chromatographic methods were used to isolate and purify the pure compounds. Along with that, structural modification of the major compound was successfully synthesized *via* Williamson etherification. The structural elucidations of these isolated and synthesized compounds were achieved through FT-IR, UV-Vis, LC-MS, 1D, and 2D-NMR. Anti-bacterial tests were conducted against various bacterial strains, including Gram-positive bacteria (*Lactiplantibacillus plantarum*) and Gram-negative bacteria (*Enterobacter cloacae*, *Pseudomonas aeruginosa*, and *Serratia marcescens*). Six compounds (**76**, **2**, **77**, **78**, **79** and **59**) were successfully isolated and five rubraxanthone derivatives (**80** – **84**) were successfully synthesized. Astonishingly, compound **76** was the first naturally occurring benzophenone isolated from the *Garcinia* genus. The anti-bacterial evaluation on crude extract, isolated, and synthesized compounds against tested bacteria demonstrated moderate to excellent activities outlining anti-bacterial potential. Structure-activity relationships (SAR) study demonstrated that xanthenes bearing prenyl and hydroxy groups exhibit excellent bacterial inhibition. However, adding an alkyl chain to the hydroxyl group reduced this effect, possibly due to steric hindrance. This study highlights *Garcinia* species as a promising source of natural anti-bacterial and further investigation is needed.

Keywords: *Garcinia dryobalanoides*, secondary metabolites, extract, anti-bacterial activity, xanthone derivatives

Metabolit Sekunder daripada Garcinia dryobalanoides dan Aktiviti Anti-bakteria

ABSTRAK

Garcinia didapati mempunyai sumber sebatian bioaktif yang sangat baik dengan nilai terapeutik yang tinggi. Oleh itu, ia telah mendapat perhatian kerana potensinya dalam bidang perubatan. Kajian ini dijalankan bagi menyiasat metabolit sekunder dan aktiviti anti-bakteria dari spesies *Garcinia*, khususnya *Garcinia dryobalanoides*. Pelbagai kaedah kromatografi telah digunakan dalam mengisolasi dan purifikasi sebatian kimia tulen dari spesies ini. Selain itu, pengubahsuaian struktur sebatian kimia utama berjaya disintesis melalui Williamson etherifikasi. Pengenalan struktur bagi sebatian kimia tulen telah dicapai melalui FT-IR, UV-Vis, LC-MS, 1D, dan 2D-NMR. Ujian anti-bakteria telah dijalankan terhadap pelbagai strain bakteria, termasuk bakteria Gram-positif (*Lactiplantibacillus plantarum*) dan bakteria Gram-negatif (*Enterobacter cloacae*, *Pseudomonas aeruginosa*, dan *Serratia marcescens*). Enam sebatian kimia tulen (76, 2, 77, 78, 79 dan 59) berjaya dipurifikasi dan lima derivatif rubraxantone (80 – 84) berjaya disintesis. Sebatian 76 ialah benzofenon yang pertama terhasil secara semula jadi yang diasingkan daripada genus *Garcinia*. Penilaian anti-bakteria ke atas ekstrak *G. dryobalanoides*, sebatian tulen dan sintesis, terhadap bakteria yang diuji menunjukkan aktiviti antibakteria yang sederhana dan tinggi. Kajian SAR menunjukkan bahawa xanton yang mengandungi kumpulan prenil dan hidroksi mempamerkan perencatan bakteria yang sangat baik. Walau bagaimanapun, penambahan rantai alkil kepada kumpulan hidroksil mengurangkan kesan ini, mungkin disebabkan oleh halangan sterik. Kajian ini telah membuktikan spesies *Garcinia* sebagai sumber anti-bakteria semulajadi yang bagus dan penyelidikan lebih mendalam amat diperlukan.

Kata kunci: Garcinia dryobalanoides, *matabolit sekunder, ekstrak, aktiviti anti-bakteria, xanton derivatif*

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LIST OF ABBREVIATIONS

NMR	Nuclear Magnetic Resonance
<i>s</i>	singlet
<i>d</i>	doublet
<i>dd</i>	doublet of doublet
<i>t</i>	triplet
<i>m</i>	multiplet
1D-NMR	One-dimensional Nuclear Magnetic Resonance
¹ H NMR	Proton Nuclear Magnetic Resonance
¹³ C NMR	Carbon Nuclear Magnetic Resonance
DEPT	Distortionless Enhancement by Polarization Transfer
2D-NMR	Two-dimensional Nuclear Magnetic Resonance
COSY	Correlated Spectroscopy
HSQC	Heteronuclear Single Quantum Coherence
HMBC	Heteronuclear Multiple Bond Correlation
UV–vis	Ultraviolet-Visible Spectroscopy
FT-IR	Fourier Transform Infrared Spectroscopy
LCMS	Liquid Chromatography Mass- Spectrometry
DMSO	Dimethyl sulfoxide
TLC	Thin Layer Chromatography
IZD	Inhibition zone diameter
MIC	Minimum Inhibition Concentration
MBC	Minimum Bactericidal Concentration

GDH	<i>Garcinia dryobalanoides</i> hexane extract
GDEA	<i>Garcinia dryobalanoides</i> ethyl acetate extract
GDM	<i>Garcinia dryobalanoides</i> methanol extract
MRSA	<i>Methicillin-Resistant Staphylococcus aureus</i>
MDRS	<i>Multidrug-Resistant Salmonella</i>
SARs	Structure-activity relationship (SAR) studies

CHAPTER 1

INTRODUCTION

1.1 Background of Study

The rise in infectious disease cases linked to anti-microbial resistance is a wake-up call for new anti-microbial agents to be discovered (Naves et al., 2019). The breakthrough of antibiotics, led by penicillin, resulted in a monumental paradigm shift in treating bacterial infections, substantially decreasing morbidity and mortality rates associated with infectious diseases (Liu et al., 2022). The medical community once hailed the discovery of antibiotics as a victory in the war against infectious diseases (Reygaert, 2018). However, misuse of the compound has increased bacterial resistance (Joana et al., 2019).

Notably, natural products (NPs) are a cornerstone of inspiration for innovative drug design and development (Davison & Brimble, 2019). Plants with medicinal benefits have recently piqued the interest of science and the pharmaceutical industry, of which natural substances account for approximately 73% of the drugs produced by this industry (Conceição et al., 2023). This is due to plants can produce secondary metabolites to resist pathogens, making them a promising source of anti-microbial leads (Liu et al., 2022). Moreover, investigating plants with medicinal properties yields valuable insights applicable to developing novel plant-based drugs and other therapeutic agents (Santo et al., 2020). Conversely, modifying the structure of the natural resources is also known to lead to novel medications (Yao et al., 2017). Several studies, including those by Lee et al. (2018), Zou et al. (2013), and Karunakaran et al. (2018), have investigated the modified structure of natural resources and evaluated their resulting biological activities which have led to new therapeutic agents.

Santo et al. (2020) stated that the former Guttiferae and Clusiaceae families stood out among all medicinal plants. Clusiaceae is a family of medicinal plants that includes approximately 50 genera and 600 species (Lim et al., 2021). It can be found in subtropical and tropical areas (Conceição et al., 2023). The Clusiaceae family is a diverse group of plants that includes lianas, woody perennials, shrubs, and trees (Mañourová et al., 2019). This family has been widely used in ethnomedicine as a remedy for various disease conditions, such as infection, inflammation, cancer, dysentery, ulcers, and wounds (Lim et al., 2021). *Garcinia*, is a plant genus that belongs to the Clusiaceae family (Santo et al., 2020), also received great attention due to its potential for a wide range of medicinal properties (Wong et al., 2017). The *Garcinia's* medicinal properties are attributed to its ample secondary metabolites (Wong et al., 2017), which include triterpenes, xanthones, flavonoids, coumarins, benzophenones, anthrones, and anthraquinones (Brito et al., 2017).

1.2 Genus *Garcinia*

Garcinia (synonym = *Rheedia*) (Gontijo et al., 2012) is a member of the Clusiaceae family, which was previously known as Guttiferae that can be found in Asia, Polynesia, Africa, and Australia (Akongwi et al., 2023). The genus was given its name after Laurent Garcin, a Swiss botanist who worked for the Dutch East India Company and released the first report on *Garcinia mangostana* (mangosteen), the most well-known fruit species in the genus of *Garcinia* (Mañourová et al., 2019). *Garcinia* is a dioecious evergreen tree (Paul & Zaman, 2022) that contains over 600 species (Akongwi et al., 2023), consists of leathery leaves, solitary flowers, and two to eight pulpy seeds with fleshy fruits (Paul & Zaman, 2022). The colour of the inner bark of *Garcinia* is occasionally white or yellow. Meanwhile, stembark can be either grey or brown. *Garcinia* branches and stems

exude “gamboge” (a gummy resin exudate). The colours of the exudates (cream, white, or yellow) play a role in figuring out the different *Garcinia* species (Paul & Zaman, 2022).

G. dryobalanoides (Figure 1.1) is one of the *Garcinia* species that grows in Malaysia. It is known as *kandis* by the locals. This botanical record, initially documented in the fifth volume of Flore Forestière de Cochinchine on page seven during the year 1883, designates the species' native plant as Borneo, Malaysia. This tree grows primarily within the wet tropical biome (POWO, 2024).



Figure 1.1: Herbarium specimen of leaves and twigs of *G. dryobalanoides*

Garcinia has been used in traditional medicine worldwide (Brito et al., 2017). The locals in regions where *Garcinia* species grow have used these compounds to heal various illnesses (Conceição et al., 2023). The leaves and stem bark of *G. fagraeoides* have been used to treat malaria and dermatitis in local traditional medicine (Thuy et al., 2022). Meanwhile, the bark of *G. kola* has been used to treat malaria and abdominal pain (Mañourová et al., 2019). *Garcinia* fruit infusions, on the other hand, were used to treat dysentery, ulcers and wounds (Santo et al., 2020). Aside from that, in Ayurvedic medicine, the fruits of *Garcinia* were used to treat infections and urinary disorders as well as to improve digestion. *Garcinia* gummy exudates as well are used as cathartics and emetics in folk medicine. For instance,

G. dulcis gummy exudates are used as an expectorant and laxative in Thai medicine (Paul & Zaman, 2022).

Conversely, the pharmaceutical industry has taken notice of the use of *Garcinia* plants to treat a variety of disorders (Brito et al., 2017). The Guttiferae family contains a variety of biologically active metabolites that have been shown to have a variety of intriguing biological properties such as anti-oxidant activity, anti-aflatoxigenic activity, anti-depressant activity, trypanocidal activity, anti-bacterial activity, cytotoxic activity, and anti-malarial activity (Gontijo et al., 2012). Khamthong and Hutadilok-Towatana (2017) also reported that several pharmacological and phytochemical studies of *Garcinia* species have revealed that the plant's various parts contain a diverse array of secondary metabolites, a great number of which have medicinal benefits.

1.3 Problem Statement

Nowadays, anti-microbial resistance (AMR) has become one of the crucial public health problems due to the emergence and spread of drug-resistant pathogens. The advent of β -lactam antibiotics, beginning with penicillin, has been instrumental in treating bacterial infections (Breijyeh et al., 2020). β -lactams were the most used anti-microbial agents (Reygaert, 2018). However, overuse and misuse of anti-microbial medications make infections more difficult to treat and potentially fatal (Liu et al., 2022). Thus, there's an urge to discover new compounds for anti-microbial research. Natural products have been an important framework for developing antibiotics. The genus *Garcinia*, for instance, has garnered significant attention due to its potential for diverse medicinal properties, particularly its anti-microbial activity (Wong et al., 2017). Malaysia has a tropical rainforest that consists of diverse tree species. However, many species, including Malaysian *Garcinia*