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Phytochemical Profiling of *Garcinia rostrata*, *Garcinia dryobalanosides* and *Garcinia cuneifolia* and Their Antibacterial Activity

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ABSTRACT

Garcinia spp. have been used in traditional medicine to treat various ailments, and recent studies have confirmed their pharmacological activities. In this context, the present study focused on three *Garcinia* spp., namely *Garcinia rostrata*, *Garcinia dryobalanoides* and *Garcinia cuneifolia*, which gain less attention in terms of their phytoconstituent and biological activity data. Methodologically, in this study, the phytochemical constituents of the three *Garcinia* sp. was determined through gas chromatography mass spectrometry (GC-MS) whereby the antimicrobial activity was evaluated using the Disc diffusion and Dilution method. The results showed that the extract from *Garcinia dryobalanoides* exhibited the most potent antibacterial activity against *Bacillus amyloliquefaciens* compared to the other species. The phytochemical analysis found that *Garcinia dryobalanoides* extract contained significant amounts of (Z)-18-Octadec-9-enolide and *n*-hexadecanoic acid, which are known to possess antibacterial properties. These major constituents were found to interact synergistically to produce the observed antibacterial activity. The findings suggested that *Garcinia dryobalanoides* could be a promising source for developing new antibiotics to combat bacterial infections. Overall, this study highlights the potential of *Garcinia* spp. for discovering new bioactivities, particularly their antibacterial properties. Further research is needed to explore the full range of phytochemical constituents and biological activities of these plants, which could lead to the development of new drugs to combat antibiotic resistance.

Keywords: Antimicrobial activity, *Garcinia*, GCMS, phytochemical analysis

INTRODUCTION

Plants have been used in medicine for a long time as natural remedies to cure diseases. Certain plant species have shown remarkable efficacy in combating microbial threats, implying a wealth of potential in the natural world (Parekh & Chanda, 2007). These properties were associated with the abundance of secondary metabolites such as phenolic compounds, terpenoids, flavonoids, and alkaloids (Othman *et al*., 2019; Kianfe *et al*., 2020). These compounds have an outstanding ability to prevent the growth of viruses, bacteria, and fungi.

Garcinia is the largest and taxonomically significant genus within the family Clusiaceae. It comprises nearly 250 species worldwide (Garden *et al*., 2020). The genus *Garcinia* is found abundantly in Malaysia (Khapare *et al*., 2020; Dominic *et al*., 2015), a tropical rainforest country that is rich with flora and fauna (Surbramaniam, 2013). The morphology of *Garcinia* varies depending on the species. Generally, *Garcinia* plants are evergreen trees or shrubs that can grow up to 20-25 m tall, although some species may be smaller (Wu *et al*., 2022). The leaves of *Garcinia* spp. are usually glossy and dark green, and they are arranged in pairs or whorls. The leaves can be simple or compound, and they range in size from 15 to 20 cm in length (Guedje *et al*., 2007). Meanwhile, its barks are usually brown or grey and are rough to the touch (Bora *et al*., 2017). *Garcinia* spp. is known for having good biological activities to combat many diseases owing to the existence of compounds that have significant therapeutic properties (Nguyen *et al*., 2017) such as oxygenated and prenylated xanthones. These compounds have excellent biological activity as

an anti-fungal (Adekunle *et al*., 2020), anti-inflammatory (Feng *et al*., 2021), anti-tumour (Jin *et al*., 2019), anti-oxidant (De Melo *et al.*, 2021), human immunodeficiency virus (HIV)-inhibitory (Corona *et al*., 2021) and antilipidemic properties (John *et al*., 2019).

To date, antibiotic resistance has been a major threat to global health, food security, and development. This escalating public health crisis crosses geographical and demographic boundaries, jeopardising the effective treatment of a wide range of infectious diseases in people of all ages and from all countries. In fact, infections such as pneumonia, tuberculosis, gonorrhoea, and salmonellosis are becoming more difficult to treat as antibiotics used to treat these diseases become less effective (WHO, 2020). This situation leads to an increased mortality rate hence, there is a need to search for a novel antibiotic to combat antibiotic resistance (Varela *et al*., 2021). Currently, there are 30,0000 antibiotic compounds that have been successfully identified from natural products (Thirumurugan *et al*., 2018). *Garcinia* is known for its anti-microbial activity in fighting various bacteria strains (Lin *et al*., 2021). For instance, *Garcinia gummi-gutta* essential oil contains high chemical constituents of (E)-β-farnesene and β-caryophyllene, which inhibits methicillin-resistant *Staphylococcus aureus* (MRSA) (Tan *et al*., 2020). Other than that, Garcigerin A and a-mangostin (xanthone) isolated from *Garcinia dulcis* show remarkable inhibition towards two pathogens, *S. aureus* and MRSA (Tamhid *et al*., 2019). Besides xanthone, bioflavonoids isolated from *Garcinia livingstonei* show excellent bacteria inhibition activity toward some nosocomial bacteria (Kaikabo and Eloff, 2011). Triterpene, namely Friedline isolated from *Garcinia latissima*, exhibits anti-bacterial activity against *Bacillus subtilis* (Ambarwati *et al*., 2019).

With this understanding, three Garcinia spp., namely *Garcinia dryobalanoides*, *Garcinia rostrata*, and *Garcinia cuneifolia* were investigated for their qualitative phytochemical analysis and their antimicrobial activity.

MATERIALS AND METHODS

Plant Material

The stem bark of *Garcinia rostrata* (UITM3022) was collected from Aluvial Forest, Jalan Sungai Moyan, Sarawak. For *Garcinia dryobalanoides* (UITM3032) and *Garcinia cuneifolia* (UITM 3025), the stem barks were collected from Semanggoh, and Keranggas Forest, Jalan Sungai Cina Matang, Sarawak, respectively. All samples were identified by a botanist.

Chemical and Solvent

A chromatography or an analytical grade solvent were used throughout this study. All chemicals were purchased from Merck (Darmstadt, Germany) and Sigma-Aldrich (Chemie, Steinheim, Germany)

Preparation of Plant Extract

The collected plant samples were air-dried and ground into fine powder. The powdered stem bark of *Garcinia rostrata*, *Garcinia dryobalanoides*, and *Garcinia cuneifolia* underwent maceration three times with increasing polarity solvents (hexane, ethyl acetate, and methanol) for 72 h. The macerated plant sample was then filtered, and the filtrates were allowed to evaporate under reduced pressure to obtain dry plant extracts of hexane, ethyl acetate, and methanol (Zamakshshari *et al*., 2016).

Anti-bacterial Assay

All bacteria stock cultures were preserved in Muller-Hinton Broth and stored at 4 °C. The antimicrobial activities were tested against two bacteria: two gram-positive (*Bacillus amyloliquefaciensand* and *S. aureus*) and two gram-negative (*Pseudomonas aeruginosa* and *Escherichia coli*). All of the bacteria strains were obtained from the Microbiology Laboratory at Universiti Malaysia Sarawak's Faculty of Resources Science and Technology. The diffusion method was applied to identify the antibacterial activities (Zamakshshari *et al*., 2022) with 1 mg/mL of concentration for each extract. The antimicrobial activities were assessed by measuring the diameter of zone inhibition after incubating the plates for 24 h at 37 °C. Positive and negative controls that were used for the antibacterial assay were streptomycin sulphate (10 µg/mL) and dimethyl sulfoxide (DMSO), respectively. Broth microdilution assay, on the other hand, were implemented to determine the minimum inhibitory concentration (MIC) the plant extract.

Serial dilutions of the plant extracts (1.0 mg/mL – 1.95 ug/mL) were used for the assay. Each well containing the diluted extract was supplemented with 20 µL of a 5 mg/mL TTC solution and incubated at 37 °C for 1 hour. Reduction of TTC to a pink formazan by viable microbes was used as an indicator of growth. The MIC value was determined from the lowest concentration that remained colourless. Then, from each MIC broth tube without visible growth, 100 µL of broth was pipetted onto Muller-Hinton agar and spread across the entire surface of the plate to determine the minimum bactericidal concentration (MBC). The plate was incubated for 18 – 24 h at 35 °C prior to examination of the colony growth on each plate.

Gas Chromatography Mass Spectrometry (GC-MS)

GC-MS was carried out using a Shimadzu GCMS-QP2010 Plus spectrometer. The constant pressure was set at 100.0 kPa and helium was used as the carrier gas. An RTX-5MS fused silica capillary column (30 m × 0.25 mm) with a film thickness of 0.25 µm was used in the GC-MS. Injection was performed in splitless mode at 300 °C in the injector. The temperature of the oven was increased by 4 °C/min from 40 to 160 °C (5-minute hold) and by 5 °C/min from 160 to 280 °C (15-minute hold). Each sample analysis was completed within a total run time of approximately 74 minutes. The GC-MS interface temperature was maintained at 280 °C. MS mode was used for analytical scanning between 45 and 500 atomic mass units (amu). The ion source temperature was set to 280 °C. Peaks were identified using the National Institute of Standards and Technology Mass Spectral Library (NIST17).

RESULTS & DISCUSSION

Extraction

The extraction of bioactive compounds from plants is essential for the nutraceutical and pharmaceutical industries. This process is critical to preserving the active ingredients in herbal plants and preventing their loss or destruction during preparation. Extracts obtained from plants provide a vast array of valuable compounds that are useful for further analysis and research. Therefore, the extraction step is vital before analysing the herbal plants for their potential benefits (Yahya *et al*., 2018). In this study, three *Garcinia* spp.: *Garcinia dyrobalanoides*, *Garcinia rostrata* and *Garcinia cuneifolia*, were extracted using maceration method with three solvents with different polarities namely ethyl acetate, methanol and hexane. The purpose of using three different polar solvents in the extraction of plants is to obtain a broad spectrum of chemical constituents that may have varying polarities and solubilities (Aissou *et al*., 2017). The results showed that *Garcinia dyrobalanoides* and *Garcinia rostrata* had a high percentage yield for the ethyl acetate extract compared to their methanol and hexane extracts (Table 1). This indicates that these two plant species are rich in semipolar compounds, which are soluble in ethyl acetate. On the other hand, the results showed that *Garcinia cuneifolia* had a high percentage yield of methanol extract compared to other extracts. This result indicated that *Garcinia cuneifolia* contains high-polar compounds that are more soluble in methanol. In addition, the findings suggested that the solvent used for plant extraction can significantly impact the yield and composition of the extract obtained.

Table 1. Percentage yield of extract obtained from *Garcinia dryobalanoides*, *Garcinia cuneifolia* and *Garcinia rostrata*

Species	Extract	Weight of plant sample (kg)	Extract weight (g)	Percentage yield (wt/wt)
Garcinia dryobalanoides	Hexane	3.40	41.40	1.22
	Ethyl Acetate		161.05	4.73
	Methanol		69.87	2.05
Garcinia cuneifolia	Hexane	0.51	2.66	0.52
	Ethyl Acetate		11.04	2.16
	Methanol		29.85	5.85
Garcinia rostrata	Hexane	1.32	3.07	0.23
	Ethyl Acetate		172.09	13.01
	Methanol		84.58	6.39

Antimicrobial Assay

The well diffusion method was used to screen the antimicrobial activity of extracts obtained from *Garcinia dryobalanoides*, *Garcinia rostrata* and *Garcinia cuneifolia* against selected bacteria. The screening results are tabulated in Table 2. A concentration of one mg/mL of the extract was used, as per the guidelines set by Pretto *et al*. (2004), which considers a plant extract with more than 1000 µg/mL as weak antimicrobial activity. All extracts of *Garcinia dryobalanoides* and the ethyl acetate extract of *Garcinia cuneifolia* showed good antimicrobial activity against *B. amyloliquefaciens* compared to other extracts. The antimicrobial activity is due to the presence of major compounds such as (Z)-18-octadec-9-enolide, n-hexadecanoic acid, 2-Propenoic acid, Octabenzone and 1,6,10,14,18,22-Tetracosahexaen-3-ol.
Meanwhile, none of the extracts demonstrated an inhibition zone against *S. aureus*. For the gram-negative bacteria, only the methanol extract of *Garcinia dryobalanoides* and the ethyl acetate extract of *Garcinia cuneifolia* showed more than a 10 mm inhibition zone against *E. coli* and *P. aeruginosa*, respectively. The extracts that showed more than a 10 mm inhibition zone were further evaluated for their minimum inhibitory concentration (MIC) value. The results showed that both ethyl acetate and methanol extracts of *Garcinia dryobalanoides* had MIC values of 500 µg/mL against *E. coli*, which are considered to have moderate antimicrobial activity (Pretto *et al.*, 2004). Moderate activity was also seen in the inhibition against *B. amyloliquefaciens* by *Garcinia dryobalanoides* and *Garcinia cuneifolia* extract. Only the ethyl acetate extract of *Garcinia dryobalanoides* gave a 250 µg/mL MIC value against *B. amyloliquefaciens*. Meanwhile, other extracts, such as hexane and methanol extract of *Garcinia dryobalanoides* and ethyl acetate extract of *Garcinia cuneifolia*, gave 500 µg/mL MIC values against *B. amyloliquefaciens*. It can be concluded that the evaluated Garcinia spp. exhibited bacteriostatic characteristics by their ability in inhibiting bacteria growth.

Table 2. Inhibition diameter of crude extract on garcinia species and positive control against selected microbes.

Plant	Extract	Inhibition zone (mm)			
		Bacteria strain tested			
		Bacillus amyloliquefaciens	*Staphyloccus aureus*	*Escherichia coli*	*Pseudomonas aeruginosa*
Garcinia dryobalanoides	Hexane	13.33 ± 0.57	NA	9.00 ± 0.00	NA
	Ethyl Acetate	14.33 ± 0.57	NA	10.00 ± 1.00	NA
	Methanol	11.66 ± 1.52	NA	11.00 ± 1.71	9.67 ± 0.57
Garcinia cuneifolia	Hexane	NA	NA	8.00 ± 1.00	NA
	Ethyl Acetate	14.00 ± 1.00	NA	9.33 ± 1.73	11.33 ± 0.57
	Methanol	NA	NA	9.00 ± 1.00	9.67 ± 0.57
Garcinia rostrata	Hexane	NA	NA	7.00 ± 1.00	NA
	Ethyl Acetate	8.67 ± 0.57	NA	9.33 ± 1.15	NA
	Methanol	NA	NA	9.00 ± 1.00	7.67 ± 0.57
Streptomycin		23.67 ± 0.57	24.33 ± 0.58	28.67 ± 0.57	26.00 ± 1.73
DMSO		NA	NA	NA	NA

GC-MS Analysis

The investigation on phytochemical was to analyse the chemical constituents present in various extracts of garcinia using GC-MS. The GC-MS is a powerful analytical technique that separates and identifies complex mixtures of chemicals present in a sample. The researchers analysed the chemical constituents of each extract and compared them to identify the common compounds present in most of the extracts. The results were documented in Table 3. Compounds with a selective index (SI) greater than 80% were identified with a compound name, whereas those with a SI less than 80% remained unclassified when their mass spectrum was compared to the NIST database. A total of 147 different chemical constituents were detected in all the *Garcinia* extracts, including phenols, terpenoids, acyclic alkene, phenylpropanoids, acid and others. Interestingly, the profiling of all *Garcinia* extracts revealed that (Z)-18-Octadec-9-enolide was present in each extract. This compound is also found as a

major component in other plant species such as *Imperata cylindrica* and *Millettia zechiana* (Lalthanpuii *et al*., 2019; Chama *et al*., 2022). The presence of this compound in *Garcinia* extracts suggests its potential as a bioactive compound in *Garcinia*. Furthermore, the GCMS analysis found nine compounds belonging to the family of terpenoids in some of the extracts. In general, terpenoids are cyclic unsaturated hydrocarbons that are linked to the basic isoprene skeleton and have constituent groups that vary in oxygen content. Terpenoids are present in most fruits and plants (Caputi & Aprea 2011). Previous research suggests that terpenoids have potential as protective agents and treatments for chronic illnesses including cancer and heart disease (Wagner, & Elmadfa, 2003).

Table 3. Forty Common chemical composition founds in three *Garcinia* spp.

No.	Compounds	% compound in extract (10mg extract)								
		Garcinia dryobalanoides			*Garcinia cuneifolia*			*Garcinia rostrata*		
		HEX	EA	MeOH	HEX	EA	MeOH	HEX	EA	MeOH
1	3H-3a,7-Methanoazulene	0.67	N.D	N.D	1.26	N.D	N.D	0.54	N.D	N.D
2	2,5-di-tert-Butyl-1,4-benzoquinone	0.32	0.17	N.D	N.D	N.D	N.D	N.D	N.D	N.D
3	1-Nonadecene	2.68	3.60	0.85	N.D	N.D	N.D	1.51	0.44	3.82
4	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	0.93	1.59	N.D	0.96	N.D	N.D	0.33	N.D	N.D
5	Benzenepropanoic acid	0.44	N.D	0.45	N.D	N.D	N.D	N.D	N.D	N.D
6	Glutaric acid	0.10	0.14	N.D	N.D	N.D	N.D	N.D	N.D	N.D
7	*n*-Hexadecanoic acid	6.71	9.27	N.D	17.33	6.41	0.23	2.57	2.88	7.03
8	9-Octadecenoic acid	0.30	0.79	1.48	N.D	N.D	0.19	N.D	N.D	N.D
9	Methyl stearate	1.24	1.46	0.52	N.D	0.65	N.D	0.49	0.48	0.97
10	(Z)-18-Octadec-9-enolide	6.61	12.86	8.21	45.26	27.01	5.04	9.57	13.42	28.04
11	Octadecanoic acid	2.05	2.21	N.D	N.D	1.35	N.D	N.D	N.D	N.D
12	1-Hexacosanol	3.60	4.67	N.D	N.D	N.D	N.D	N.D	N.D	N.D
13	2-Propenoic acid	2.80	2.52	1.12	N.D	6.47	N.D	1.13	3.04	6.55
14	Bis(2-ethylhexyl) phthalate	0.63	N.D	N.D	N.D	0.42	N.D	N.D	N.D	N.D
15	Tetrapentacontane	0.19	0.21	N.D	N.D	N.D	N.D	N.D	N.D	N.D
16	Octabenzone	7.26	8.31	2.20	N.D	10.48	N.D	0.70	2.45	5.16
17	alpha.-Tocospiro B	1.15	0.61	N.D	N.D	N.D	N.D	N.D	N.D	N.D
18	2,6,10,14-Hexadecatetraen-1-ol	3.22	3.85	N.D	N.D	N.D	N.D	0.78	N.D	N.D
19	(R)-2,8-Dimethyl-2-((3E,7E)-4,8,12-trimethyltrideca-3,7,11-trien-1-yl)chroman-6-ol	3.74	0.56	0.95	N.D	N.D	N.D	N.D	N.D	N.D
20	beta.-Sitosterol acetate	0.88	N.D	1.09	N.D	1.97	N.D	0.76	2.41	4.64
21	1,6,10,14,18,22-Tetracosahexaen-3-ol	22.82	14.00	18.00	N.D	10.66	N.D	4.19	N.D	N.D
22	Stigmasterol	1.70	1.90	3.78	N.D	N.D	N.D	N.D	N.D	N.D
23	Cyclopentadecanone	N.D	0.99	N.D	5.38	N.D	0.29	0.24	N.D	N.D
24	alpha.-Amyrin	N.D	1.90	3.69	N.D	N.D	23.26	N.D	N.D	N.D
25	gamma.-Sitostenone	N.D	0.53	N.D	N.D	N.D	7.97	N.D	N.D	N.D
26	Friedelan-3-one	N.D	2.27	8.88	N.D	N.D	32.99	10.07	9.26	11.19
27	beta.-Amyrone	N.D	0.53	N.D	N.D	N.D	0.64	N.D	N.D	N.D
28	(E)-9-Octadecenoic acid ethyl ester	N.D	N.D	0.57	N.D	2.56	N.D	N.D	N.D	N.D
29	1-Heptacosanol	N.D	N.D	2.26	N.D	1.77	N.D	2.18	1.90	4.59
30	24-Norursa-3,12-diene	N.D	N.D	4.66	N.D	N.D	N.D	0.52	N.D	N.D
31	Linoleic acid	N.D	N.D	6.72	1.49	1.40	N.D	3.07	N.D	N.D
32	Thunbergol	N.D	N.D	0.67	N.D	N.D	N.D	0.54	N.D	N.D
33	Tricyclo[20.8.0.0(7,16)]triacontane	N.D	N.D	28.68	N.D	N.D	N.D	52.29	38.52	13.37
34	Copaene	N.D	N.D	N.D	1.02	N.D	N.D	0.22	N.D	N.D
35	Caryophyllene	N.D	N.D	N.D	0.68	N.D	N.D	0.79	N.D	N.D
36	Pentadecanoic acid	N.D	N.D	N.D	N.D	0.43	N.D	N.D	0.36	N.D
37	Heptadecanolide	N.D	N.D	N.D	N.D	1.07	N.D	N.D	0.27	N.D
38	11,14-Eicosadienoic acid	N.D	N.D	N.D	N.D	0.78	N.D	N.D	0.28	0.80
39	28-Norolean-17-en-3-one	N.D	N.D	N.D	N.D	1.72	N.D	N.D	3.07	6.74
40	Stigmasta-5,22-dien-3-ol	N.D	N.D	N.D	N.D	1.67	N.D	N.D	0.85	N.D

HEX = hexane extract, EA= ethyl acetate extract, MeOH = methanol extract and N.D = not detected.

Correlation of Anti-BacteriaL Activity with Chemical Constituents of the Extract

The chemical constituents present in an extract contribute to the anti-bacterial activity. The results showed that the presence of major constituents such as (Z)-18-octadec-9-enolide, *n*-hexadecanoic acid, 2-Propenoic acid, Octabenzone and 1,6,10,14,18,22-Tetracosahexaen-3-ol in all *Garcinia dryobalanoides* extracts and the ethyl acetate extract of *Garcinia cuneifolia* has led to inhibition of *B. amyloliquefaciens*. These major compounds are interacted synergistically among them and increase their anti-microbial activity. Previous research reported that the presence of

n-hexadecanoic acid can moderately antibacterial activities against several bacteria strains, such as *K. pneumoniae, E. coli, B. subtilis*, and *S. aureus* at low maximum concentrations (Ganesan *et al*., 2022). Besides that, the presence of more than 30% of (Z)-18-Octadec-9-enolide in the extract is reported to lead to good antimicrobial activity (El-Sayed *et al*., 2023). However, even though some extracts contain those compounds as major constituents, the weak anti-bacterial activity shown might be due to antagonistic interactions between major and minor compounds. The synergistic and antagonistic interactions of chemical constituents in a plant extract refer to the way these constituents can interact with each other to produce either a greater or a lesser effect than expected. Synergistic interactions occur when two or more constituents work together to produce a stronger effect than each constituent could produce on its own (Caeser & Cech., 2019). Meanwhile, antagonistic interactions occur when two or more constituents work against each other, resulting in a weaker effect than expected (Guo *et al*., 2019).

CONCLUSION

The qualitative phytochemical analysis and antibacterial activity of three Garcinia spp., namely *Garcinia dryobalanoides*, *Garcinia rostrata*, and *Garcinia cuneifolia* towards four bacteria strains were fully established. Among all, *Garcinia dryobalanoides* extract demonstrated good biological activities compared to others owing to the synergistic interactions of chemical constituents present in the extract. This study is crucial as the identification of extract activities and phytochemical analysis is vital for herbal product development and acts as a bioactive marker or fingerprint for herbal standardisation. Despite that, further study on the isolation and extraction of chemical constituents from Garcinia species, specifically *Garcinia dryobalanoides* is essential to determine their potential as drug candidates to combat the antibiotic resistance that has become one of the most serious threats to world health, food security and development.

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