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Research Article

Garcinia parvifolia Miq. Dried Pericarp Phytochemical Screening and Antibacterial Activity

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ABSTRACT

The reports of drug resistant microbes are increasing and will significantly affect the current available antimicrobial drugs. Plants have been widely used since ancient time and may provide the solution to solve this problem. Moreover, modern medicine has also used plants as preparatory material in producing synthetic drugs. There are still plants that are widely used with much bioactive potentials but unknown to the society. *Garcinia parvifolia* is a commonly used plant of Sabah, Malaysia in flavouring local dishes, with no known medicinal values. Locally, it is known as takob-akob. The Genus Garcinia is prominent for their phytochemicals and bioactives. Therefore, current study is conducted to determine the phytochemical constituents and antibacterial potential of *G. parvifolia*, emphasizing on the dried fruit pericarp of *G. parvifolia*. Through this study, phytochemical screening of the methanolic dried pericarp of *G. parvifolia* fruit has revealed the presence of alkaloid, carbohydrate, flavonoid, steroid, terpenoid and phenolic compounds. The antibacterial activity was determined by the disc diffusion evaluation, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MAB) evaluation. The Disc diffusion evaluation showed that the extract is resistant against *Staphylococcus aureus* (ATCC 14756) with inhibition zone of 10.67±0.88 mm and susceptible against *Serratia mercescens* (ATCC 43300) with the inhibition zone of 17.00±0.58 mm. Whereas, the MIC and MBC is identified at 250µg/ml. This study has revealed the potential of *G. parvifolia* as natural antibacterial agents besides being used only as food additives. However, further studies are needed to evaluate the medicinal properties efficacy of this plant.

Keywords: *Garcinia parvifolia*, takob-akob, phytochemical, antibacterial

INTRODUCTION

There are reported cases of drug resistant microorganism, especially bacteria that will consequently decrease the effect of treatment from antimicrobial drugs[1]. Whilst it is well-known that plants are one of the natural suppliers of drugs both in traditional and conventional medicine[2]. The modern medical pharmaceutical industry has used plant parts as starting material for the synthesis of drugs[3]. Therefore, it is eminent that plants are rich with compounds that will affect the human health. New discoveries of natural antimicrobial agents are invaluable and will be useful to produce alternative or synergism with present drugs[4]. *Garcinia parvifolia* Miq. belongs to the family Guttiferae[5,6]. The Guttiferae family comprises of 1350 spesies, while the genus Garcinia comprises of 250 spesies[5,7]. The genera are confined in tropical countries such as Malaysia, Thailand, Indonesia and others. In Malaysia, it is best known as genus of fruit tree. In Borneo, *Garcinia parvifolia* (Miq.) is called Kundong, Kumanjing, Kedundong, Kandis, Gandis, and Enteleng. In Sabah, Malaysia, *Garcinia parvifolia* (Miq.) is known as takob akob by the locals[8]. Other common names are Cherry mangosteen, Kandis, and Yellow candies[9].

The Genus Garcinia is well known and reported to comprise diverse phytochemical properties with significant bioactives[10-12]. Most of plants in *Garcinia* genus produce an array of bioactive compound such as xanthone, despidones and phloroglucinols for treatment of diseases and traditionally used for other medicinal purposes[13,14]. *Garcinia parvifolia* (Miq.) is recognized as rich resources with high pharmaceutical and medical potential[9]. Prominent *Garcinia* genus such as *G. mangostana, G. kola, G. cowa* and *G. atroviridi*s were also reported to have significant biological activities[8,15]. The fruit of *G. parvifolia* is small, green and round. The flowers are pale yellow-coloured and the fruits are also yellow coloured with small seeds enwrapped in thin-layered skin[9]. The natives used to mixed young leaves with other ingredients to form a jelly-like food. Dried fruits are consumed raw or added in curries as additional flavour[9]. In Sabah, Malaysia, the dried fruit is widely used in local dishes. The fruit is green coloured when young and change to rusted orange colour when ripened. It is also a seasonal fruit. So, when it is abundant, the fruits are collected, pitted and dried by local Sabahan. Due the tremendous potential of the Garcinia genus and other plant parts of *G. parvifolia*, current study is done to determine the potential of the dried pericarp of *G. parvifolia* as antibacterial agent through its phytochemical constituents and antibacterial evaluations.

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Additionally, there is also lack of reported studies pertaining to the fruit of *G. parvifolia*. Locals only used the fruit for flavouring in their dishes with no known nutritional or medicinal values.

MATERIALS AND METHODS

Sample Collection & Preparation

Dried fruit pericarp of *Garcinia parvifolia* was purchased from local market of Dongongan in Penampang District, Sabah, Malaysia. The dried fruit pericarp of *G. parvifolia was* grounded and extracted (40g) by maceration in 200ml of 80% methanol. The crude extract was then filtered using Whatman No. 1 filter paper and evaporated using rotary evaporator at 40˚C. The crude extract was kept in a dark bottle and stored at 4˚C until further analysis[8,16].

Phytochemical Screening

Qualitative tests were implemented in this study. The tests are based on visual observation of changes in color and also the observation of formation of precipitation after the addition of the specific reagents. The methanolic extract was subjected to different chemical tests according to standard procedures[17,2,16,18].

Detection of carbohydrates (Benedict's test)

1 ml of the extract added with few drops of Benedict's reagent and heated gently for a few minutes. The presence of orange-reddish precipitate indicated the presence of carbohydrate.

Detection of alkaloids (Dragendroff's test)

3ml of crude extract was mixed with 1ml HCl and heated for 20 minutes. The mixture was allowed to cool and then filtered with Whatmann No.1 filter paper.1ml of filtrate was then added with Dragendorff's reagent. The yellow creamy precipitate indicated the presence of alkaloids.

Detection of phenol (ferric chloride test)

1ml of the crude extract was added with few drops of $FeCl_3$. The changes of color into bluish black indicated the presence of phenols.

Detection of flavonoid (alkaline reagent test)

3ml of crude extract was added with 1 ml of 10% of NaOH solution along with addition of dilute HCL. The colour changes of the mixture from yellow to colorless marked the presence of flavonoid.

Detection of Terpenoid

3ml crude extract was mixed with 2 ml of chloroform and allowed to evaporate. Then, 2ml of sulphuric acid was added. The mixture was heated for two minutes. A positive test indicated by the appearance of grayish color

Detection of steroids

3ml crude extract was mixed with few drops of sulphuric acid. The appearance of red color marked the presence of steroid.

In vitro Antibacterial Activity

Disc Diffusion Evaluation.

Paper disc diffusion used to test the susceptibility of the extract of the pericarp of *G. parvifolia* against *Staphylococcus aureus* (ATCC 14756) and *Serratia mercescens* (ATCC 43300). Sterile paper discs of 6mm diameter were impregnated with 10µl of concentrated extract of *G. parvifolia.* While 30µg disc of tetracycline (Oxoid) used as standard positive control and blank disc as control negative. All assays were done in tri-replicates, the inoculated agar plates were then incubated at 37˚C for 18 hours[19,20].

Minimum Inhibition Concentration (MIC)

Two-fold serial microdilution was performed to determine the MIC values. The assay was done in 96 wells 2 ml of microtiter plate. The extract was diluted with sterile distilled water from 4000µg/ml to achieve 2000, 1000, 500, 250, 125, 62.5, 31.25, 15.63, 7.81, 3.91, and 1.96 µg/ml solution of 100µl respectively in a well. 100µl standardized suspension of the microorganisms then transferred to each well. The broth cultures incubated for 18 hours at 37°C for both *S. marcescens* and *S. aureus*. After incubation, the cloudy formation observed indicated the ineffective concentration to inhibit the bacterial growth. The first clear well after the turbidity formation observed, indicated the lowest dilution effective to inhibit the bacterial growth[20,21].

Minimum Bactericidal Concentration Evaluation (MAB)

The minimal bactericidal concentration were determined by touching the loop from each well of the MIC plate from 4000, 2000, 1000, 500, 250, 125 and 62.5µg/ml and then streaked on a nutrient agar and incubated for 18 to 24 hours for *S. marcescens* and *S. aureus* respectively. Clear agar cultures indicated the lowest concentration that killed the bacteria[22].

RESULTS AND DISCUSSION

Phytochemical Screening

The qualitative phytochemical screening has revealed that the methanolic dried pericarp extract of *G. parvifolia* contained phytochemical constituents as shown in Table 1. The results have revealed that the extract contained many potential bioactive compounds as reported in other Garcinia species. The extract especially has the potential as antimicrobial agent. However, the amount of phytochemical constituents may vary in amount.*In vitro Antibacterial Activity*

Disc Diffusion Evaluation

Agar disc diffusion evaluation is a common method to test potential antimicrobial agents. The parameter used is the zone of inhibition by impregnated disc that placed on an agar bacterial culture[20,28]. The susceptibility of microorganism against antimicrobial agents are categorised into resistant for weak agent (inhibition zone ≤11mm), intermediate for stronger agent (inhibition zone between 12 to 14 mm) and susceptible for strongest antimicrobial agent (inhibition zone ≥15mm)[20]. The results showed that the methanolic dried pericarp extract of *G. parvifolia* has antibacterial properties. However, the extract is resistant towards *S. aureus*, which is a gram positive bacterium. *S. aureus* is also notoriously described as antibiotic resistant pathogen worldwide[29]. Nonetheless, the extract is 53% as effective as the standard antibiotic tetracycline. The result against *S. mercescens* has shown that the extract is susceptible. The bacterium *S. mercescens* is a gram negative bacterium[30]. This suggests that the extract showed better antibacterial activity against gram negative bacterium. The extract may have different effect on different organisms as investigators had also observed

Table 1: Phytochemical Constituents of *G. Parvifolia* dried pericarp methanolic extract.

Phytochemicals	Detection	Bioactivities
Alkaloid	Detected	Antimicrobial, antidiarrhoeal, antihelminthic, antibacterial[2,23,24]
Carbohydrate	Detected	Source of energy; nutrient absorbent and colonic health[25,26]
Phenol	Detected	Anti-apoptosis, anti-aging, anti-inflammatory, antimicrobial, antidiarrhoeal, antihelminthic[2,18,24]
Flavonoid	Detected	Antibacterial, antimicrobial, antidiarrhoeal, antioxidant[2,27,24,24]
Terpenoid	Detected	Antiproliferative, antidiabetic, antimicrobial, anticarcinogenic[2,24]
Steroid	Detected	Antibacterial, antidiarrhoeal[2,18]

Table 2: Diameter zone of G. *Parvifolia* against *S. aureus* and *S. marcescens.*

Test organisms	Diameter of inhibition zone (mm)		
	G. parvifolia	Tetracycline	Distilled water
S. aureus	10.67±0.88 (Resistant)	20.00±0.57 (Susceptible)	0
S. marcescens	17.00±0.58 (Susceptible)	20.00±0.58 (Susceptible)	0

Table 3: Minimum inhibition concentration (MIC) of methanolic dried pericarp extract of *G. parvifolia* against *S. aureus* and *S. mercescens.*

Extract concentration (µg/ml)	Growth of *S. aureus*	Growth of *S. mercescens*
4000	Negative	Negative
2000	Negative	Negative
1000	Negative	Negative
500	Negative	Negative
250	Negative	Negative
125	Positive	Positive
62.5	Positive	Positive
31.25	Positive	Positive
15.63	Positive	Positive
7.81	Positive	Positive
3.91	Positive	Positive
1.96	Positive	Positive

Table 4: Minimum bactericidal concentration (MBC) of methanolic dried pericarp extract of *G. parvifolia* against *S. aureus* and *S. mercescens.*

Extract concentration (µg/ml)	Growth of *S. aureus*	Growth of *S. mercescens*
4000	Negative	Negative
2000	Negative	Negative
1000	Negative	Negative
500	Negative	Negative
250	Negative	Negative
125	Positive	Positive
62.5	Positive	Positive

that sensitivity of microorganisms towards antimicrobial agents differ based on the strain types[31].

Minimum Inhibition Concentration (MIC)

The two-fold serial dilution of the extract has resulted in 12 different concentrations. By referring to Table 3, it is evident that the lowest concentration effective to inhibit both *S. aureus* and *S. mercescens* is 250µg/ml. MIC less than 5000µg/ml is interpreted to be strong antimicrobial agent[32]. Therefore, the extract is a strong antimicrobial agent as the MIC is 20 times more diluted than 5000µg/ml.*Minimum Bactericidal Concentration (MBC)*

The minimal bactericidal concentration was determined by the minimum inhibition concentration results. The evaluation is based on the presence of bacterial growth on a sterile agar plate. The absence of bacterial growth indicates that the particular concentration is sufficient as bactericidal agent and vice versa[20,33]. The MBC of *G. parvifolia* extract is identified at 250µg/ml. Therefore, the extract not only inhibits the growth of bacteria but also causes bactericidal.

CONCLUSION

In conclusion, phytochemical screening of the dried pericarp of the fruit has revealed the presence of alkaloid, carbohydrate, flavonoid, phenolic compound, steroid and terpenoid. All the detected compounds attributed the antimicrobial and antibacterial properties. Thus, indicating the potential of the extract as antimicrobial agent. The extract also exhibited antibacterial activity against *Staphylococcus aureus* (ATCC 14756) and *Serratia mercescens* (ATCC 43300). The diameter of inhibition in the disc diffusion evaluation was 10.67±0.88mm and 17.00±0.58 mm respectively. This indicated that the extract is resistant towards *S. aureus* and susceptible against S. *marcescens*. The Minimum Inhibitory Concentration and Minimum Bactericidal Concentration was 250µg/ml which indicated strong antimicrobial agent. This study shows the potential of *G. Parvifolia* as antibacterial agent apart of being used just as food additives. However, further studies are needed to evaluate its potential and efficacy.

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