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Short communication

Antioxidant and Antimicrobial Activity of Different Plant Parts of Garcinia prainiana- an Endangered Plant

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Abstract

Garcinia prainiana is a critically endangered tree belongs to the family Clusiaceae found native in Asia and yet not many studies have been conducted to scientifically study its medicinal potential. The present study was conducted to evaluate potential antioxidant, antimicrobial and to screen the phytochemical screening of *Garcinia prainiana* methanolic extract. The solvent used for extraction was methanol for dried leaves, twig and fruit sample. Antioxidant activity was tested using free radical diphenylpicrylhydrayl (DPPH) assay. While for antimicrobial activity, the disc diffusion method was carried out against six types of pathogenic bacteria from Gram-positive and Gram-negative bacteria. In addition, quantitative phytochemical screening was carried out to determine total phenolic and flavonoid content of the methanolic extract. The plant showed moderate antioxidant activity. *Garcinia* leaves extract showed the highest antioxidant activity (70.43%) followed by twig extract (47.46%) and fruit extract (44.45%). The IC_{50} value managed to be obtained from leaves extract at 0.26 mg/ml. Meanwhile, for antimicrobial activities, zone of inhibition was determined at 100 mg/ml for leaves extract (9 ± 0.73 mm) and twig extract (7 ±0.5 mm) against *Salmonella typhimurium* ATCC 14028. Quantitative phytochemical screening showed that total phenolic content was the highest in leaves extract at 51.83mg GAE/g, while for total flavonoid content, fruit extract showed the highest value at 80.93 mg QE/g. Total phenolic content showed a good correlation with the antioxidant activity, but not with total flavonoid content.

Keywords: *Garcinia prainiana; antioxidant; antimicrobial; methanolic extract .*

1. Introduction

Garcinia prainiana (button mangostene) comes from the family of Gruttiferae (Clusiaceae) [1]. Guttiferae family is mainly distributed in tropical regions and consists of 40 genera with 1200 species [2]. The plants are broadly distributed in tropical Africa, Asia, New Caledonia, and Polynesia [3]. Most of the species variety were found in Asia with about 400 species and around 49 species have been recorded in Malaysia. The plant is a small to medium-sized tree, up to 18 m high with a narrow, dense and bushy crown. It produces white latex, and local people in Malaysia usually known *Garcinia prainiana* as cerapu, cerpu, mencupu and kecupu [4]. The use of *Garcinia prainiana* is well known traditionally as medicinal plants since it was used as expectorant, treatment of lymphatitis, parotitis and struma by local people in Thailand and Indonesia [5].

This plant has been listed in IUCN Red List of Threatened Species in 1998 and has become one of the endangered plants in Malaysia. But the study on its potential biological activities are still scarce. There were some study on pytochemical compounds reported by Ee and Mong [6] and Jabit et al. [7] on other *Garcinia* species found in Malaysia. Another study done by Mawa and Said [5] and On et al. [4] managed to identify chemical constituents from this species such as xanthones, biflavanoids, benzophenones and triterpenoids [4, 5]. But, to date, there is no report on its biological activities were carried out so far for this species. Hence, in this paper we reported its bioactivities (antioxidant and antimicrobial) including total phenolic and total flavonoid content from different parts (leaves, twig and fruit) of *Garcinia prainiana*.

2. Methodology

2.1. Plant Materials

The fresh leaves, fruits and twigs (Fig. 1) of *Garcinia prainiana* were collected from a fruit farm at Kampung Chegar Bedil, Jeli, Kelantan, Malaysia. The samples were cleaned thoroughly using running water to remove debris.

2.2. Preparation of plant samples

Various plant parts were collected such as fruit, twig, and leaves (Fig.1). The samples were washed, chopped into small pieces and dried in the oven at 60 ˚C for a few days until the weight was constant. The dried samples were then grounded into powder using an electric grinder. The powdered samples were stored at dry area for next subsequent extraction.

Fig. 1: *Garcinia prainiana* leaves, twigs and fruits part.

2.3. Methanol extraction

About 200 g of powdered samples was soaked in 500 ml 100 % methanol until super saturation for 72 hours at room temperature [8]. The sample extract was kept away from sunlight and were stirred several times with a sterile glass rod. The supernatant was filtered through Whatman No.1 filter paper. The collected extracts were added to a rotary evaporator at 40 °C to evaporate the methanol. Finally, the extracts were re-suspended in 15 % dimethyl sulfoxide (DMSO) and kept at -20 °C for storage.

2.4. Antioxidant test

Using method by Wang et al. [9], free radical scavenging activity was measured using DPPH free radical. About 0.1 M DPPH stock solution was prepared by dissolving 1.5 mg of DPPH in 100 ml methanol. Next, 40 µL of sample extract at different concentrations (1-20 mg/mL)were mixed with 200 µL of 0.1 M of DPPH solution. Then, the mixture was incubated in dark condition for 15 minutes at room temperature. After that, the absorbance was measured at 517 nm. Trolox was used as a standard with different concentrations ranging from (0.005 to 0.05 mg/ml).
The inhibition percentage of the radical scavenging was calculated using the equation below:

Inhibition (%) = 100 – 100 ($A_s \div A_o$)
Where (A_o) is absorbance of the control and (A_s) is absorbance of standard and samples. IC_{50} value (sample concentration that is required to scavenge 50% of free radical) was estimated from the percentage inhibition graph and compared with standard. Three replicates of each samples were tested for the test and results were expressed in mg/mL.

2.5. Antimicrobial test

2.5.1. Preparation of bacterial strain

About six bacteria strains were used in this study, which include Gram positive; *Bacillus substilis* ATCC 14579, *Staphylococcus aureus* ATCC 33591 and *Enterroccus faecalis* ATCC 29212 and Gram negative bacteria; *Pseudomonas aeruginos*a ATCC 27853, *Saccharomyces cerevisiae* ATCC 70892 and *Salmonella typhimurium* ATCC 14028. These bacteria were cultured from the glycerol stock available in Microbiology Laboratory, Faculty of Bioresources and Food Industry, UniSZA,Malaysia. Reviving process begin by thawing the bacteria at room temperature, followed by transferring into Mueller Hinton broth (MHB) and incubated overnight at 37 °C for growth. After overnight incubation, the bacteria broth was streaked on Nutrient Agar (NA) for single colony formation. The plates were kept at 4 °C. For long term storage, 20 % glycerol stocks were prepared for each bacteria strain and stored at -80 °C.

2.5.2. Disc diffusion assay

Antibacterial activity of *Garcinia prainiana* extract was conducted using standard procedure of disc diffusion method by Nostro et al. [10]. A concentration of 0.5 mg/mL plant extract was prepared from the stock solution. Cultures of the bacteria tested were inoculated on the surface of Mueller Hinton agar plates using sterile cotton swab and were allowed to stand for a few minutes to enable pre-diffusion of the inoculated organisms. Each bacteria strain was evenly spread over the entire surface of agar to obtain a uniform inoculum. Disc (6 mm diameter) was sterilized by autoclaving. The blank sterile disc was dropped with 10 µL of different concentrations of stock solutions (500 mg/ml, 250 mg/ml and 100 mg/ml) before placing aseptically on the inoculated Mueller Hinton Agar surface using a sterile forceps and was gently pressed to enable even contact. Gentamycin was used as control at a concentration of 50 µg/ml. The plates were incubated at 37 °C for 24 hours. All the tests were performed in triplicate. After incubation, zone of inhibition was measured using metric ruler by taking the measurement from the edge of the zone of inhibition to the other edge. The diameter of zone of inhibition represents antibacterial activity.

2.6. Quantitative Phytochemical Analysis

2.6.1. Total Phenol Content

Using method Lister et al. [11], about 100 ml of sample were dissolved in 500 ml of Folin-Ciocalteu reagent and 1 ml of distilled water. Then, the mixture was incubated at room temperature for 1 minute and later, 1.5 ml of 20% sodium carbonate solution was added. This mixture was shaken and incubated for 2 hours in dark at room temperature. Finally, the results were expressed in milligrams gallic acid equivalents per gram of dry plant extract (mg GAE/g).

2.6.2. Total Flavonoid Content

Using method Quettier-Deleu et al. [12], about 1 ml of diluted sample was mixed with 1 ml of 2 % aluminium chloride ethanolic solution. Then, the sample was incubated at room temperature for 15 minutes. The absorbance of the mixture was measured at 430 nm. The flavonoid content was expressed in milligrams quercetin equivalents per gram of dry plant extract (mg QE/g).

2.7. Data analysis

Experimental data were analyzed using statistical data analysis for Microsoft Excel. All the results represented as mean values of triplicates ± standard deviation.

3. Results and Discussion

3.1. Antioxidant Activity

Different plant parts (leaves, twig and fruit) of *Garcinia prainiana* were dried and macerated using methanol due to its ability to dissolve polar and phenolic compound completely [13]. Molecule that neutralizing free radicals intermediate by removing the chain reaction is an antioxidant molecule [14]. A protonated of DPPH which has the maximum absorbance at 517 nm can decrease the scavenging activity of proton radical. Hence, antioxidant activity can be evaluated from the effect of free radical scavenging activities. Moreover, the presence of free radical scavenger, vanished the DPPH by absorbing the number of electrons taken up is stoichiometric with the resulting decolourization [15]. In this experiment, methanolic extract of leaves, twig and fruit of *Garcinia prainiana* were used to evaluate *in vitro* potential of antioxidant activity through the free radical scavenging assay. All the tests were done in triplicate.

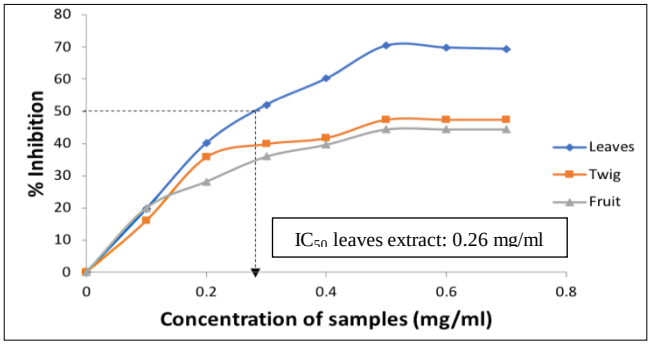
Table 1: Percentage inhibition (%)of *Garcinia prainiana* methanolic extracts by DPPH scavenging ability assay.

Trolox (Standard)		***Garcinia prainiana* Methanolic Extracts**			
Concentration (mg/ml)	**% Inhibition (Mean ± SD)**	**Concentration (mg/ml)**	**% Inhibition (Mean ± SD)**		
			Leaves	**Twig**	**Fruit**
0.01	28 (±0.027)	0.1	19.76 (±0.009)	16.12 (±0.008)	20.01 (±0.032)
0.02	52.94 (±0.039)	0.2	40.11 (±0.046)	35.80 (±0.052)	28.24 (±0.018)

0.03	67.35 (±0.031)	0.3	51.99 (±0.02)	39.89 (±0.040)	35.99 (±0.025)
0.04	83.34 (±0.013)	0.4	60.21 (±0.14)	41.78 (±0.031)	39.70 (±0.01)
0.05	86.34 (±0.004)	0.5	70.43 (±0.007)	47.46 (±0.012)	44.45 (±0.083)

Each value is expressed as a mean ± SD of triplicates.

Result for antioxidant activity was presented in Table 1. The highest percentage of inhibition for *Garcinia prainiana* methanolic extract was recorded at 70.43% for 0.5 mg/ml leaves extract while for standard Trolox, the highest inhibition percentage was at 86.34% for 0.04 mg/ml extract. However, for twig and fruit extract, the highest percentage of inhibition were 47.46% and 44.45% respectively at the concentration of 0.5mg/ml. A study by Ismail and Shaari [16] as also reported that leaves of *Garcinia* plant have higher inhibition percentage than the plant stems.



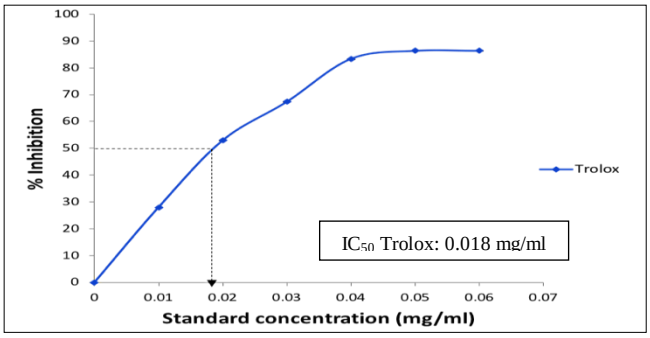


Fig. 2: Antioxidant activity and IC_{50} value of methanolic extract of leaves, twig and fruit of *Garcinia prainiana* (A) and Trolox as standard (B).

Based on Fig. 2, IC_{50} value of the *Garcinia prainiana* methanolic extract was identified for leaves extract at concentration of 0.26 mg/ml, whereas IC_{50} value for twig and fruit extract cannot be determined due to low antioxidant activity profiles showed. Trolox was used since it is a natural antioxidant and widely used as a standards drug for many assays [17]. The IC_{50} value for Trolox was determined at 0.018 mg/ml (Fig. 2). From this study, it showed that different parts of *Garcinia prainiana* exhibit different antioxidant profiles. Results showed that methanolic extract of the leaves (70.43%) have better potential as antioxidant agent compared to others. While, for both twig and fruit extract were identified contain low antioxidant properties (Fig. 2). Kruawan and Kangsadalampai (2006) stated that if the inhibition percentage is more than 90%, the sample can be reported to have a great potential antioxidant activity. While, for 60%-90% inhibition percentage, the sample is under moderate potential of antioxidant activity whereas the value below 60% inhibition, the sample has low potential antioxidant activity [18].

Study done by Bendary et al. [19] stated that antioxidant activity is influenced by the presence of hydroxyl group. The antioxidant activity is also very dependent on the number and position of the hydroxyl group in the molecules [19]. IC_{50} is a measure of compound effectiveness in inhibiting biological or biochemical function and also defined as the amount of antioxidant substance required to scavenge 50 % of free radical present in the assay system. Previous study by Rajkumar et al. [20] showed that IC_{50} of leaves extract from Garcinia imberti was found the highest compared to stem bark [20]. Another study by Abdullah et al. [21] on Garcinia atroviridis has also reported that the leaves of the plant have higher inhibition percentage to scavenge free radical molecule as its inhibition percentage is the highest compared to stems.

The presence of phenolic compounds such as flavonoids, phenolic acids, topopherols and others are in the form of natural antioxidant in plants enable them to involve in free radical scavenging assay [22]. Polyphenol compounds are more stable while, phenolic, flavonoid and tannin however are unstable compounds [21, 23]. The temperature during drying process is one of the factor for the degradation of some of the polyphenol substituents [24]. The overall antioxidant activity of the plant extract could be effected by climatic growth conditions, duration of storage and the cultivation of the plant [25].

3.2. Antimicrobial Activity

Antimicrobial activity is the ability to produce certain killing effect upon bacteria cultivated in a liquid medium by using antimicrobial agent. Six bacteria strains were used in this study to evaluate its potential antimicrobial activity. They include Gram positive; Bacillus cereus ATCC 14579, Staphylococcus aureus ATCC 33591 and Enterroccus faecalis ATCC 29212 and Gram negative bacteria; Pseudomonas aeruginosa ATCC 27853, Acinetobacter baumamni ATCC 19606 and Salmonella typhii. The result of antimicrobial activity of Garcinia prainiana methanolic extract was presented at Table 2. Results showed that the Gram-negaitive bacteria were more sensitive to inhibition by leaves and twig extract, the highest zones of inhibition were observed on leaves extract against Salmonella typhimurium ATCC 14028 with 9 ± 0.73 mm zone of inhibition, followed by twig extract at 7± 0.5 mm against S. typhimurium ATCC 14028 . All inhibition occur at the highest concentration of 100 mg/ml of the plant extracts used. Gram-positive bacteria were less susceptible, this could be related to the bacteria outer membrane which contains lipopolysaccharide and peptidoglycan which enrich the bacterial cell surface with strong hydrophilicity which acts as strong permeability boundary [26]. Some of the organism show negative results which means that there were no inhibition effect towards the tested plant extracts especially at lower concentration.

Results showed no inhibition zone observed for fruit extract at various concentrations tested against Gram positive; *Bacillus cereus* ATCC 14579, *Staphylococcus aureus* ATCC 33591 and *Enterroccus faecalis* ATCC 29212 and Gram negative bacteria; *Pseudomonas aeruginos*a ATCC 27853, *Acinetobacter baumamni* ATCC 19606 and *Salmonella typhimurium* ATCC 14028. This might due to low concentration of plant material (lack of plant resources availability) during extraction. Besides, poor release of compounds from the plants during extraction might be one of the reasons to be considered [27]. Gram positive bacteria acquire a thick (20–80 nm) cell wall as exterior shell of the cell. In contrast Gram negative bacteria have a relatively thin (<10 nm) layer of cell wall, but harbour an additional exterior membrane with several pores and appendices. These properties of the cell envelope promote different responses to external stresses, including heat, UV radiation and antibiotics [28].

Table 2: *In vitro* antimicrobial activity of *Garcinia prainiana* methanolic extracts against pathogenic bacteria strains.

		Microorganisms/Zone of Inhibition (mm)	
Concentra-	**Sam-**	**Gram-Positive**	**Gram-Negative**

tion (mg/ml)	ple	BC	EF	SA	AB	PA	ST
25	Leaves	-	-	-	-	-	-
	Twig	-	-	-	-	-	-
	Fruit	-	-	-	-	-	-
50	Leaves	-	-	-	-	-	-
	Twig	-	-	-	-	-	-
	Fruit	-	-	-	-	-	-
100	Leaves	-	-	-	-	-	9 ±0.73
	Twig	-	-	-	-	-	7 ±0.51
	Fruit	-	-	-	-	-	-
Gentamycin (50 µg/ml)		21 ±0.21	17 ±0.13	22 ±0.12	19 ±0.13	20 ±0.12	22 ±0.11

Abbreviations : BC = *Bacillus cereus* ATCC 14579, EF = *Enterroccus faecalis* ATCC 29212, SA= *Staphylococcus aureus* ATCC 33591 , AB = *Acinetobacter baumamni* ATCC 19606, PA= *Pseudomonas aeruginos*a ATCC 27853, ST = *Salmonella typhimurin* ATCC 14028

3.3. Quantitative Phytochemical Analysis

3.3.1. Total Phenolic Content (TPC)

In this study, *Garcinia prainiana* methanolic extracts from leaves, twig and fruit were oxidized by Folin-Ciocalteu reagent and neutralized by addition of sodium carbonate (Na_2CO_3). Then, the results were expressed as milligram of Gallic Acid equivalent (GAE) in 1 gram of sample (mg GAE g-1 DE).

The content of total phenolic was determined by extrapolation from the calibration curve prepared from gallic acid concentrations and expressed in milligram of gallic acid. Based on the Fig. 3, total phenolic content in leaves extract was remarkably high (51.83 ± 0.04 mg GAE/g), followed by twig extract (14.73 ± 0.44 mg GAE/g). In comparison, there was no phenolic content found in the fruit methanolic extract which is as predicted due to lacking of anthocyanins compound presence in the fruit which give the fruit orange color. This statement is in agreement with a report by Galván et al. [29] which stated anthocyanins are water-soluble plant pigments which contributed to the difference colors of many plant tissue such as blue, purple, and red color. *Garcinia* is a rich source of xanthones, flavonoid, benzophenone,lactones and phenolic acid [30]. Xanthones are known for their antibacterial, antidiabetic, antiplasmodial, anticancer and antihypertensive while benzophenones have been studied to possess antimicrobial, antioxidant, cytotoxic activity and cryoprotection against HIV-1 *in vitro* [31].

Different levels of phenolic content were reported in this study may be attributed to the different part of *Garcinia prainiana* plant tested. The usage of Folin-Ciocalteu reagent was also measured based on the colour measurement which was non-specific to certain compound such as phenol and there were other components that can react with the reagent such as ascorbic acid [32]. Besides, different compounds of phenolic have different response to this assay. Phenol are compounds that have the ability to destroy the radical due to the presence of hydroxyl groups. The hydrogen atoms from the hydroxyl group of phenol compounds are given up to radicals and form stable phenoxyl radicals, then its play important roles in antioxidant activity.

In fact, total phenolic content is significantly different among the three samples of *Garcinia prainiana*, following the order: leaves > twig > fruit. High amount of phenolic compounds in leaves methanolic extract was probably because of leave samples was collected at fully matured stages compared to twig and fruit. In related, total phenolic compounds generally increased with maturation stages of plant regardless of the analytical method employed.

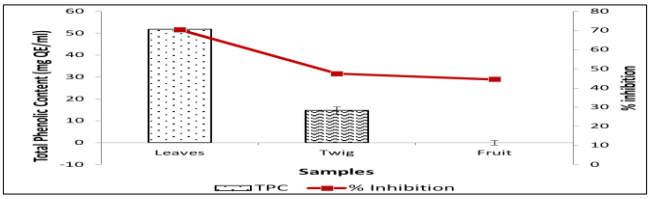


Fig. 3: Total phenolic content (mg GAE/g) in different parts of *Garcinia prainiana* methanolic extract. Each value is expressed as a mean ± SD of triplicates. All data were significantly different at $p<0.05$.

Several studies have reported strong relationship between total phenolic content and antioxidant activity in selected grains, herbs and vegetables [33, 34, 35]. Some authors found a correlation between the phenolic content and the antioxidant activity, while others found no such relationship. However, studies conducted by Pontis et al [36] on honey found that no correlation between phenolic content and antioxidant activity. Fig. 3 shows that methanolic leves extract of *Garcinia prainiana* was found to have the highest antioxidant activity and also highest content of phenolic compounds. This finding shows correlation between antioxidant ability with total phenolic content. This finding was in agreement with the above authors.

3.3.2. Total Flavonoid Content (TPC)

Flavonoids are plant secondary metabolites which can determine by the principles of Aluminium chloride ($AlCl_3$) colorimetric method. The content of total flavonoid was determined by extrapolation from the calibration curve prepared from quercetin concentration and expressed in milligram of quercetin.

Based on Fig. 4, total flavonoid content in fruit extract (80.93 ± 0.25 mg QE/g) was significantly higher compared to twig extract (57.78 ± 0.23 mg QE/g) and leaves extract (34.42 ± 0.25 mg QE/g) respectively. The flavonoid content in leaves extract was found to be significantly lower compared to other parts of the plant. In fact, total flavonoid content is significantly different among all plant parts following the order: fruit > twig > leaves. Result from total flavonoid content also revealed that there are no relationships between antioxidant activity with total flavonoid content as the highest flavonoid content was observed in fruit contain the lowest antioxidant activity (Fig. 4). Differences in the reported values could be due to many factors such as growing and processing conditions which can influence the concentration of flavonoids in vegetables and foods [37].

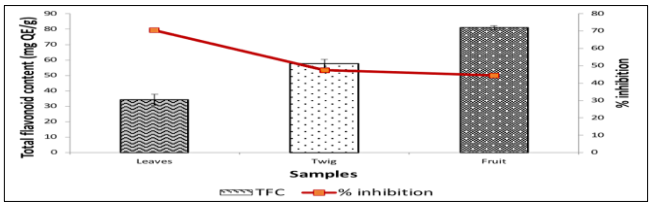


Fig. 4: Total flavonoid content (mg QE/g) in different part of *Garcinia prainiana* methanolic extracts. Each value is expressed as a mean ± SD of triplicates. All data were significantly different at $p<0.05$.

4. Conclusion

All plant parts of *Garcinia prainiana* tested showed different level of antioxidant and antimicrobial properties. The leaves extract showed moderate potential of antioxidant activity with the inhibition percentage of 70.43%. The IC_{50} value of leaves extract was recorded at 0.26 mg/ml with no IC_{50} value recorded for other plant parts. Antimicrobial assay showed zone of inhibition against *Salmonella typhimurium* ATCC 14028 for leaves and twig extracts at 9 ± 0.73 mm and 7 ± 0.5 mm respectively. For quantitative phytochemical analysis, results showed that there is a correlation between total phenolic content to the inhibition percentage.

While, no close correlation was observed between total flavonoid content and antioxidant inhibition percentage, but more in-depth study is required to identify the active compounds presence. As overall, *Garcinia prainiana* can be a potential plant for a source of natural antioxidant. Details of this information are very important in order to provide awareness to others about this endangered plant.

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