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Original Article

Evaluation and Comparison of Antioxidant Activity of Leaves, Pericarps and Pulps of Three Garcinia Species in Malaysia

Yik-Ling Chew[1,*], Yau-Yan Lim[2]

ABSTRACT

Background: Garcinia genus plants are a group of well-known fruit trees in Malaysia.The botanical parts of the trees are used for medicinal purposes, where they are prepared as decoction, pastes for wound infections, and some have been consumed as food. Objectives: This paper focused on the assessment of antioxidant potential of three selected Garcinia species commonly used in Malaysia. **Materials and Methods:** Fresh plant materials were extracted using maceration, and various antioxidant activities of the extracts were evaluated. *In vitro* antioxidant properties studied were total phenolic and anthocyanin content, free radical scavenging (FRS), ferric reducing power (FRP) and ferrous ion chelating (FIC) activities. **Results:** *G. mangostana* leaves and pericarps were found to consist of the highest TPC, FRS and FRP activities, followed by *G. hombroniana* and *G. atroviridis*. TAC was found only in *G. mangostana* pericarps. For fruit pulps, *G. hombroniana* exhibited the highest antioxidant activities (TPC, FRS and FRP), while *G. mangostana* had the lowest activities. FIC activity of the Garcinia samples behaved in dose-dependent manner and not correlated with TPC and other antioxidant activities. **Conclusion:** In general, *G. mangostana* and *G. hombroniana* leaves and pericarps exhibited good antioxidant activities, but the antioxidant activities in their fruit pulps were relatively low. *G. atroviridis* leaves and pericarps generally have lower antioxidant activities than the other species.

Key words: Antioxidant activity, Free radical scavenging, Garcinia, Pericarp, Total phenolic content.

Key message: Garcinia species have been used widely in Asia countries, especially countries in Southeast Asia. The investigation of various antioxidant activities could potentially identify the suitable candidates as sustainable source of antioxidative agents.

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INTRODUCTION

Excessive free radicals such as reactive oxygen species and reactive nitrogen species may be harmful because they can initiate biomolecular oxidations which lead to cell injury and death, and create oxidative stress which results in numerous diseases and disorders, such as cancer, ageing, neurodegenerative disease, arteriosclerosis, and gastric ulcer. Uptake of antioxidants from plant source could help to reduce the harmful effect of excessive free radicals.[1] Many antioxidant compounds present in plant have been identified as free radical scavengers.[2] There is an inverse relationship between the dietary intake of antioxidant-rich food and prevalence of human diseases.[3] Antioxidants from plants are effective in health and disease prevention.[4] Antioxidants can be found abundantly in fruits. Some fruits are rich in antioxidants, such as blueberries, kiwis, grapefruits, prunes and mangosteens.[5]

Garcinia (Family: Guttiferae) is a large genus of polygamous trees or shrubs, distributed in the tropical Asia, Africa, and Polynesia. Plants from Garcinia genus are a group of well-known fruit trees in Malaysia. The fruits of Garcinia species are mostly edible and some are served as a substitute for tamarinds in curries. Botanical parts of Garcinia trees have been used as traditional medicines by the Malaysian community. *Garcinia mangostana*, also known as mangosteen, is a slow-growing tropical tree, which can grow up to 25 m in height.[6] Its pericarp is dark purple to red-purple in colour while the edible pulp is white, soft, and juicy and sweet. Burkhill[6] reported that the pulp is believed to have detoxification properties. It could relieve thirst in fever and used as an astringent. The Malays make a conserve called *halwa manggis* by boiling the arils of unripe fruits with sugar. Decoction made from the leaves and bark is used to treat fever, urinary disorders, dysentery and opened wound.[6-7] *G. hombroniana* (Malay: *beruas, manggis hutan*) is a small or medium tree which produces globose fruit in bright red colour. It is found near coastal primary and

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secondary forest of tropical countries. Their roots are used to make herbal decoction for women after childbirth and the leaves are used to relieve itching.[6] *G. atroviridis* (Malay: *asam gelugor*) is a medium-sized tree up to 25 - 27 m tall and grows wildly throughout Peninsular Malaysia lowland forest. Its sourish fruit is used as seasoning. (-)-Hydroxycitric acid (HCA) in the fruit is proved to be effective towards weight management.[8] The juice from the leaves is given as a health tonic to woman after childbirth by the Malays to aid recovery.[6] Decoction prepared from the leaves and roots is used to treat ear-ache.[6]

These three Garcinia species are commonly consumed by Malaysian community as food, seasonings and used as traditional medicines. In view of the potential benefits listed above, it is therefore of great interest to evaluate the antioxidant activities of leaves, pericarps and fruit pulps and perform the statistical comparison.

MATERIALS AND METHODS

Plant Materials

Fresh leaves of *G. mangostana* were collected from a farm at Semenyih. The leaves of *G. atroviridis* were collected from Rimbun Dahan Arts Centre at Kuang, Selangor. The plant materials were gifted by the owner, Mrs. Angela Hijjas. The leaves and fruits of *G. hombroniana* were collected from Kepong, Kuala Lumpur, Malaysia. The fruits of *G. mangostana* were purchased from a night market in Kuala Lumpur. The fruits of *G. atroviridis* were purchased from a farmers' market in Seri Gombak, Selangor. All samples were collected or purchased on the day when the extraction was performed to preserve freshness and ensure the reliability of results. The plant materials were first washed and dried with clean tissue paper.

Preparation of extracts

Fruit pulp extracts were prepared as described in Lim, Lim[9] while pericarp and leaf extracts were prepared as described in Chew, Goh[10] In brief, 30.0 g of the fruit pulps were weighed and ground manually using mortar and pestle, followed by extraction using 100 mL 50% v/v ethanol with continuous shaking in room temperature for 60 min. For pericarps and leaves, 1 g of each sample were frozen with liquid nitrogen and ground into fine powder in a mortar. The powdered materials were extracted with 50 mL 75% v/v methanol, which was determined as the best solvent, with continuous shaking in room temperature for 60 min. The extracts were then filtered under vacuum and stored at -20˚C until further analysis was performed.

Total phenolic content (TPC)

The total phenolic content (TPC) of extracts was measured quantitatively using Folin–Ciocalteu method as described previously.[10-11] Briefly, Folin–Ciocalteu's phenol reagent (1.5 mL, 10% v/v) and 1.2 mL of 7.5% w/v sodium carbonate were added to the 0.3 mL extract. The reaction mixture was thoroughly mixed and was then incubated in the dark for 30 min, followed by measurement of the absorbance at 765 nm. TPC was expressed in terms of mg gallic acid equivalents (GAE) per 100 g sample.

Total anthocyanins (TAC)

The total anthocyanins (TAC) were determined by the pH differential method.[10] One mL of 0.2 M potassium chloride solution (pH 1.0) and 1.0 mL 1 M sodium acetate buffer (pH 4.5) were added into 2.0 mL of extracts respectively. The absorbance was then measured at 520 and 700 nm. The results are expressed as the amount of cyanidin-3-glucoside (cy-3-glu) equivalents per 100 g of sample (mg cy-3-glu/100 g sample), which are calculated as $A/\varepsilon L \times MW \times 10^3 \times$ Dilution factor,[4] where A is the difference of absorbance between pH 1.0 and 4.5; ε is molar extinction coefficient for cy-3-glu (26,900); L is the path length of the spectrophotometer cell (1.0 cm), and MW is molecular weight of cy-3-glu (449.2 g/mol).

Free radical scavenging (FRS) activity

Free radical scavenging (FRS) activity was assessed based on the method previously described.[11] Two mL of 0.15 mM 2, 2-diphenyl-1-picrylhydrazyl (DPPH) (in methanol) was added to the 1.0 mL serially diluted extracts. The reaction mixture was incubated for 30 min after which its absorbance was measured at 517 nm. Methanol was used as blank. Negative control was prepared by replacing the extract with methanol. The decrease in absorbance was calculated as an IC_{50}, concentration of extract required to reduce the absorbance by 50% and the results were expressed as mg ascorbic acid (AA) equivalents per 100 g sample (AAE) as follow.

$$AAE\ (mg\ AA/100\ g) = IC_{50\ (ascorbate)}/IC_{50\ (sample)} \times 10^5$$

Ferric reducing power (FRP)

The ferric reducing power (FRP) was evaluated as previously described.[11] Potassium phosphate buffer (2.5 mL, 0.1 M, pH 6.6) and 1% w/v potassium ferricyanide (2.5 mL) were mixed with 1.0 mL of extracts of varying dilutions. The reaction mixture was incubated at 50˚C for 20 min, followed by the addition of 2.5 mL of 10% w/v trichloroacetic acid. Water (2.5 mL) and 0.1% w/v ferric chloride (0.5 mL) were then added to 2.5 mL of the reaction mixture, and the solution was incubated at 28˚C for 30 min to facilitate colour development. The absorbance was measured at 700 nm and the results were expressed as amount of gallic acid equivalents in mg per gram sample (mg GAE/g).

Ferrous ion chelating (FIC)

The ferrous ion chelating (FIC) assay was performed as described in Chew, Goh.[10] 0.1 mM ferrous sulphate (1 mL) was added to various dilutions of the extracts (1 ml; concentration between 1.0 – 7.0 mg/mL), followed by the addition of 0.25 mM ferrozine (1 mL). The reaction mixtures were allowed to stand for 10 min and the absorbance was then taken at 562 nm. The ferrous ion chelating property of extracts was expressed as percentage chelating activity using the following formula.[12] Chelating activity (%) = $(A_{control} - A_{sample})/A_{control} \times 100$; where $A_{control}$ and A_{sample} are the absorbance of the control and extract, respectively. Extraction solvent was used to replace the extract in control.

Statistical analysis

All assays were carried out in triplicates. The experimental results were expressed as mean ± standard deviation. The data were analyzed using one-way analysis of variance (ANOVA) using SPSS version 20.

RESULTS

Antioxidant properties of leaves, pericarps and pulps of the three Garcinia species were evaluated by determination of TPC, TAC, FRS, FRP and FIC.

It was noticed that *G. mangostana* leaves and pericarps had the highest TPC (4360 ± 230 mg GAE and 10600 ± 984 mg GAE/100 g, respectively), followed by *G. hombroniana* (2910 ± 361 mg GAE/100 g and 1840 ± 256 mg GAE/100 g, respectively), and *G. atroviridis* (273 ± 44 mg GAE/100 g and 33.3 ± 4.7 mg GAE/100 g). On the other hand, *G. hombroniana* pulps possessed the highest TPC (152 ± 4 mg GAE/100 g), followed by *G. atroviridis* and *G. mangostana* (Table 1). It is to be noted that FRS and FRP antioxidant activities of leaves, pericarps and pulps followed the similar pattern as TPC (Table 1).

TAC was only present in *G. mangostana* pericarps, where 29.7 ± 2.9 mg cy-3-glu/100 g was found.

FIC activity of the leaves, pericarps and pulps were examined using three increasing concentrations. It was noticed that the FIC activity of the

Table 1: Total phenolic content (TPC), total anthocyanin content (TAC) and antioxidant activities of leaves, pericarps and pulps of *G. mangostana, G. atroviridis and G. hombroniana*.

Plant parts	Species	TPC (mg GAE/100 g)	TAC (mg cy-3-glu/100 g)	FRS (mg AA/100 g)	FRP mg GAE/g
Leaves	*G. mangostana*	4360 ± 230[a]	ND	4240 ± 493[a]	20.9 ± 2.4[a]
	G. atroviridis	273 ± 44[b]	ND	166 ± 37[b]	2.93 ± 1.33[b]
	G. hombroniana	2910 ± 361[c]	ND	2290 ± 166[c]	12.6 ± 3.5[c]
Pericarps	*G. mangostana*	10600 ± 984[a]	29.7 ± 2.9	12700 ± 2490[a]	56.4 ± 7.2[a]
	G. atroviridis	33.3 ± 4.7[b]	ND	19.5 ± 4.5[b]	0.139 ± 0.049[b]
	G. hombroniana	1840 ± 256[c]	ND	2050 ± 406[c]	9.54 ± 1.30[c]
Pulps	*G. mangostana*	83.8 ± 8.7[a]	ND	35.3 ± 5.0[a]	0.400 ± 0.058[a]
	G. atroviridis	122 ± 12[b]	ND	100 ± 15[b]	0.589 ± 0.096[b]
	G. hombroniana	152 ± 4[c]	ND	78.5 ± 10.1[c]	0.800 ± 0.153[c]

Results are expressed as means ± SD (n = 3). For each column, values followed by the same letter (a-c) are statistically insignificant (P > 0.05), as determined using ANOVA, and this does not apply for different plant parts 'ND' represents 'not detected'

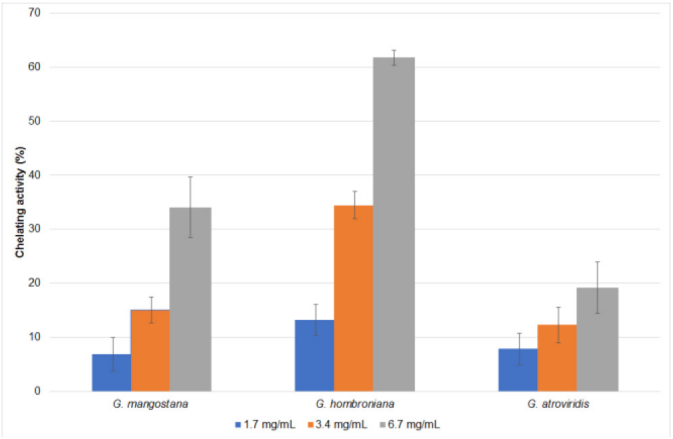

Figure 1: Ferrous ion chelating activity of *G. mangostana, G. atroviridis and G. hombroniana* leaves.

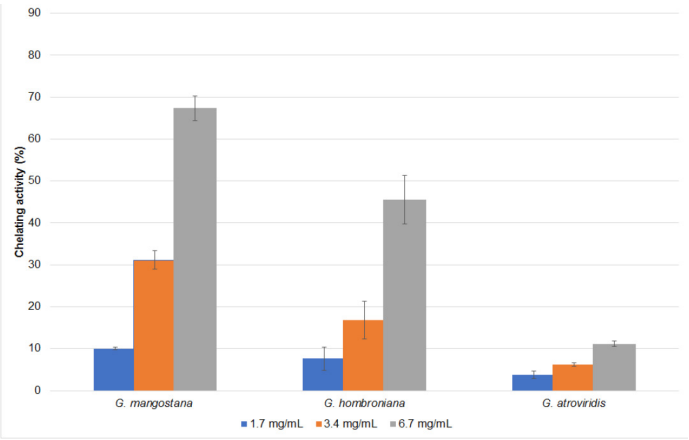

Figure 2: Ferrous ion chelating activity of *G. mangostana, G. atroviridis and G. hombroniana* pericarps.

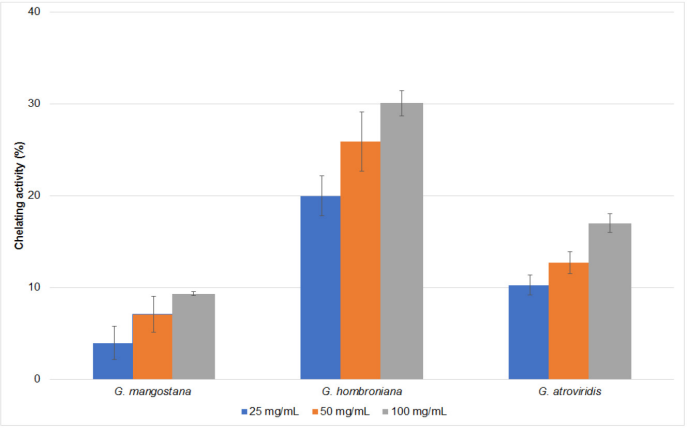

Figure 3: Ferrous ion chelating activity of *G. mangostana, G. atroviridis and G. hombroniana* pulps.

Garcinia samples behaved in dose-dependent manner. FIC activity of the leaves could be ranked as follows: *G. hombroniana* > *G. mangostana* > *G. atroviridis* (Figure 1). Statistical significance was noticed in the chelating activity of the Garcinia leaf samples. Garcinia pericarp samples exhibited similar chelating activity at lower concentration (1.7 mg/mL). However, *G. mangostana* pericarps exhibited significantly higher activity than *G. hombroniana* and *G. atroviridis* at higher concentrations (Figure 2). *G. hombroniana* pulps exhibited the highest chelating activity (30% at the highest concentration), followed by *G. atroviridis* and G. *mangostana* (Figure 3).

DISCUSSION

High amount of TPC was observed in leaves and pericarps of *G. mangostana* and *G. hombroniana* (> 1000 mg GAE/100 g). Pulps for the three species were found to be relatively low compared to leaves and pericarps. When compared to other Malaysian fruits, *G. hombroniana* pulp is similar to guava (138 – 179 mg GAE/100 g) while *G. atroviridis* is similar to star fruit (131 mg GAE/100 g), but it is higher than orange.[9] Xanthones are present in *G. mangostana, G. hombroniana* and other Garcinia species. Numerous benzophenones, flavonoids and benzoic acid derivatives were isolated from *G. hombroniana*,[13-14] would correspond positively in the assay. To date, there are more than fifty xanthones isolated from *G. mangostana*[15-16] and thirteen xanthones have been isolated from *G. hombroniana*.[13-14,17-19] Xanthones have been widely reported to exhibit diverse pharmacological activities, such as antioxidant, antifungal, antibacterial, anti-inflammatory, antihistamine and antiviral activities.[15,20] Antioxidative properties exhibited by xanthones, benzophenones and flavonoids would correspond to the high FRS and FRP in *G. mangostana* and *G. hombroniana*. Xanthones could scavenge free radicals by transferring labile H-atoms to radicals. The number and location of the hydroxyl group in xanthones could exhibit varying degree of FRS activity. Xanthones with dihydroxyl groups in the B-ring, such as γ-mangostin and garcinone E found in *G. mangostana* were potent free radical scavengers. If the hydroxyl groups in xanthones were protected or

shielded, the FRS activity exhibited would be lower.[21] This was noticed in the reported FRS activity of α-mangostin, β-mangostin and 9- hydroxy-calabaxanthone.[21]

Our previous studies have shown that TPC, FRS and FRP correlated positively with each other.[10-11,22-23] The positive correlation was also noticed in this study. Significant correlation between TPC and FRS was shown using Pearson correlation analysis.[24] However, there was slight variation in the relationship between TPC, FRS, and FRP with FIC activities, as reported in malting barley[25] and seaweed.[24] *G. hombroniana* exhibited the strongest chelating activity, followed by *G. mangostana* and *G. atroviridis*. Although *G. mangostana* leaves exhibited the stronger antioxidant activities (TPC, FRS and FRP) than *G. hombroniana* leaves, its FIC activity was significantly lower than *G. hombroniana* leaves. The iron binding ability of compounds was related to the presence of certain functional groups.[26] For instance, hydroxamate compounds, i.e. desferroxamine B and benzohydroxamic acid are good iron chelators, while catecholate chelators, such as caffeic acid, 2, 3-dihydroxybenzoic acid exhibited weaker iron binding ability. The authors also discovered that the FRS activity of the compounds did not correlate with their iron chelating ability. This was explained that the three bidentate ligand structure of desferroxamine B is crucial in iron binding. Roosenberg, Lin[27] discovered that compounds with siderophores and three bidentate ligands inside their structure are most effective in forming hexadentate complexes with smaller entropic variations which are caused by chelating one ferric ion with separate ligands. No correlation was noticed between TPC and FIC in the principal component analysis (PCA) reported as not all phenolic compounds were effective metal chelators.[24]

Recent study reported that *G. mangostana* pericarp extract (GME) could prevent the development of hypertension and cardiovascular using animal model.[28] They reported that GME could reduce the vascular superoxide production and plasma malondialdehyde in hypertensive induced rats and lower the systolic blood pressure of induced rat without exhibiting hypotensive effect on normal control rats. Furthermore, the treatment of GME in induced rats also showed significantly lower mean arterial pressure, diastolic blood pressure, pulse pressure, and heart rate.

Anthocyanins were only detected in *G. mangostana* pericarp. Three anthocyanins have been reported, namely chrysanthemin, cyanidin-3-sophoroside, and cyanidin-3-glucoside.[15] Cyanidin-3-sophoroside gives the purple-brown colour of the pericarps.

Although the occurrence of xanthones, benzophenones and biflavonoids is common in the *Garcinia* genus, exception is noticed in *G. atroviridis*. To date, only atroviridin, garcinia acids, i.e. HCA, 2- (butoxycarbonylmethyl)-3-butoxycarbonyl-2-hydroxy-3-propanolide, 1′,1″-dibutyl methyl-hydroxycitrate and its γ-lactone have been reported in *G. atroviridis*[29] HCA was proven to be effective in the treatment of obesity. It inhibits lipogenesis, lowers cholesterol and fats, increases glycogen production in the liver, suppresses appetite, and increases the thermogenesis process. These series of metabolic changes would thereby promote weight reduction by decreasing the conversion rate of carbohydrates to fats. HCA exhibited antioxidative properties by increasing the level of non-enzymatic and enzymatic antioxidants and reduction in lipid peroxidation product, malondialdehyde in HCA treated non-alcoholic steatohepatitis induced rats.[30]

CONCLUSION

In conclusion, our findings showed that Garcinia species, especially leaves and pericarps of *G. mangostana* and *G. hombroniana* have very great potential to be developed into nutraceutical products since they are very high in antioxidant activities. This study has identified new sustainable source of antioxidative agents. The extracts of Garcinia samples could be partially purified, and the fractions should be explored further to discover their potential to be used as alternative treatments for diseases which were related to oxidative stress.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interests.

ABBREVIATIONS

A: Difference if absorbance between pH 1.0 and 4.5; **AA:** Ascorbic acid; **AAE:** Ascorbic acid equivalent; **Acontrol:** Absorbance of the control; **Asample**: Absorbance of the extract; **Cy-3-glu:** Cyanidin-3-glucoside; **DPPH:** 2,2-diphenyl-1-picrylhydrazyl; **ε:** Molar extinction coefficient; **FIC:** Ferrous ion chelating; **FRP:** Ferric reducing power; **FRS:** Free radical scavenging; **GAE:** Gallic acid equivalents; **GME:** Garcinia mangostana pericarp extract; **HCA:** (-)-Hydroxycitric acid; **IC_{50}:** Concentration of extract required to reduce the absorbance by 50%; **L:** Path length of spectrophotometer; **MW:** Molecular weight; **PCA:** Principle component analysis; **TAC:** Total anthocyanin content; **TPC:** Total phenolic content.

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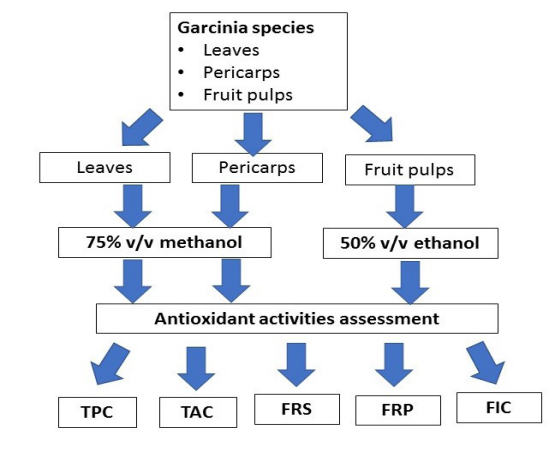
GRAPHICAL ABSTRACT



SUMMARY

G. mangostana, G. hombroniana and *G. atroviridis* leaves, pericarps and pulps were evaluated for antioxidant activities, namely TPC, TAC, FRS, FRP and FIC. Leaves and pericarps of *G. mangostana* and *G. hombroniana* exhibited good antioxidant activities, but the antioxidant activities in their fruit pulps were relatively low. G. atroviridis leaves and pericarps generally have lower antioxidant activities than the other species. leaves and pericarps of G. mangostana and G. hombroniana have very great potential to be developed into new sustainable source of antioxidative agents.

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