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Total phenolic content and ferric reducing antioxidant power of the leaves and fruits of *Garcinia atrovirdis* and *Cynometra cauliflora*

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Abstract

In this study, two types of plants materials were used namely *Garcinia atrovirdis* and *Cynometra cauliflora* to determine the proximate composition, mineral content and antioxidant activities. Total phenolic content (TPC) and ferric reducing antioxidant power (FRAP) assay had been used to determine antioxidant activity in both samples. The moisture, ash, fiber, fat, protein and carbohydrate content in both samples were determined by using Association of Official Analytical Chemists (AOAC) methods. Mineral content in the sample was determined using Atomic absorption spectrophotpmetry (AAS). The results showed higher TPC and FRAP values in *Cynometra cauliflora* compared to *Garcinia atrovirdis*. Methanol extractions gave higher TPC and FRAP values compared to water extraction. The results obtained indicated that both samples have the potential to be as a source of natural antioxidants. Further study should be conducted to explore the benefits of underutilized fruits not only in antioxidant activity but other usages as well.

Introduction

Many food industries used synthetic antioxidants to preserve food namely butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate (PG), and tert – butyl hydroquinone (TBHQ) as a food additive. Previous studies shown that the use of BHA and BHT as antioxidant could be toxic, increase manufacturing costs, lower the efficiency of natural antioxidants, and increasing consciousness of consumers with regard to food additive safety as well (Sherwin, 1990; Wanasundara and Shahidi, 1998). Replacement of synthetic with natural antioxidants may be beneficial due to health implications and functionality.

Malaysia is rich with local fruits and vegetables that are relatively high in antioxidants activities (Amin *et al*., 2004; Amin and Lee, 2005; 2006). Besides that, Malaysia is also rich with diversity underutilized fruit such as Asam Gelugor (*Garcinia atrovirdis*), Nam-nam (*Cyanometra cauliflora*), Pulasan (*Nephelium mutabile*), and others that wildly grown in region of Peninsular Malaysia, Sabah, and Sarawak.

Garcinia atrovirdis grow wildly throughout Peninsular Malaysia is also widely cultivated especially in the northern states owing to its economic and medicinal value (Mackeen *et al.*, 2000). Locally, Asam Gelugor also known as 'asam keping' when undergoes sun dried slices. This are commonly use in cooking as adding ingredients to give sour taste such as in seasoning in curries, sour relish and also for dressing fish (Burkill, 1966; Corner, 1988).

Cynometra cauliflora is from Fabaceae family which locally known as Nam-nam fruit and some places also call 'katak puru' for this fruit because of the skin look like which is rough and wrinkled. Usually, this plant growth in wet tropical lowlands, but may also grow well in climates with a more distinct dry season and resistant to wind.

However, all of these fruits were not given much attention as antioxidant sources compared with commercial fruits such as guava, papaya and pineapple (Ikram *et al*., 2009). The main objective of this study are to evaluate the antioxidant activity in both fruits using ferric reducing antioxidant power (FRAP) assay and total phenolic content (TPC) analysis and to determine the proximate composition and mineral content in both plants as well. Two types of underutilized fruits; asam gelugor (*Garcinia atrovirdis*) and nam-nam (*Cyanometra Cauliflora*) were used as the samples.

Materials and Methods

Sample preparation

The fruits and leaves of *Garcinia atrovirdis* and *Cyanometra cauliflora* were collected by hand in October 2010 from Kg. Gajah, Perak, Malaysia. The samples were identified by the Herbarium Unit of School of Biological Sciences, Universiti Sains Malaysia. The leaves of Garcinia atrovirdis were cleaned with tap water and dried. The skin for both

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samples was removed using stainless steel knife before the pulp was blended into small pieces and stored at -20˚C, prior to further use.

Sample extraction

The samples were extracted using 80% methanol (v/v) and distilled water. Each sample of 1 g was weighed into conical flask that was wrapped with aluminum foil and 50 mL 80% methanol solution was added. For water extraction, 50 mL of deionized water was added into the conical flask. The mixtures were then placed in an incubator shaker at temperature 30˚C and 150 rpm for 24 hours. The samples were then centrifuged at 3200 rpm for 20 minutes to obtain a clean solution.

Proximate composition

Chemical composition of the samples namely moisture (Method 977.11), ash (Method 923.03), crude protein (Method 960.52), fat (Method 920.39) and crude fibre (Method 935.53) were determined according to the Association of Official Analytical Chemist (AOAC), (1990) methods. The results were expressed in wet basis. All analyses were done in duplicate and averaged.

Mineral content determination

Mineral content that was determined in both samples were sodium (Na), calcium (Ca), iron (Fe), zinc (Zn) and cadmium (Cd). Series of standard were prepared for all elements with different concentration. The concentration standard for elements Ca, Fe, and Cd are 1.0, 2.0, 3.0, and 4.0 mg/L (ppm) meanwhile for elements Na and Zn are 0.5, 1.0, 1.5, and 2.0. Results obtained were expressed as mg/100 g sample.

Total phenolic compounds determination

Total phenolic compounds (TPC) of samples were determined using Folin-Ciocalteau (FC) assays as described by Singleton and Rossi (1956) with slightly modification. Extracted samples of 400 µl were pipette into test tubes. FC reagent (2 ml) was added into each test tube and was vortexed. Then, the mixtures were left standing at room temperature for 5 minutes. An amount of 1.6 ml 7.5% Na_2CO_3 were added into the mixture and vortexed again. The mixtures were allowed to stand for 1 hour in dark at room temperature (20°C ± 5°C). The absorbance was measured at 765 nm using UV-visible spectrophotometer and calibration curve was prepared using gallic acid at the concentration of 0,0.1, 0.3, 0.5, 0.7, 0.9 and 1.0 mg/ml ($r^2 = 0.999$). Results were expressed as mg gallic acid equivalents (GAE)/100g of freeze dried sample.

Ferric reducing antioxidant power (FRAP) assay

FRAP assay was performed according to the methods of Benzie and Strain (1999) with slightly modification. An amount of 200 µl extracted samples were mixed with 3 mL FRAP reagent in test tubes and undergoes vortex. Blank samples were prepared for both methanol and deionized water extracted samples. Both samples and blank were incubated in water bath for 30 minutes at 37°C and the absorbance of the samples was determined against blank at 593 nm. Series of stock solution at 200, 400, 800, 1200 and 1600 µM were prepared (r^2= 0.9944) using aqueous solution of $FeSO_4.7H_2O$ as standard curve. The values obtained were expressed as µM of ferrous equivalent Fe (II) per gram of freeze dried sample.

Statistical analysis

Results obtained were reported as mean + SD of triplicate measurements. Significance differences for multiple comparisons were determined by one way analysis of variance (ANOVA) followed by Duncan test with $\alpha = 0.05$ using SPSS (version 17).

Results and Discussions

Proximate composition

Oven drying method was used for moisture content determination. Table 1 showed the moisture content in all of the samples. *Garcinia atrovirdis* fruit has the highest moisture content at 89.45% followed by *Cyanometra cauliflora* at 87.27% and *Garcinia atrovirdis* leaves at 78.52%. There was a significance different at $p < 0.05$ between all samples for moisture content.

Table 1. Moisture, ash, crude protein, fat, crude fiber and carbohydrate content in *Garcinia atrovirdis* and *Cynometra cauliflora*

Plant *species*	Part used	Moisture	Ash	Crude protein	Fat	Crude Fiber	Carbohydrate
Garcinia	Fruit	89.48+0.04^c	0.18+0.01^a	0.56+0.00^a	0.18+0.01^a	1.44+0.05^a	8.18+0.01^a
atrovirdis	Leaves	78.52+0.03^a	5.19+0.01^c	1.03+0.04^b	0.54+0.03^b	2.84+0.13^b	11.88+0.17^c
Cynometra cauliflora	Fruit	87.27+0.09^b	1.41+0.13^b	0.66+0.04^a	0.18+0.01^a	1.72+0.13^a	8.77+0.21^b

* Data were expressed in mean values + SD with n=3 according to Duncan's Multiple- Range test. Values with different superscript are significantly different at $p < 0.05$

Total ash content was higher in *Cyanometra cauliflora* (1.41%) compared to *Garcinia atrovirdis* fruit with total ash of 0.18%. The highest percentage of total ash was in *Garcinia atrovirdis* leaves with the amount of 5.19% (Table 1). There was a significance difference at $p < 0.05$ between all samples for ash content.

Protein content was determined using Micro-Kjeldahl Method. Micro-Kjeldahl method is based on the principle of conversion of nitrogen in food product into ammonia. Only crude protein were determined as it determines the total organic nitrogen content in samples which include all nitrogen from protein and non-protein source producing results that are estimates of actual protein content. Based on the result, fruits for both samples have lower protein content compared *Garcinia atrovirdis* leaves. *Garcinia atrovirdis* fruit contained only 0.56% protein while the leaves have 1.03%. On other hand, *Cynometra cauliflora* fruit has 0.66% protein. There was a significance difference at $p < 0.05$ between both fruits with *Garcinia atrovirdis* leaves for protein content.

Results in Table 1 also showed that fat content in both *Garcinia atrovirdis* fruit and *Cynometra cauliflora* fruit were the same at 0.18%. However, *Garcinia atrovirdis* leaves contained higher fat content of 0.54%. There was no significance difference at $p > 0.05$ between both fruits but there was significance ($p < 0.05$) between both fruits with *Garcinia atrovirdis* leaves for fat content.

Dietary fiber is frequently defined as polysaccharides and lignin that are not digested by human enzymes. *Garcinia atrovordis* fruit gave 1.44% of crude fiber while *Cynometra cauliflora* fruit with 1.72%. Value of crude fiber in both samples were almost same, but were higher in *Garcinia atrovirdis* leaves with 2.84%. There was no significant differences at $p > 0.05$ for fiber content between both fruits but significant difference at $p < 0.05$ existed between both fruits with *Garcinia atrovirdis* leaves.

From proximate analysis that had been done, carbohydrate contain in the samples can be calculated using formula [100%-Moisture-ash–fat-protein-crude fiber]. Carbohydrate content was highest in *Garcinia atrovirdis* leaves (11.88%) followed by *Cynometra cauliflora* fruit (8.77%) and *Garcinia atrovirdis* fruit (8.18%). There was significant difference at $p < 0.05$ between both fruits in carbohydrate content.

Minerals determination

In this study, calcium (Ca), sodium (Na), iron (Fe), zinc (Zn), cadmium (Cd), and lead (Pb) were determined using atomic absorption spectrophotometer (AAS). According to Natow and Heslin (2004), minerals can be classified in two types which are major and minor minerals. Major minerals are minerals that our body need in high amounts such as calcium, potassium, magnesium, sodium, and phosphorus. As for minor minerals, our body just needs a little amount which is less than 100 mg/day. Zinc, iron, selenium, manganese, and copper can be classified as minor mineral or trace minerals. Table 2 showed the mineral content in all of the samples. Results were express as mg/100 g of wet samples.

Table 2. Minerals content in *Garcinia atrovirdis* and *Cynometra cauliflora*

Plants	Part used	Calcium (Ca)	Zinc (Zn)	Iron (Fe)	Sodium (Na)	Cadmium (Cd)	Lead (Pb)	Copper (Cu)
		Minerals content (mg/100 g, wet basis)						
Garcinia	Fruit	6.65 ± 0.07^{b}	0.32 ± 0.02^{a}	1.14 ± 0.13^{a}	0.38 ± 0.08^{b}	nd	nd	nd
atrovirdis	Leaves	4.02 ± 0.11^{a}	0.55 ± 0.09^{b}	2.14 ± 0.15^{b}	0.16 ± 0.02^{a}	nd	nd	nd
Cynometra cauliflora	Fruit	6.14 ± 0.28^{c}	0.48 ± 0.04^{b}	1.01 ± 0.02^{a}	0.55 ± 0.05^{c}	nd	nd	nd

* Data were expressed in mean values + SD with n = 3 according to Duncan's Multiple- Range test. Values with different superscript are significantly different at $p < 0.05$. nd: not detected

Garcinia atrovirdis fruit showed the highest calcium content (6.65 mg/100 g samples) compared to *Cynometra cauliflora* fruit with 6.14 mg/100 g samples and *Garcinia atrovirdis* leaves with 4.02 mg/100 g samples. There was a significance different at $p < 0.05$ between *Garcinia atrovirdis* fruit with its leaves and with *Cynometra cauliflora* fruit in calcium content. Calcium is an important mineral as it plays a role in mediating the constriction and relaxation of blood vessels, nerve impulse transmission, muscle contraction, and secretion of hormone such as insulin. Besides that, calcium is involved in producing stronger bones and teeth (Natow and Heslin, 2004).

From the result, zinc content in *Garcinia atrovirdis* leaves was highest with 0.55 mg/100 g samples followed by *Cynometra cauliflora* fruit (0.48 mg/100 g samples) and *Garcinia atrovirdis* fruit (0.32 mg/100 g samples). There was a significance difference at $p < 0.05$ between *Garcinia atrovirdis* fruit with its leaves, while an insignificant different at $p > 0.05$ between both fruits in zinc content. Usually zinc is higher in green peas, fresh oysters, spinach and ginger root (Natow and Heslin, 2004).

The iron (Fe) content was highest in *Garcinia atrovirdis* leaves with 2.14 mg/100 g samples followed by its fruits (1.14 mg/100 g samples) and *Cynometra cauliflora* fruit at 1.01 mg/100 g samples. Significant different existed at $p < 0.05$ between *Garcinia atrovirdis* fruit with it leaves, while no significant different existed at $p > 0.05$ between both fruits in iron content.

The results obtained showed that all samples contained low natrium content. The highest natrium content was in *Cynometra cauliflora* fruit with 0.55 mg/100 g samples followed by *Garcinia atrovirdis* fruit (0.38 mg/100 g samples) and its leaves at 0.16 mg/100 g samples. There was a significant different

at $p < 0.05$ between all of the samples. There were some elements that were not detected such as plumbum and cadmium, thus making the plants safer for consumption.

Total phenolics content

The amount of TPC in samples was reported as mg of gallic acid equivalent (GAE) per 100 g freeze dried sample (Table 3). According to Nazck and Shahidi (2004), Follin-Ciocalteau reagent was not specific and can detects all phenolic groups found in the samples including those found in the extractable proteins. This method can cause interference of reducing substances such as ascorbic acid as well. Extract of *Cynometra cauliflora* was diluted 10 times as it contains high concentration of TPC.

Table 3. Comparison between total phenolic content in *Garcinia atrovirdis* (fruit and leaves) and *Cynometra cauliflora* using methanol and water extraction

Solvent extraction	Plants	* mg GAE/100g samples
Methanol	*Garcinia atrovirdis* (fruit)	62.34 ± 3.07^{b}
	Garcinia atrovirdis (leaves)	29.93 ± 0.43^{a}
	Cynometra cauliflora (fruit)	847.31 ± 26.82^{c}
Water	*Garcinia atrovirdis* (fruit)	32.53 ± 0.56^{b}
	Garcinia atrovirdis (leaves)	18.69 ± 0.60^{a}
	Cynometra cauliflora (fruit)	98.79 ± 6.19^{c}

*Data were expressed in mean values + SD with n=3 according to Duncan's Multiple- Range test. Values with different superscript are significantly different at $p < 0.05$

Table 3 showed that *Cynometra cauliflora* fruit exhibited the highest total phenolics content compared with fruit and leaves of *Garcinia atrovirdis* in both types of extraction. Extraction using methanol gives higher total phenolics content compared to distilled water. There was a significant different at $p < 0.05$ for total phenolics compounds in methanol and water extraction in all of the samples. On other hand, the TPC that were obtained from both plants in methanol extracts were 847.31 mg gallic acid/100 g sample in *Cynometra cauliflora* (fruit) compared to 62.34 mg gallic acid/100 g sample in *Garcinia atrovirdis* fruit and 29.93 mg GAE/100 g sample in *Garcinia atrovirdis* leaves. The total phenolics compound in both plants were in the order of *Cynometra cauliflora* (fruit) > *Garcinia atrovirdis* (fruit)> *Garcinia atrovirdis* (leaves).

The same trend was observed in water extraction where *Cynometra cauliflora* fruit gave the highest amount of TPC at 98.79 mg GAE/100 g sample compared to *Garcinia atrovirdis* fruit (32.53 mg gallic acid/100 g sample) and 18.69 mg GAE/100 g sample in *Garcinia atrovirdis* leaves. The result obtained was related with the study done by Ikram *et al.* (2009) where *Cynometra cauliflora* has higher TPC compared to *Garcinia atrovirdis*. The low phenolic content in *Garcinia atrovirdis* suggests that the presence of other compounds that may act as antioxidant properties (Moure *et al*., 2001). Based on the same study, *Cynometra cauliflora* contained TPC of 1868.94 mg GAE/100 g sample while *Garcinia atrovirdis* only 68.41 mg GAE/100 g sample. There was some difference in the value of TPC from previous study in *Cynometra cauliflora* with this study due to different cultivated plant, different storage condition and loss of some phenolic compounds during sample preparation. There was a significant difference at $p < 0.05$ between all three types of samples for both methanol and water extraction in the TPC.

A study by Gorinstein *et al.* (2004) and Sellappan *et al*. (2002) showed strong correlations between TPC and antioxidant activity in various kinds of fruits. Hodzic *et al.* (2009) also stated that the amount of TPC will affect the antioxidant capacity. In the study, different types of grain were used as sample to determine the TPC and FRAP assay to determine the antioxidant capacity. The result showed linear correlation between the amount of TPC and antioxidant capacity where high TPC gives high antioxidant capacity.

Ferric reducing antioxidant power (FRAP) assay

The results (Table 4) showed that FRAP values were higher in methanol extracted samples compared to water extraction. This showed that methanol extraction was more efficient in extracting antioxidants in plant materials compared to water. *Cynometra cauliflora* fruit gave higher FRAP values than *Garcinia atrovirdis* (fruit and leaves). Methanol extracted *Cynometra cauliflora* fruit has FRAP value of 19397.22 μM/g freeze dried samples followed by Garcinia atrovirdis fruit with 624.166 μM/g freeze dried samples and *Garcinia atrovirdis* leaves with 325.85 μM/g freeze dried samples. There was a significant difference at $p < 0.05$ exist between *Cynometra cauliflora* fruit with *Garcinia atrovirdis* (fruit and leaves). However, no significance difference at $p > 0.05$ between the FRAP values of methanol extracted of Garcinia atrovirdis fruit and leaves.

For water extraction, *Cynometra cauliflora* fruit also had the highest FRAP value of 7197.22 μM/g freeze dried sample, followed by *Garcinia atrovirdis* fruit with 434.17 μM/g freeze dried sample and *Garcinia atrovirdis* leaves with 109.40 μM/g freeze dried sample. The same trend can be observed

Table 4. FRAP values in *Cynometra cauliflora* (fruit) and *Garcinia atrovirdis* (fruit and leaves) (μM/g dried samples)

Solvent extraction	Plants	* FRAP μM/g samples (dw)
Methanol	*Garcinia atrovirdis* (fruit)	624.17 ± 12.58[a]
	Garcinia atrovirdis (leaves)	325.85 ± 18.05[a]
	Cynometra cauliflora (fruit)	19397.22 ± 1296.29[b]
Water	*Garcinia atrovirdis* (fruit)	434.17 ± 28.34[a]
	Garcinia atrovirdis (leaves)	109.40 ± 8.80[a]
	Cynometra cauliflora (fruit)	7197.22 ± 1267.14[b]

* Data were expressed in mean values + SD with n=3 according to Duncan's Multiple- Range test. Values with different superscript are significantly different at $p < 0.05$

between the fruit and leaves of *Garcinia atrovirdis* and *Cynometra cauliflora* where significant different existed at $p < 0.05$ between both the samples. However, there was insignificant difference at $p > 0.05$ between *Garcinia atrovirdis* fruit and *Garcinia atrovirdis* leaves in FRAP values for water extraction. A study by Ikram *et al.* (2009) showed that the FRAP value for *Garcinia atrovirdis* was about 0.67 mM/g samples (dried). This value was slightly similar with the FRAP value for *Garcinia atrovirdis* in this study (624.166 μM/g sample).

According to Hodzic *et al.* (2009), FRAP assay had been used to determine antioxidant activity as it is simple and quick. Besides that, the reaction is reproducible and linearly related to molar concentration of the antioxidants. However, some disadvantage was found in this method as FRAP assay does not react fast with some antioxidants such gluthathione (Guo *et al*., 2003). Schafer and Buettner (2001) stated that FRAP assay still can be used for assessment of antioxidant activity in plants materials as humans only absorb limited amount of gluthathione. Higher FRAP values give higher antioxidant capacity because FRAP value is based on reducing ferric ion, where antioxidants are the reducing agent. Antioxidants are compounds capable of donating a single electron or hydrogen atom for reduction.

There are some factors that not only affect the extracted compounds from plant materials but antioxidant activity as well. According to Moure *et al.* (2001), quality of natural extracts and antioxidant activities does not only depend on storage time, geographic origin, harvesting date but also environment and technological factors as well. Besides that, solvent used is one of the important factors to extract antioxidant compound in plant materials due to the different antioxidant potential of compound with different polarity. Temperature and light also contribute to antioxidant activity change during storage.

Conclusion

In TPC determination, *Cynometra cauliflora* fruit showed the highest TPC followed by *Garcinia atrovirdis* fruits and its leaves. *Cynometra cauliflora* has higher levels of phenolic compounds than does *Garcinia atrovirdis*, which may act as antioxidant properties. Range for TPC in the samples for methanol extraction was from 62.34 to 847.31 mg GAE/100 g freeze dried samples. As for water extraction, the TPC was in between 322.53 to 98.79 mg GAE/100 g freeze dried samples. Methanol extraction was more efficient compared to water extraction. For FRAP assay, *Cyanometra cauliflora* fruit has the highest value followed by *Garcinia atrovirdis* fruit and its leaves. Methanol extraction gave a value of between 624.17 to 19397.22 μM/g freeze dried samples. For water extraction, the range was between 434.17 to 109.40 μM/g freeze dried samples. Higher FRAP values were obtained in methanol extraction. It can be seen that higher TPC gave higher FRAP values. *Garcinia atrovirdis* leaves had higher value in total ash, crude protein, crude fiber and fat content while *Garcinia atrovirdis* has high moisture content. Further study should be conducted to explore the benefits of underutilized fruits not only in antioxidant activity but other usages as well.

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