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Nutritional Content and *in vitro* Antioxidant Potential of *Garcinia atroviridis* (*Asam gelugor*) Leaves and Fruits

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ABSTRACT

Introduction: The objective of this study was to determine antioxidant potential of *Garcinia atroviridis* leaves and fruits extracts *in vitro*. **Methods**: Antioxidant activity was assessed using 1,1-diphenyl-2-picrylhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP) assays. Total phenolic content (TPC) of the extracts was estimated as gallic acid equivalent by Folin-Ciocalteau method. Proximate analysis was determined based on the Association of Official Analytical Chemists (AOAC) procedures. **Results**: *Garcinia atroviridis* leaves extracted at 100°C/15 min demonstrated the highest TPC value (21.21 ± 0.28 mg GAE/mg) and was significantly different (p<0.05) from that of leaves extracted at 60°C/6 h and 40°C/12 h. On the other hand, the fruit extracted at 60°C/6 h showed the highest TPC value (16.23 ± 0.18 mg GAE/mg) (p<0.05) compared to the fruit extracted at 40°C/12 h and 100°C/15 h respectively. The antioxidant activities of both samples were positively correlated with the TPC values based on DPPH-radical-scavenging activity and ferric reducing power estimation. *Garcinia atroviridis* leaf extract contained significantly higher proteins, carbohydrate and ash contents (2.16% ± 0.08; 15.98% ± 0.12 and 0.72% ± 0.07 respectively) than its fruit extract (0.46% ± 0.08, 8.64% ± 0.06 and 0.15% ± 0.06) respectively). The energy content was also found to be higher in the leaf (73.64% ± 2.15) compared to the fruit (38.38% ± 1.72) (p<0.05). **Conclusion**: The findings indicate that *G. atroviridis* leaves and fruits have potential for use as a source of natural antioxidants and nutrients for therapeutic purposes against free radical mediated health conditions.

Keywords: *Garcinia atroviridis*, antioxidants, nutrient content, total phenolics

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INTRODUCTION

The term antioxidant is commonly used in scientific literature and has been defined in multiple ways. The definition proposed for an antioxidant is “any substance that delays, prevents or removes oxidative damage to a target molecule”, based on the physiological role of the compound in preventing cellular components damage caused by free radicals (Halliwell & Gutteridge, 1998).

Oxidative damage has been known to affect human health and cause various diseases but it may be prevented or limited by dietary antioxidants (Jantan *et al.,* 2011). The synthetic antioxidant butylated hydroxytoluene (BHT) has been used in the food processing industry as a preservative to prolong storage stability of foods and reduce oxidative damage to the human body. However, the use of synthetic antioxidants has been restricted following evidence of harmful side effects such as liver damage and carcinogenesis (Wichi, 1988). Therefore, plants and fruits have been receiving attention for their potential role in food quality improvement as well as human disease prevention. The interest in health benefits of plants and herbs has increased owing to their antioxidant and free radical scavenging activities observed *in vitro.*

Garcinia atroviridis is an endemic fruit tree species in Peninsular Malaysia. Various parts of this tree have been utilised by ethnobotanists and ethno-pharmacists as a natural preservative, seasoning and for medicinal purposes as well (Mackeen *et al.*, 2002). Dried fruit has been used to improve blood circulation, treat coughs, and as an expectorant and laxative (Amran *et al.,* 2009). Previously, *G. atroviridis* fruits and leaves extracts demonstrated various *in vitro* physiological functions including antioxidant, antimicrobial and antibacterial and antitumour-promoting activities while being non-toxic (MacKeen *et al*., 2012, Wen *et al.,* 2012, Mackeen *et al.*, 2002). The drying characteristic along with some physical and chemical properties of the fruit have been previously described (Rittirut & Siripatana, 2006).

The present study was undertaken to determine the antioxidant activity and total phenolic contents of *G. atroviridis* fruits and leaf extracts. The study also aimed at determining the optimum sample operating parameters such as extraction time and temperature settings for the fruit and leaf. Energy and selected nutrient contents were also determined.

METHODS

The chemicals and reagents used were butylated hydroxytoluene (BHT), 1,1-diphenyl-2-picrylhydrazyl (DPPH), L-ascorbic acid (Vitamin C), ferric chloride, hydrochloric acid, ferrous sulphate, acetic acid, sodium acetate and gallic acid, all of which were from Sigma Chemical Co. (USA). The reagent 1,1- Folin-Ciocalteu and sodium carbonate were from Merck (Germany). The reagent 2,4,6-tris(2-pyridyl)-1,3,5-triazine (TPTZ) was from Fluka (Switzerland).

The proportion of carbohydrate, moisture, crude protein, fat and ash in fresh samples of *G. atroviridis* fruits and leaves were estimated in accordance with the standard methods described by the Association of Official Analytical Chemists (AOAC) (AOAC, 1995). The analyses were carried out in triplicates and are reported as percentages. The mineral content was determined by atomic absorption spectrophotometry.

Samples of authenticated fruits and leaves of *G. atroviridis* were collected fresh from Kedah, Malaysia. The samples were cut into pieces, dried in an oven at 30°C for 2 days and ground into fine powder. The aqueous extracts (10% w / v) of the samples were prepared by soaking 100 g of either powdered fruits or leaves in 1000 ml distilled water, and then incubated in a shaking water bath at various temperatures and time setting: 40 °C / 12 h, 60°C / 6 h and 100°C / 15 min. The variation in temperature and incubation time was proposed with the goal of optimising the yield of potential biological compounds from the extract since no data on the optimisation of the extraction procedure of this plant has been reported. The crude extracts were then filtered and the supernatants were dehydrated using a spray dryer to produce powdered extracts while preserving the quality. The extracts were subsequently subjected to antioxidant activity assessments.

DPPH radical scavenging

The antioxidant activity of GAFE and GALE was assessed on the basis of scavenging activity against a stable free radical 1,1-diphenyl-2-picrylhydrazyl (DPPH), as previously described (Yen & Hseih, 1998). The activity was compared against the standard antioxidants BHT and vitamin C. Briefly, 1 ml of 0.45 mM DPPH was added to 0.5 ml absolute ethanol to prepare the control solution. As for the sample solution, 1 ml of 0.45 mM DPPH was added to 0.5 ml of the extract (5 ml / ml). This step was repeated by replacing the extract with BHT or vitamin C (5 mg / ml). Then, the control and sample solutions were incubated for 30 min. Following incubation, the absorbance was recorded at 517 nm. The inhibition percentage which represents the scavenging activity of the sample against DPPH was calculated as per the following equation:

$$\text{Inhibition (\%)} = \frac{[\text{Absorbance of control} - \text{Absorbance of test sample}] \times 100\%}{\text{Absorbance of control}}$$

Ferric reducing antioxidant power

The reducing ability of GAFE and GALE was evaluated on the basis of ferric reducing antioxidant power (FRAP) assay as described by Benzie & Strain (1996). The FRAP reagent was freshly prepared by mixing 10 mM 2, 4, 6-tripyridyl triazine (TPTZ) and 20 mM ferric chloride in 0.25M acetate buffer (pH 3.6). Then, 100 μl of extract was added to 300 μl of distilled water, followed by 3 ml of FRAP reagent. The absorbance was recorded at 593 nm spectrophotometrically after 4 min of incubation at room temperature. The reducing ability of the extracts was compared with BHT. The results are expressed as the concentration of antioxidants having ferric reducing ability equivalent to that of 1 mM $FeSO_4$, expressed in milimolar per litre.

Total phenolic content

The total phenolic concentration of GAFE and GALE were determined using Folin–Ciocalteu reagent and gallic acid as standard (Velioglu *et al*., 1998). Briefly, 200 μl (0.2 mg / ml) of extract was added to 0.75 ml of 10-fold diluted Folin–Ciocalteu reagent. Following 5 min of incubation, 0.75 ml of 6% Na_2CO_3 solution was added. The mixture was allowed to stand for 90 min at room temperature. The absorbance was measured at 725 nm. The same procedure was repeated to all standard gallic acid solutions (0.005–0.050 mg / ml) and a standard curve was obtained. All the results are expressed in mg of Gallic Acid Equivalent (GAE) per mg of sample.

Statistical analysis

All data were analysed using the Statistical Package for Social Science (SPSS) program version 15. After confirming the normality of data and the homogeneity of variance of data, the significance of the differences between means of test and control studies was established by one-way analysis of variance (ANOVA) coupled with post hoc Tukey HSD for multiple group comparison. All data are presented as means $\pm$ SD. All analyses with value of $p < 0.05$ indicate statistical significance.

RESULTS

DPPH radical scavenging

Figure 1 represents the scavenging activity of BHT, vitamin C, *G. atroviridis* fruit extract (GAFE) and *G. atroviridis* leaf extract (GALE) against free radical 1,1-diphenyl-2-picrylhydrazyl (DPPH). The standard antioxidant BHT demonstrated 94.6% ± 0.21 scavenging ability and represents the highest scavenging activity compared to the other groups. GALE prepared at 100°C for 15 min (GALE100) demonstrated 89.2% ± 0.27 scavenging ability and was significantly higher than other GALE variants ($p<0.05$). On the other hand, the scavenging activity of GALE100 was comparable with vitamin C. Meanwhile, the highest scavenging ability amongst GAFE was observed in GAFE60 (52% ± 0.11) and was found to be significant compared to the other leaf extracts ($p<0.05$). In addition, higher scavenging activity in all samples of leaf extracts was observed than in the fruit extract groups ($p<0.05$).

Ferric reducing antioxidant power

Figure 2 represents the ferric reducing antioxidant power (FRAP) of BHT, GAFE and GALE prepared at various temperatures and incubation times. The FRAP value of all leaf extracts was found to be significantly higher than groups of the fruit extracts ($p<0.05$). The standard antioxidant BHT demonstrated the highest antioxidant power with FRAP value of 2.29 ± 0.04 mmol / L but was not significantly different when compared to GALE100 (2.24 ± 0.02 mmol / L). The antioxidant power in GALE100 was significantly higher than other leaf extracts ($p<0.05$). No significant difference of antioxidant power between GALE40 and GALE60 was observed.

Total phenolic content

Figure 3 represents the total phenolic content of GAFE and GALE extracts prepared at various temperature and incubation time. The test revealed that GALE100 extracted at 100°C for 15 min demonstrated the highest

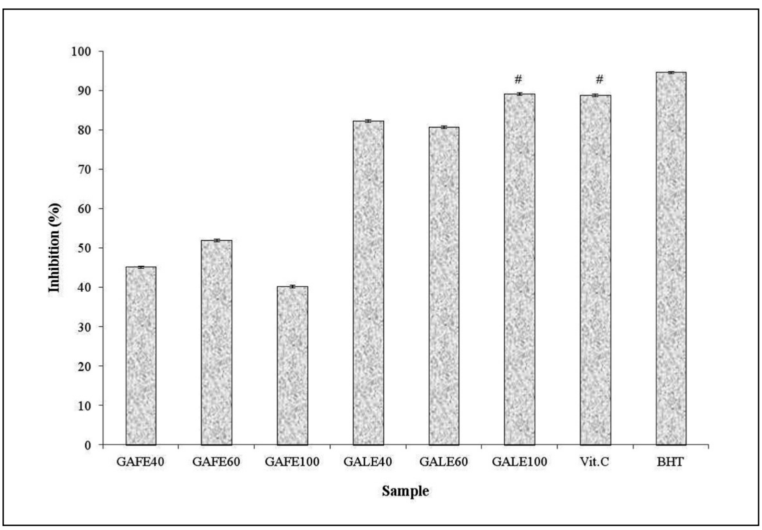

Figure 1. DPPH free radical scavenging of BHT, vitamin C, *G. atroviridis* fruit (GAFE) and leaf (GALE) extracts prepared at various temperatures and incubation times (GAFE40 and GALE40: 40°C, 12 h; GAFE60 and GALE60: 60°C, 6 h; GAFE100 and GALE100: 100°C, 15 min). Samples with (#) are not significantly different ($p\geq0.05$). Values are expressed as mean ± SD.

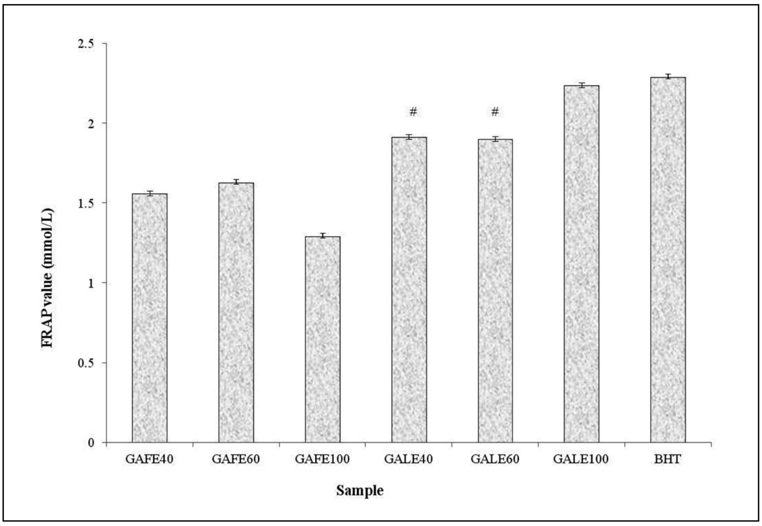

Figure 2. FRAP value (mmol/L) of BHT, *G. atroviridis* fruit (GAFE) and leaf (GALE) extracts prepared at various temperatures and incubation times (GAFE40 and GALE40: 40°C, 12 h; GAFE60 and GALE60: 60°C, 6 h; GAFE100 and GALE100: 100°C, 15 min). Samples with (#) are not significantly different ($p \geq 0.05$). Values are expressed as mean ± SD.

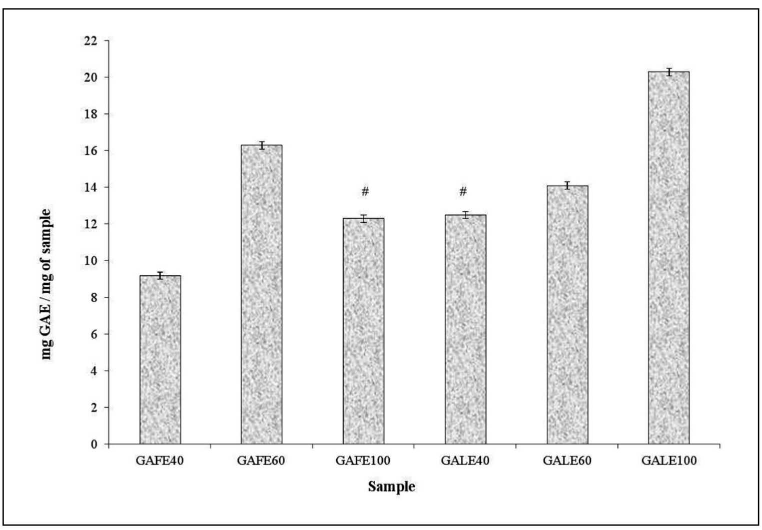

Figure 3. Total phenolic content of *G. atroviridis* fruit (GAFE) and leaf (GALE) extracts prepared at various temperatures and incubation times (GAFE40 and GALE40: 40°C, 12 h; GAFE60 and GALE60: 60°C, 6 h; GAFE100 and GALE100: 100°C, 15 min). Samples with (#) are not significantly different ($p \geq 0.05$). Values are expressed as mean ± SD.

concentration of phenolic (20.21 ± 0.21 mg GAE/mg) ($p<0.05$) compared to other extract groups followed by GAFE60 (16.23 ± 0.18 mg GAE/mg). There was no significant difference in phenolic contents in GALE40 and GAFE100. Besides these two samples, the phenolic content in all other extract samples are significantly different ($p<0.05$) from each other. The lowest phenolic content was observed in GAFE40 (9.25 ± 0.12 mg GAE/mg).

Proximate analysis and mineral contents

The proximate and mineral composition of *G. atroviridis* fruit and leaf are presented in Table 1. The analysis revealed that moisture represents the utmost portion of proximate weight for the leaf and fruit (81.03% ± 8.79 and 90.52% ± 5.12); however, no significant difference in moisture content was observed between the samples. Other nutritional components contributed approximately 10 to 20% of the fresh sample total weight. Protein, carbohydrate and ash were found to be significantly higher in the leaf (2.16% ± 0.08; 15.98% ± 0.12 and 0.72% ± 0.07) than in the fruit (0.46% ± 0.08, 8.64% ± 0.06 and 0.15% ± 0.06) respectively ($p<0.05$). Contradicting earlier results, no significant difference in the fat content was found between the leaf and fruit. On the other hand, the energy content in the leaf was higher than in the fruit ($p<0.05$) by 35.26 Kcal/100g.

Mineral analysis revealed that both the leaf and fruit samples contain calcium, potassium and other minerals in trace amounts (Table 1). There was no significant difference in the mineral contents of the leaf and fruit samples. No heavy metal elements such as arsenic and lead were detected.

DISCUSSION AND CONCLUSION

The stable free radical DPPH assay has been used for decades to assess the free radical-scavenging ability of various samples owing to its simplicity and ease of conducting in a relatively short time compared to other methods (Bahramikia & Yazdanparast, 2010). With a difference of only 5.4%, the scavenging ability of GALE100 was comparable to the standard antioxidant BHT. In fact, the ability of GALE100 was 0.4% higher than vitamin C. This data was in agreement with previous reports that

Table 1. Proximate and mineral composition of fresh *G. atroviridis* leaf and fruit

Constituents	*Leaf*	*Fruit*
Moisture, % (w/w)	81.03 ± 8.79	90.52 ± 5.12
Carbohydrate, % (w/w)	15.98 ± 0.12	8.64 ± 0.06
Protein, % (w/w)	2.16 ± 0.08	0.46 ± 0.08
Fat, % (w/w)	0.12 ±0.02	0.22 ± 0.05
Ash, % (w/w)	0.72 ± 0.07	0.15 ± 0.06
Energy, Kcal/100g	73.64 ± 2.15	38.38 ± 1.79
Carbon, mg/100g sample	50.23 ± 5.10	42.51 ± 3.23
Oxygen, mg/100g sample	41.98 ± 4.59	55.52 ± 6.34
Aluminium, mg/100g sample	0.25 ± 0.01	0.96 ± 0.01
Potassium, mg/100g sample	1.03 ± 0.01	0.86 ± 0.01
Calcium, mg/100g sample	0.37 ± 0.01	0.18 ± 0.01
Sulfur, mg/100g sample	0.15 ± 0.01	0.10 ± 0.01
Phosphorus, mg/100g sample	0.19 ± 0.01	ND
Bromide, mg/100g sample	0.24 ± 0.01	ND
Molybdenum, mg/100g sample	ND	0.39 ± 0.01

Note: Energy was calculated by summation of (fat x 9 kcal) + (protein x 4 kcal) + (carbohydrate x 4 kcal). ND: not detected.

conclude that the antioxidant capacities of several flavonoids are stronger than vitamins C (Prior & Cao, 1999). The effect of antioxidants on DPPH is due to their hydrogen donating ability when antioxidants donate protons to free radicals (Baumann, Wurn & Bruchlausen, 1979). This illustrates the proton-donating ability of *G. atroviridis* extracts which could serve as free radical inhibitor and act as a primary antioxidant.

The analysis of ferric reducing ability in the present study recorded that GALE100 possessed the highest FRAP value amongst all extracts and was comparable to the standard antioxidant BHT followed by GALE60 and GALE40. The reducing power of all leaf extracts was superior to the fruit extracts. Our data supports earlier findings whereby extracts composed of various fractions have different levels of antioxidants and scavenging activity in each tested system and serve as one of the methodological limitations for antioxidant determination (Kaur & Kapoor, 2001). The reducing potential of the extracts proves the presence of polyphenolic compounds as electron donors capable of neutralising free radicals (Bahramikia &Yazdanparast, 2010).

The analysis of total phenolic content in the present study revealed that both the leaf and fruit samples were rich in phenolics with higher concentrations obtained when extracted at 100°C for 15 min for the leaf and 60°C / 6 h for the fruit. The increased total phenolic concentrations in the leaf and the fruit were proportional to increased antioxidant activities (DPPH and FRAP). Phenolic compounds found in various natural sources are consistently correlated with important physiological functions, particularly antioxidant activity (Kono *et al.*, 1995; Nagai *et al.*, 2003). However, increased phenolic content does not necessarily reflect its antioxidative activity (Kubola & Siriamornpun, 2008).

Previous studies documented that *G. atroviridis* fruit acids, including citric acid, tartaric acid, ascorbic acid and hydroxycitric acid (HCA) (Jena *et al.*, 2002) contribute to its antioxidant activity (Rittirut & Siripatana, 2006). The strong antioxidant activity of the leaf has been attributed to the presence of flavonoids (Mackeen *et al.*, 2002; Cooks & Samman, 1996). Xanthone and hydroquinone which include several potential antioxidants such as atroviridin, atrovirisidone, atrovirinone and garcinol have also been isolated from parts of *G. atroviridis* (Permana *et al.*, 2001; Sang *et al.*, 2001; Kosin *et al.*, 1998; Minami, Miho & Fukuyama, 1994).

The present findings illustrate the effects of temperature and incubation time on the antioxidant ability and phenolic content of the extracts. For the leaves, a high extraction temperature with a short incubation time was required to attain maximum antioxidant ability. Conversely, the fruit required medium temperatures with longer incubation times. Exposure of biological samples to high temperatures during extraction may have important implications on bioactivity of the extracts (Güçlü-Üstündað & Mazza, 2009). Heat treatment may affect the interaction of bioactive components with the medium, with the antioxidant activity and total phenolic content of extracts increasing as phenolic compounds are released from their bound states (Jeong *et al.*, 2004). Furthermore, increased bioactivity could also be due to formation of new compounds following heat treatment (Kitts & Hu, 2005). On the other hand, extraction of plant materials at high temperature may also cause degradation of bioactive compounds (Ju & Howard, 2003). This study reveals that the extraction parameters of 60°C for 6 h and 100°C for 15 min of the respective *G. atroviridis* fruits and leaf samples produce extracts with optimum antioxidant activity and proportionate to the phenolic concentrations. The radical scavenging and ferric reducing ability of the extracts verified the presence of polyphenolic compounds that stabilised free radicals and acted as electron donors. This clearly specifies the antioxidant potential of *G. atroviridis* extracts against *in vitro* oxidative systems.

The proximate and mineral analyses of *G. atroviridis* fruits and leaves in the present study revealed that both samples had high moisture content. This agrees with previous reports on the physical and chemical properties of the fruit which recorded an average moisture content of 86.47% and total soluble solid of 6.34% (Rittirut & Siripatana, 2006). The leaf contains higher amounts of carbohydrate, protein, ash, energy, potassium and calcium than the fruit, while the leaf has higher fat content. The absence of heavy metal elements such as arsenic and lead suggests that *G. atroviridis* leaf and fruit could be safe for consumption.

The study findings indicate the potential use of *G. atroviridis* leaves and fruits as a source of natural antioxidants and nutrients for therapeutic purposes against free radical mediated health conditions.

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