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Antioxidant activity of *Garcinia atroviridis* Griff. ex T.Anderson

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ABSTRACT

This study aimed to investigate and evaluate the amount of antioxidants activity and total phenolic contents of the plant *Garcinia atroviridis* Griff. ex T.Anderson. Only the leaves and fruit were used for this study. These samples were washed, dried and grinded, then Soxhlet apparatus and methanol were used for extraction, and the end products were used for the study. Two methods were used for this study, 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) assay was used to evaluate the antioxidant activity and the second assay was total phenolic content (TPC) to measure the quantity of phenols in leave and fruit extracts. Four standards were used for DPPH assay including ellagic acid, tannic acid, gallic acid and rutin. Their results were then compared with the two samples results. Twelve concentrations were prepared for the standards and samples. The antioxidant activity was expressed as the efficient concentration needed to scavenge 50% of free radicals. For TPC assay, gallic acid was the standards and six concentrations were prepared for the standards and samples. In both methods, microplate reader machine or spectrophotometer was used to read the absorbance. According to the results of both assays, the fruit extract exhibited higher antioxidant activity and higher total phenolic content compared to the leaves extracts. The efficient concentration of fruit extract was 4 mg mL^{-1}, while in the case of leaves extract, it could not be attained. The mean of gallic acid in 1 mg of fruit extracts was 0.070, while in leaves extract was 0.069. It was also concluded that when the concentration increases, antioxidant activity and total phenolic content elevates as well.

Keywords: *Garcinia atroviridis* Griff. ex T.Anderson, Antioxidant, Phenolic, 2,2-Diphenyl-1-Picrylhydrazyl (DPPH).
Article type: Research Article.

INTRODUCTION

Thousands or millions of years ago or even more, mankind was created on this earth together with other living things such as bacteria, plants, viruses, and animals. These things can either be harmful or useful to mankind. Those which are harmful can disturb the biological system of the human body. As a result, illnesses developed and the man started to use his skills and knowledge to explore, observe and use the raw materials in his surroundings which are usually plants to maintain a healthy lifestyle and to prevent diseases. This kind of treatment is called traditional, folk, or indigenous medicine. The most common example of traditional medicine is herbal medicine. Nowadays, traditional medicine is widespread and plants present a large source of natural antioxidants which might serve as the development of novel drugs. Several anti-inflammatories, digestive, anti-necrotic, neuroprotective, and hepato-protective drugs have shown an antioxidant and/or antiradical scavenging mechanism as part of their activity (Gupta *et al.* 2020). Recently, the interest in using herbal medicine is growing as people are recalling the history of its uses by their ancestors. Thus, the studies about them are increasing and are been taken more into concern compared to modern medicine. It was believed that herbal medicine has curative power and many studies nowadays are identifying the active components in plants that can play a vital role in human health. Typically, antioxidant molecules are the main components that investigators look for in plants. However, the substitution of synthetic antioxidants with natural antioxidants is still debatable, and the health implications of antioxidants as nutritive, have enhanced and reinforced research on fruits and vegetable sources,

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also the examination of raw materials for identifying different kinds of antioxidants (Lourenço *et al.* 2019; Manessis *et al.* 2020; Bayas-Morejón *et al.* 2020; Khademian Amiri *et al.* 2022). Antioxidants are compounds that can delay or inhibit the oxidation process of biomolecules by inhibiting the oxidation chain reactions using free radicals and thus they will reduce oxidative damage to the human body (Santos-Sánchez *et al.* 2019). Therefore, many herbal medicines are screened for their possible antioxidant effects against various chemical-induced tissue damages in animals. It is estimated that there are 250,000 to 500,000 species of plants on Earth. Relatively small percentages (1 - 10%) of these are used as food by both humans and other animal species, and lower percentages for medicinal purposes (Aejazuddin 2016). There are high numbers of species belonging to the genus *Garcinia* or *Rheedia* as the largest genus found in the Guttiferae family. It comprises around 400 species widespread in tropical Asia, Africa, New Caledonia, Polynesia, and Brazil. Some of them are well known such as the *G. mangostana* and the fruits of most *Garcinia* species are edible. However, their effectiveness at normal consumption levels is still unknown clearly (Espirito Santo *et al.* 2020). This genus has been used for ages in folk medicine, and it is known to be rich in oxygenated and prenylated phenol derivatives. A great variety of compounds have been isolated from the Guttiferae family, mainly polyisoprenylated benzophenones, flavonoids, and xanthones (Espirito Santo *et al.* 2020). *G. atroviridis* Griff. ex T. Anderson has been proved to be a rich resource of compounds with important therapeutic properties. However, only a few investigations have been conducted so far on the chemical composition of the volatile oils and active compounds from this species (Tavares *et al.* 2020). *G. atroviridis* Griff. ex T. Anderson is not a very popular plant. However, it is a native plant in Asian countries including Thailand and Indonesia. It has been used widely in traditional herbal medicine and as a food flavouring. Even though it is an endemic plant, the information on the active compounds of this species is limited. Thus, in this context, the present work would contribute new and additional knowledge on its bioactivities. The objective of this study was to evaluate the antioxidant activity of *G. atroviridis* extract, to determine which part of the tree (leave or fruit) has higher antioxidant activity and to determine at which concentration the sample yields better antioxidant activity.

MATERIALS AND METHODS

Plant material preparation

The leaves and fruit were washed using deionized water. The fruit was immediately cut into slices and exposed to the sun for a few days to dry and then put in an oven at 50 ºC for one week to dry completely. The leaves were then dried in an oven at 40 ºC for 4 days. The dried fruit slices and leaves were then ground into powder using a centrifuge mill.

Methanol extracts preparation

The powdered samples were extracted with methanol using Soxhlet apparatus. One hundred grams of the sample were placed in the Soxhlet together with one litre of methanol solution, for two weeks at 40 ºC. Then the extracts were separated from methanol using a vacuum rotary evaporator and the crudes were collected and stored at room temperature until further use.

DPPH scavenging assay

The DPPH free radical scavenging assay was performed according to Le *et al.* (2007). The assay was initiated by diluting 20 µL of sample extracts in 180 µL methanol, then 12 concentrations were prepared by serial dilution (5, 2.5, 1.25, 0.625, 0.3125, 0.156, 0.8, 0.4 0.02, 0.01, 0.005 and 0.0025 mg mL^{-1}). In this experiment, absolute methanol was used as blank. The assays were performed in almost dark area to avoid light reaction. At first, 100 µL DPPH solution was poured in the 96-well microplates. Then each extract fruit and leave was diluted in methanol (0.01 g extract with 1000 µL methanol), 100 µL of diluted extract were loaded into the 96 well. Thus, the 96 wells were filled with 200 µL DPPH and the sample extract. This mixture was allowed to incubate at room temperature for 30 minutes. The control was only a mixture of DPPH and methanol. Afterward, the microplate reader was used to measure the absorbance at 517 nm. The microplates were placed into the microplate reader machine, and all extracts were analysed in triplicate. The following equation was used to calculate the DPPH radical scavenging activity:

$$\text{Radical scavenging activity (\%)} = \frac{(\text{Absorbance control} - \text{Absorbance sample})}{\text{Absorbance control}} \times 100$$

The scavenging activity of extract was expressed as the concentration necessary to scavenge free radical by 50% (EC_{50}). Four standards with the concentrations of 5, 2.5, 1.25, 0.625, 0.3125, 0.156, 0.8, 0.4 0.02, 0.01, 0.005 and 0.0025 mg mL^{-1}, including ellagic acid, tannic acid, rutin and gallic acid were used to compare them with the samples. These four are various types of natural phenolic acids that are found in plants and have good antioxidant properties. These standards were mixed with the DPPH substituting the sample extract.

Determination of total phenolic content

According to Krishnaraju *et al.* (2005) with few modifications, Folin-Ciocalteu's phenol reagent was used to quantify the total phenolic content of the extracts. Each extract of fruit and leaves were mixed with this reagent, and the mixture turned blue colour when the phenolic compounds in the extract mixed with the reagent. The blue colour complex will be formed after adjusting with alkali. Briefly, 40 µL extract in methanol with different concentrations (0.03, 0.06, 0.12, 0.25, 0.5 and 1 mg mL^{-1}) were pipetted into the 96-well microplate, followed by 50 µL of 15% Folin-Ciocalteau. Then, 10 µL distilled water was added to adjust the volume to 100 µL. The mixture was left for 5 minutes before the addition of 100 µL Na_2CO_3 aqueous (0.105 g mL^{-1}). The absorbance of extract was then measured at 756 nm after incubation at room temperature for 60 minutes. The method was performed in triplicate. Different concentrations of gallic acid (0.03, 0.06, 0.12, 0.25, 0.5 and 1 mg mL^{-1}) were used to prepare a standard graph. The concentrations of total phenolic compounds in all extracts were expressed as mg of gallic acid equivalents per gram of dry weight of extract using a linear equation.

RESULTS

DPPH assay

In this experiment, leaves and fruit of *Garcinia atroviridis* Griff. ex T. Anderson were studied along with rutin, ellagic acid, tannic acid and gallic acid as standards that shown in Fig. 1. The EC_{50} value of *G. atroviridis* was determined by the DPPH assay. It exhibited a significant scavenging activity on DPPH free radical which increases with elevating the extract concentration. The fruit extract scavenged 50% of the free radical by only using 4 mg mL^{-1}. In the case of leaves extract, its scavenging activity was much less than the fruit and the EC_{50} could not be attained. The four standards showed a very high scavenging activity. Ellagic acid, tannic acid and gallic acid displayed the same EC_{50} which was around 0.3 mg mL^{-1}, while rutin around 0.6 mg mL^{-1}.

Total phenolic content assay

Table 1. The amount of gallic acid (phenols) in the leaves and fruit extracts.

Sample	Mean of absorbance	Mean of gallic acid (mg mL^{-1})
Leave	1.933	0.069
Fruit	1.969	0.070

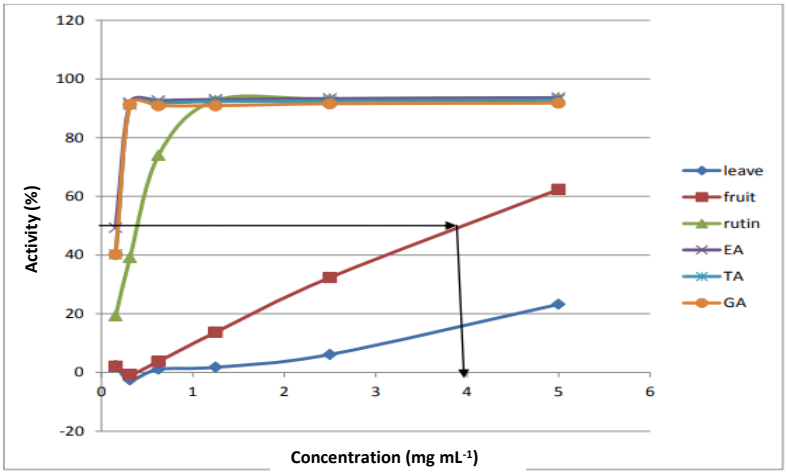

Fig. 1. The antioxidant activity by the various concentrations of extracts via the DPPH radical scavenging method.

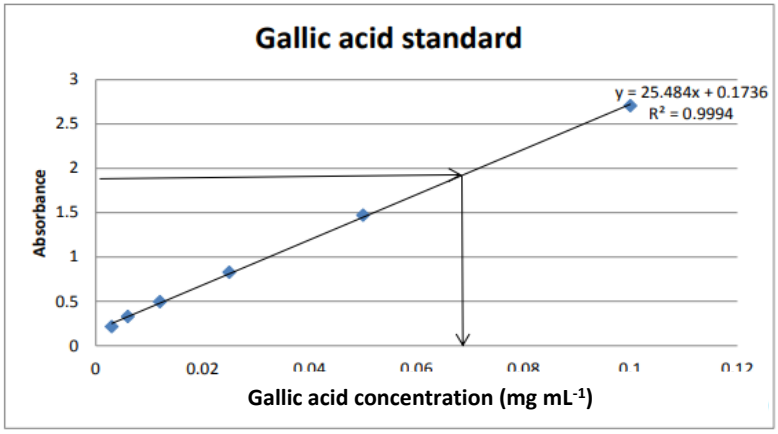

Fig. 2. Various concentrations of the standard gallic acid versus absorbance.

In Fig. 2, total phenolic content is expressed as gallic acid equivalents per gram. The total phenolic contents, calculated using the standard curve of gallic acid (R^2 = 0.999). It was found that the fruit extract contains higher phenolic contents (0.070 mg mL^{-1}) compared to leaves (0.069 mg mL^{-1}).

DISCUSSION

In this study, two methods were used to evaluate the antioxidant activity of *G. atroviridis* in methanolic extract, including DPPH and total phenolic content assays. The reason behind choosing these assays was because they have been used vastly and their methods are established by many papers before. Besides, they were good enough to evaluate the antioxidant activity and exhibited satisfying results in a relatively short period of time at low cost (Aryal *et al.* 2019). These methods do not need any special preparation and can be performed at room temperature with avoiding cell degradation. In addition, DPPH is a highly sensitive assay (Kumar *et al.* 2014) and is independent of substrate polarity which means that DPPH is able to accept hydrogen radicals or electron to become stable diamagnetic molecule (Kedare & Singh 2011; Santos-Sánchez *et al.* 2019). The concentrations in this study play a vital role as it was the most important factor that determines the best result of antioxidant activity. Fig. 1 shows six different curves with each using six different concentrations (5, 2.5, 1.25, 0.625, 0.3125, 0.156 mg mL^{-1}). These concentrations determine the percentage of antioxidant activity of the sample that can cease or inhibit the reaction of the free radical chain.

The EC_{50} value is a parameter mostly used to interpret DPPH results. It stands for the "efficient concentration" which means the concentration of the substrate needed to inhibit 50% of the damage caused by free radicals (Olszowy-Tomczyk 2021). Ellagic acid, tannic acid, and gallic acid exhibited the highest antioxidant scavenging activity. The graph shows between the concentrations of 0.1 and 0.3. They displayed a significant increase and reached their highest antioxidant activity at around 91%. Then with upraising the concentration, their activity becomes steady at that point (91%). Afterward, rutin becomes in the second place, exhibiting a significant rise between 0.1 and 0.6 mg mL^{-1} reaching the highest antioxidant activity at 74%, and then becomes steady with elevating the concentration. Fruit and leaves revealed a relatively low antioxidant activity compared to the standards, however, their antioxidant activity was elevated, as the concentration increased. Thus using a high concentration (more than 5 mg mL^{-1}) yields a better antioxidant activity. Gangwar *et al.* (2014) also observed the same result in their study.

Nevertheless, the fruit sample showed a better antioxidant activity than the leaves sample. At 5 mg mL^{-1}, the scavenging activity of the fruit was around 62% while the leaves were around 23 % only. The reason behind this result is that the fruit contains much more active compounds that are able to scavenge the free radicals in its surrounding, e.g., butanol, HCA, γ-lactone, atroviridin, atrovirisidone and atrovirinone as well as organic acids, octadecanoic, dodecanoic, nonadecanoic, pentadecanoic, and citric acids. The metabolites also give the fruit a higher antioxidant activity. However, these antioxidants compound can be also found in the leaves, albeit in a lower amount. In the present study, the fruit samples showed a higher absorbance compared to the leaves, reflecting that the higher amount of active compounds in the fruit sample absorbs the light, thus less light was transmitted. Meanwhile, the lesser amount of active compounds in the leaves sample can only absorb little light

and more light has been transmitted. The decreasing intensity of the purple colour is a good sign that the scavenging activity was increasing. It is because the purple colour of DPPH turns yellow, only when an antioxidant scavenges the free radical by hydrogen donation, and this activity was monitored and evaluated using a spectrophotometer at 517 nm (Rahman *et al.* 2015). Previously, it has been proved that almost all plants are potential sources of bioactive compounds such as organic acids and metabolites, as they play an important role in plant defence system (Lautié *et al.* 2020).

Flavonoids and phenols are the primary compounds that are found in vegetables and fruits. They are highly potent antioxidants, since they can reduce the occurrence of cancer and chronic inflammatory disease (Panche *et al.* 2016). The second method is total phenolic content which is basically evaluating the quantity of phenol molecules in each sample extract (fruit and leaves) and is commonly used in conjunction with the DPPH method. Phenolic molecules are usually a part of plant metabolites, since they possess an excellent redox property, which gives the plant a great antioxidant activity. The phenol molecule has a hydroxyl group on its aromatic ring which gives them the ability to be hydrogen donors, reducing agents, and singlet oxygen quenchers (Kumar & Goel 2019). Few methods are available to estimate the phenol quantity, including permanganate titration, colorimetry with iron salts, ultraviolet absorbance, and Folin–Ciocalteu. The latter is widely used in most antioxidant studies. In the TPC assay, it was observed that the solution turned blue colour, since the phenols form phosphomolybdic–phosphotungstic phenol complex in alkaline solution which appear blue in colour. The total phenolic content assay showed quite a high content of total polyphenols in *G. atroviridis* extract. This observation indicates that this extract has a good antioxidant activity, therefore, may play a role in the prevention of some diseases such as atherosclerosis (Morago *et al.* 2020). It was proved by many authors that the two assays, DPPH and total phenolic content were highly correlated (Kumar *et al.* 2014; Mohyelden *et al.* 2021) and in the present study, this extract has also been proven to have the same observation.

This uniformity is due to the principle of the two essays that share a similar mechanistic basis, which is the transfer of electrons from the antioxidant molecule to reduce an oxidant, as explained by Aryal *et al.* (2019). In addition, other studies on plant extracts have shown that using both DPPH and TPC methods will upgrade the overall evaluation result. However, each method reflects a different aspect of the antioxidants manner (Hatami *et al.* 2014; Nirmala *et al.* 2020). Thus, the outcomes from the present and previous studies, together with the similar mechanistic basis of the assays, indicate a high degree of estimation in the employment of both assays for screening plant extracts. The result of the TPC assay shows that high gallic acid in 1 mg of the extract is associated with high phenolic contents. This finding was reported previously many times by different scientists (Zhang 2011; Hafiza *et al.* 2017; Aryal *et al.* 2019).

CONCLUSION

On the basis of the outcome and observations presented in this study, it can be concluded that *Garcinia atroviridis* Griff. ex T. Anderson is a good source of natural antioxidants as it exhibited high antioxidant activity and good total TPC. Between these two methods DPPH and total phenolic content, it was observed that DPPH method showed higher records, since DPPH is more general and evaluates the content of all the antioxidants molecules in a sample, such as phenols, flavonoids, and ascorbic acid content, while TPC is only for phenolic compound evaluation. The end outcome of this study has also demonstrated the accuracy of the hypothesis which is that concentration has a significant effect on the result. In both methods, DPPH and TPC, the readings of antioxidant activity and phenolic content increase by elevating its concentration in the sample. It has also been proved that the fruit extract yields better scavenging activity and higher phenolic contents compared to leaves extract. However, we can conclude that both samples displayed good antioxidants behaviour in both methods, and the activity of both fruit and leaves extracts exhibit similar results.

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