ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 13, Issue 8, 2020

Online - 2455-3891
Print - 0974-2441

Research Article

DETERMINATION OF THE TOTAL PHENOLICS AND ANTIOXIDANT ACTIVITY IN THE RIND EXTRACTS OF *GARCINIA MANGOSTANA* L., *GARCINIA COWA* ROXB., AND *GARCINIA ATROVIRIDIS* GRIFF. EX T. ANDERS.

REGINA ANDAYANI*, FITHRIANI ARMIN, AINUL MARDHIYAH

Department of Pharmacy, Faculty of Pharmacy, Andalas University, Limau Manis Campus, Padang, West Sumatera, 25163, Indonesia.
Email: reginaandayani@phar.unand.ac.id

Received: 01 December 2019, Revised and Accepted: 06 June 2020

ABSTRACT

Objectives: *Garcinia atroviridis* Griff. ex T. Anders., *Garcinia mangostana* L., and *Garcinia cowa* Roxb. are plants of the genus Garcinia that has been widely used by the community as a food flavoring, spices, and also as a herbal medicinal ingredient. This research aimed to evaluate the total phenolics and antioxidant activity from three species of Garcinia (*G. atroviridis* Griff. ex T. Anders., *G. mangostana* L., and *G. cowa* Roxb.)

Methods: The total phenolic content (TPC) of the extracts was estimated as Gallic Acid Equivalent by the Folin-Ciocalteu method. Antioxidant activity was assessed using Ferric Reducing Antioxidant Power assay.

Results: The TPC of *G. mangostana* L. rind extract is higher (31.83±3.70%), than *G. cowa* Roxb.(4.35±0.17%) and *G. atroviridis* Griff. ex T. Anders. (2.47±0.42%). Based on the antioxidant activity, *G. mangostana* L. rind has a higher total antioxidant activity (24.68 μmol Fe(II)/g) than *G. cowa* Roxb. (18.88±0.12 μM Fe (II)/g and *G. atroviridis* Griff. ex T. Anders.(17.61±0.05 μM Fe(II)/g).

Conclusion: The results showed that *G. mangostana* L rind extract contains a higher level of TPC and antioxidant activity among the other rinds. The results obtained indicate that the three samples have the potential to be a source of natural antioxidants. Further studies must be carried out to isolate compounds that have antioxidant activity.

Keywords: Total phenolic, Antioxidant activity, *Garcinia atroviridis* Griff. ex T. Anders., *Garcinia mangostana* L., and *Garcinia cowa* Roxb.

 DOI: http://dx.doi.org/10.22159/ajpcr.2020.v13i8.36525

INTRODUCTION

Antioxidants are substances that can prevent and protect against diseases related to oxidative stress through preventing the free-radical formation, scavenging and neutralizing reactive oxygen species, and inhibiting oxidative reactions [1]. Therefore, widespread interest has been recently concerned about the evaluation of antioxidant plants and phytochemicals for reducing the risk of various diseases and improving the quality of life [2,3]. Several plants have significant antioxidant activity due to the presence of certain natural products responsible for scavenging the excess free radicals from the system [4-6].

Garcinia atroviridis Griff. ex T. Anders. has also been attributed with anti-inflammatory properties and has been found useful in cases of acne. *G. atroviridis* Griff. ex T. Anders., *Garcinia mangostana* L., and *Garcinia cowa* Roxb. are plants of the genus Garcinia which is widely used by people as a food flavoring, spices, and also as herbal medicinal ingredients [4].

The *G. atroviridis* Griff. ex T. Anders fruit contains citric acid, tartaric acid, malic acid, ascorbic acid, γ-lactone compounds, atroviridin, atrovirisidone, atrovirinone, pentadecanoic, octadecanoic, nonadecanoic, dodecanoic acid, and flavonoids [7]. *G. mangostana* L. contains xanthone compounds, mangostin, garsinon, flavonoids, and tannins in the fruit rind [8]. Whereas *G. cowa* Roxb. contains secondary metabolites, especially triterpenoids, flavonoids, xanthone, and florogusinol [9]. The plants contain many antioxidant compounds, such as phenolics. Many of the isolated compounds have a full range of pharmacological activities, including anticancer, anti-inflammatory, antibacterial, antiviral, antifungal, anti-HIV, antidepressant, and antioxidant [7-9].

The main objective of this study is to evaluate the antioxidant activity in three Garcinia rind extracts using total phenolic content (TPC) analysis and ferric reducing antioxidant power (FRAP) assay.

METHODS

Chemicals

About 70% ethanol (Merck), gallic acid (Sigma-Aldrich), Folin-Ciocalteu (FC) (Sigma-Aldrich) reagent, sodium carbonate (Sigma-Aldrich), ferroin (Sigma-Aldrich), iron (III) chloride (Sigma-Aldrich), aquadest (Brataco), iron (II) sulfate heptahydrate (Sigma-Aldrich), sodium acetate trihydrate (Sigma-Aldrich), and iron (III) chloride hexahydrate (Sigma-Aldrich).

Plant material and preparation of extracts

The sample used was fruit rinds of *G. atroviridis* Griff. Ex T. Anders. from Medan, North Sumatra, *G. mangostana* L. from the Batusangkar, West Sumatra, and *G. cowa* Roxb. from Padang, West Sumatra. Plant identification was carried out at Andalas University Herbarium (ANDA) Department of Biology FMIPA Andalas University, Padang, West Sumatera.

Fresh samples were collected and cleaned with water, then drained. Then, the samples were dried in an oven at 50°C for 72 h and ground to powder using an electric grinder. Each powder was weighed 300 g, and then extracted using 3 l of 70% ethanol. Soak for the first 6 h, stirring occasionally, then allowed to stand for 18 h. After that, the extract was separated, and then evaporated with a rotary evaporator so that a thick extract was obtained [10].

Screening of phytochemical of rind extracts

Qualitative phytochemical tests on *G. atroviridis* Griff. ex T. Anders, *G. cowa* Roxb, and *G. mangostana* L. rind extracts were carried out

to identify the availability of the main phytoconstituents including alkaloids, tannins, saponins, flavonoids, steroids, and terpenoids [11].

The TPCs

The TPC of samples were determined using FC assay as described by Singleton and Rossi (1956) with slight modification. Extracted samples of 0.2 mL 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 8 min. An amount of 1.6 ml 7.5% Na_2CO_3 was added into the mixture and vortexed again. The mixtures were allowed to stand for 2 h in the dark at room temperature (20±5°C). The absorbance was measured at 751 nm using a ultraviolet (UV)-visible spectrophotometer, and a calibration curve was prepared using gallic acid at the concentration of 300, 400, 500, 600, and 700 mg/L (r^2=0.9998). The results were expressed as mg gallic acid equivalents (GAE)/100 g of dried samples [12].

FRAP assay

The ability to reduce ferric ions was measured using the method described by Benzie and Strain (1996) with slight modification. The FRAP reagent was produced just before use by mixing 10 mL of 0.3 M sodium acetate buffer (pH 3.6), 1 mL of 10 mmol ferroin solution, and 1 mL of 20.0 mM $FeCl_3.6H_2O$ solution in a ratio of 10:1:1 in volume. The samples were then added to 3 ml of FRAP reagent, and the reaction mixture was incubated at 37°C for 30 min. Absorbance was read at 510 nm. The FRAP value of Garcinia rind extracts was equated with that of L-ascorbic acid. The values obtained were expressed as µM of ferrous equivalent Fe (II) per gram of dried sample [13]. New working solutions of $FeSO_4$ were used for calibration. Series of stock solution at 0.3, 0.4, 0.5, 0.6, and 0.7 mM were prepared (r^2=0.9996) using aqueous solution of $FeSO_4.7H_2O$ as standard curve.

Statistical analysis

The experiments were done 3 times, and the result was evaluated as a mean, standard deviation. The data obtained are displayed in the form of bar charts and linear graphs to see the relationship of total phenolic levels with the antioxidant activity of the extracts of each sample.

RESULTS

The TPC assay

For calibration curves used standard solution of gallic acid with various concentrations of 300 mg/L; 400 mg/L; 500 mg/L; 600 mg/L; and 700 mg/L. The relationship between concentration and absorbance of gallic acid standard solution obtained a regression equation Y=0.0009X+0.1384 with r=0.9999. A value of r close to 1 proves that the regression equation is linear. Gallic acid calibration curves are shown in Fig. 1.

TPC of the selected rind extracts was expressed with GAEs, and the contents were obtained using the regression calibration curve Y=0.0009x+0.1384 with r=0.9999. TPC obtained from *G. mangostana* L. rind extract were 31.83±3.70%, *G. cowa* Roxb. rind extract were 4.35±0.17%, and *G. atroviridis* Griff. Ex T. Anders. rind extract were 2.47±0.42 % (Table 1). This showed that *G. mangostana* L. rind extract has the highest phenolic content ability.

FRAP assay

Determination of antioxidant activity is carried out using the FRAP method. The maximum absorption wavelength of iron (II) sulfate heptahydrate solution with UV-visible spectrophotometry was 510 nm with an absorbance of 0.306.

For the calibration curve, a standard solution of iron (II) sulfate heptahydrate was made with various concentrations of 0.3, 0.4, 0.5, 0.6, and 0.7 mmol/l. From the measurement of the iron (II) sulfate heptahydrate standard solution, the regression equation y=1.333x−0.137 with r=0.9996 was obtained. This correlation coefficient (r) shows a linear result because the value of r is close to 1. The calibration curve of the standard iron (II) sulfate heptahydrate solution is shown in Fig. 2.

The antioxidant activity of *G. mangostana* L. rind extract was 24.68±0.19 µM Fe(II)/g, and *G. cowa* Roxb. rind extract was 18.88±0.12 µM Fe(II)/g, and *G. atroviridis* Griff. Ex T. Anders rind extract was 17.61±0.05 µM Fe(II)/g. This showed that *G. mangostana* L. rind extract had the highest FRAP value among others. However, this value is lower than Vitamin C (Table 2 and Fig. 3).

Fig. 4 shows that *G. mangostana* L. rind extract had the highest TPC and antioxidant activity compared to rind extracts of *G. atroviridis* Griff. Ex T. Anders and *G. cowa* Roxb. It can be seen that the higher TPC gave higher FRAP values.

DISCUSSION

Screening of phytochemical of rind extracts

The presence of alkaloids, flavonoids, tannins, saponins, and terpenoids in all the extracts of *G. atroviridis* Griff. ExT. Anders, *G. cowa* Roxb., and *G. mangostana* L. is shown in Table 3. Ethanolic extracts of *G. atroviridis* Griff. ExT. Anders, *G. cowa* Roxb., and *G. mangostana* L., fruit rinds contain flavonoids and terpenoids as the main phytochemical compounds. The rich flavonoid plants could manifest themselves as good sources of antioxidants that would assist in the enhancement

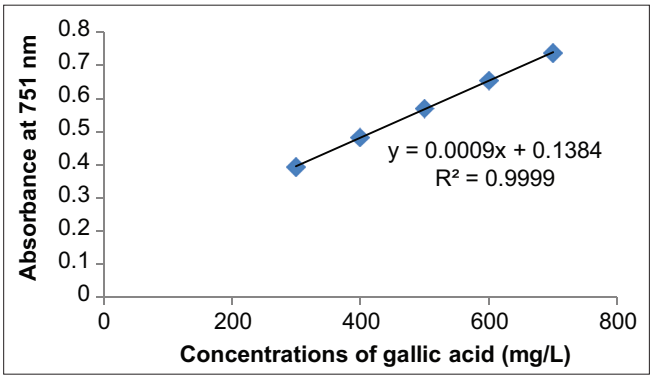

Fig. 1: Standard curve of the total phenolic assay using gallic acid

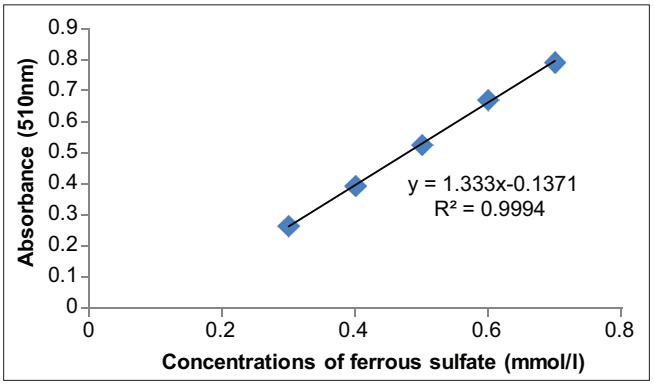

Fig. 2: Standard curve of ferric reducing antioxidant power assay using ferrous sulfate

Table 1: Total phenolic contents of *Garcinia atroviridis* Griff. Et Anders., *Garcinia mangostana* L., and *Garcinia cowa* Roxb. rind extracts with the ultraviolet-visible spectrophotometer at λmax 751 nm

Plants	g GAE/100 g sample
Garcinia mangostana L.	31.83±3.70
Garcinia cowa Roxb.	4.35±0.17
Garcinia atroviridis Griff. Et Anders	2.47±0.42

*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

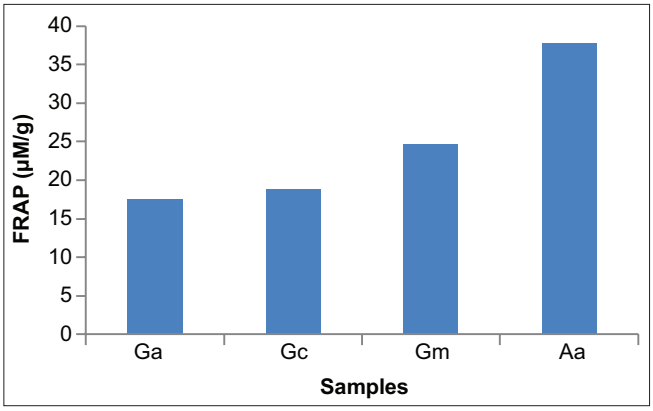

Fig. 3: Antioxidant activity of Garcinia rind extracts and standard controls (ascorbic acid). Ga*: Garcinia atroviridis* Griff. Et Anders, Gc: *Garcinia cowa* Roxb., Gm: *Garcinia mangostana* L., Aa: Ascorbic acid

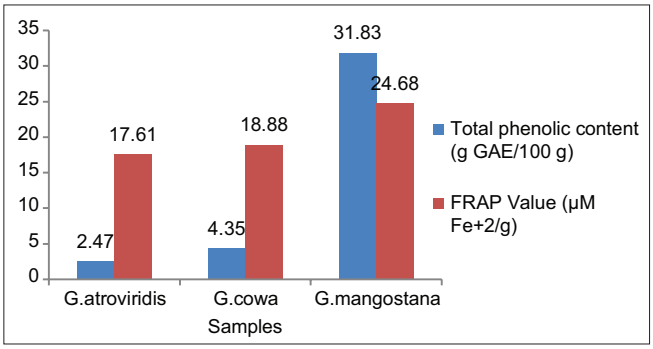

Fig. 4: Total phenolic content and ferric reducing antioxidant power value of *Garcinia atroviridis* Griff. Et Anders., *Garcinia mangostana* L., and *Garcinia cowa* Roxb. rind extracts

Table 2: Antioxidant activity of *Garcinia atroviridis* Griff. ex T. Anders., *Garcinia mangostana* L., and *Garcinia cowa* Roxb. rind extracts with ultraviolet - visible spectrophotometer at λmaks 510 nm

Plants	FRAP (µM $Fe^{+}2$/g)
Garcinia mangostana L.	24.68±0.19
Garcinia cowa Roxb.	18.88±0.12
Garcinia atroviridis Griff. ex T. Anders	17.61±0.05
Ascorbic acid	37.77±0.01

*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: Screening of major phytochemicals in fruit rind extracts

Rind extracts major phytochemicals	*Garcinia atroviridis*	*Garcinia cowa*	*Garcinia mangostana*
Alkaloid	–	–	+
Flavonoid	+	+	+
Steroid	+	+	–
Terpenoid	+	+	+
Saponin	–	–	+
Tannin	+	–	+

+ Positive – Negative

of the overall antioxidant capacity of an organism and protection against lipid peroxidation [14,15]. Hydroxycitric acid and flavonoids presence in *G. atroviridis* fruit extract contributed to its hypolipidemic properties [16]. In recent years, research and isolation of phytochemical compounds that have antioxidant activity are highly developed due to its potential in the therapy of chronic and infectious diseases.

From the data obtained, it can be seen that the mangosteen rind extract has the highest TPC. This data show that the extract of *G. mangostana* L. rind contains more phenolic compounds than extracts of *G. atroviridis* Griff. Ex T. Anders. and *G. cowa* Roxb.

The phenolics or polyphenols are very important secondary metabolites, judging from the virtue of their antioxidant activities by chelating redox-active metal ions, inactivating lipid free radical chains, and avoiding the hydroperoxide conversions into reactive oxyradicals [17].

The FRAP assay measures the reducing potential of an antioxidant reacting with a ferric- ferroin (Fe^{3+}-Ferroin) complex and produce a colored ferrous ferroin (Fe^{2+}-Ferroin) [13]. In general, the reducing properties are linked with the presence of compounds which exert their action by breaking the free radical chain by donating a hydrogen atom. 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 [18].

The results showed that *G. mangostana* L. rind extract has higher antioxidant activity than extracts of *G. cowa* Roxb. and *G. atroviridis* Griff. Ex T. Anders. It shows that *G. mangostana* L. rind extract contains many compounds that act as antioxidants. The previous studies documented that there were 40 xanthones present in the pericarp of the fruit, the most abundant xanthones found are α-mangostin, β-mangostin, and γ-mangostin [19] contribute to its antioxidant activity.

However, the increase in TPC in mangosteen rind extract was not comparable to the increase in antioxidant activity. This can be due to the extraction solvent of 70% ethanol which cannot extract the α-mangostin compound (as the abundant antioxidant) completely. In earlier researcher reported, the ethyl acetate was the best solvents capable of extracting the highest concentration of α-mangostin, followed by dichloromethane, ethanol, and water [20].

It can be concluded that the extraction solvent significantly affects the yield and antioxidant activity of mangosteen rind extract.

CONCLUSION

The results showed that *G. mangostana* L. rind extract contained total phenolic level and antioxidant activity which was higher than those of *G. cowa* Roxb. and *G. atroviridis* Griff. ex T. Anders. The three samples have the potential to be a source of natural antioxidants. Further studies must be carried out to isolate compounds that have antioxidant activity.

AUTHORS FUNDING

Authors are grateful to the Dean of the Faculty Andalas University Pharmacy, which has funded this research is through the DIPA funds from the Faculty of Pharmacy for the fiscal year 2019.

CONFLICTS OF INTEREST

The authors declare that they have no competing interests, and also, there are no conflicts of interest among them.

AUTHORS CONTRIBUTION

The author declares that this work was done by the authors named in this article.

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