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Research Article

Comparative Antioxidant Capacity of Seed and Fruit Ethanol Extracts of Bitter Melon (*Momordica charantia* L.) Using FRAP Assay

Luysi Ariani, Faradiba Abdul Rasyid*, Dewi Yuliana, Rahmawati, Andi Muflihunna, Asni Amin and Rezki Amriati Syarif

Faculty of Pharmacy, Universitas Muslim Indonesia, Makassar, Indonesia

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***Corresponding Author:**
Faradiba Abdul Rasyid,
Faculty of Pharmacy,
Universitas Muslim
Indonesia, Makassar,
Indonesia
E-mail addresses:
faradiba.faradiba@umi.ac.id

ABSTRACT

Momordica charantia L. (bitter melon) is traditionally consumed as both food and medicine in Indonesia. Its fruit and seeds contain bioactive compounds such as saponins, flavonoids, polyphenols, and unsaturated fatty acids with recognized antioxidant potential. This study compared the antioxidant activity of ethanol extracts from bitter melon seeds and fruit using the Ferric Reducing Antioxidant Power (FRAP) assay. Extraction was conducted by ultrasound-assisted extraction (UAE) with ethanol as solvent, and antioxidant activity was expressed as quercetin equivalents (mg QE/g extract). The seed extract showed an antioxidant value of 8.680 mg QE/g, while the fruit extract exhibited 8.626 mg QE/g. The statistical analysis showed that the p-value was greater than 0.05, indicating no significant difference between the two extracts. This suggests that both extracts exhibit comparable antioxidant activity. These results highlight that both seeds and fruit of *M. charantia* may serve as promising natural antioxidant sources for functional food and pharmaceutical applications

Keywords: *Momordica charantia*; bitter melon; seeds; fruit; FRAP; antioxidant

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INTRODUCTION

Free radicals are unstable molecules that can damage important biomolecules, such as lipids, proteins, and DNA, causing oxidative stress and various degenerative diseases, including kidney failure, diabetes mellitus, and stroke (Arnanda and Rina Fajri Nurwanda 2019). Antioxidants are compounds that are able to inhibit free radicals, so that they are able to prevent diseases caused by these free radicals (Munadi 2020). The body actually produces endogenous antioxidants, but the amount is often insufficient so it requires an intake of antioxidants from outside, one of which is through medicinal plants (Tambunan, Nadia and Siregar 2024).

Bitter melon (*Momordica charantia* L.) is one of the herbal plants that are widely consumed by the Indonesian people, both as a vegetable and as traditional medicine. Bitter melon is known to contain bioactive compounds such as saponins, flavonoids, polyphenols, and vitamin C that act as antioxidants in warding off free radicals (Sriwijayanti et al. 2024). Bitter melon is widely used by the community as a medicinal plant, especially as an antidiabetic and antipyretic (Yuda Kusuma and Maesaroh 2020). In addition to the fruit, bitter melon seeds also have pharmacological potential, including as an anticancer agent and a source of unsaturated fatty acids that are antioxidants (Ratnasari et al. 2022).

According to Hussain and colleagues, significant differences were observed among the peel, flesh, and seeds of *Momordica charantia*. The authors reported that seed powder contained higher levels of fat and protein, while peel powder showed greater ash and fiber content. The study also demonstrated that microminerals such as iron, zinc, copper, and manganese were significantly higher in the seeds, whereas the flesh was richer in macrominerals, including calcium and magnesium. Furthermore, the seeds exhibited the highest total phenolic and total flavonoid contents, as well as the highest antioxidant activity. In contrast, the flesh had the greatest carotenoid and β-carotene concentrations. HPLC analysis confirmed that bioactive compounds were unevenly distributed across the three fractions, indicating a distinct organ-specific phytochemical profile (Hussain et al. 2024).

Previous findings have demonstrated that the peel, flesh, and seeds of *Momordica charantia* exhibit distinct phytochemical profiles, with the seeds showing higher total phenolic and flavonoid contents and stronger antioxidant potential than the other fractions (Hussain et al. 2024). These differences suggest that each anatomical part may exhibit varying antioxidant capacities, thereby warranting comparative evaluation. The extraction of active compounds from bitter melon can be effectively performed using ethanol, as it dissolves a broad spectrum of bioactive constituents, has a relatively low boiling point (79°C), and is considered safe for laboratory use (Hasanah 2020). To assess antioxidant capacity, several analytical methods are available, among which the Ferric Reducing Antioxidant Power (FRAP) assay is widely applied due to its simplicity, rapid

procedure, cost-effectiveness, and minimal instrumentation requirements (Aminah, Muflihunna, and Abidi 2016). Therefore, this study aimed to compare the antioxidant activity of ethanol extracts derived from bitter melon seeds and fruit using the FRAP method.

MATERIALS AND METHODS

Equipment

The equipment used in this study includes a micropipette, an oven, a rotary evaporator, a centrifuge, a set of UAE tools, a UV-Vis spectrophotometer, an analytical scale, and a vortex.

Material

The materials used in this study included bitter melon (*Momordica Charantia* L.), bitter melon seed (*Momordica Charantia* L.), Quercetin, Aquades, Trichloroacetic Acid/TCA ($C_2HCl_3O_2$), Iron III Chloride ($FeCl_3$), dapar phosphate pH 6.6, ethanol 96%, Potassium ferrisianide ($K_3Fe(CN)_6$).

Data Analysis

Data analysis was first carried out by measuring the maximum wavelength absorbance on the UV-Vis spectrophotometer, then testing antioxidant activity using the FRAP (Ferric Reducing Antioxidant Power) method so that a value in the form of absorbance was obtained. After obtaining the maximum absorbance value, it is calculated by the regression equation formula of the standard quercetin curve through a linear equation, y = bx + a.

Determination of antioxidant activity:

$$Antioksidant\ activity = \frac{Sample\ concentration \times\ sample\ vol}{sample\ weight} \times Fp$$

The antioxidant activity of the ethanol extracts from the seeds and fruit of *Momordica charantia* was evaluated using the FRAP method, with triplicate measurements (n = 3) per sample. The results were expressed as mean ± standard deviation (SD). Statistical analysis was performed using an independent-samples t-test to compare antioxidant activity between the two groups.

Methods

Preparation of Extract

Fruit

The sample used in this study is bitter melon (*Momordica charantia* L.) obtained from Makassar City, South Sulawesi Province. The plant material was identified and authenticated at the Department of Pharmacognosy and Phytochemistry, Faculty of Pharmacy, Universitas Muslim Indonesia. A voucher specimen was deposited for future reference. The collected bitter melon fruit is then cleaned of the dirt attached to the fruit by washing it with running water. The sample is then thinly sliced after which it is dried using an oven at 50^{o}C and blended until a sample is obtained in powder form. Bitter melon simplicia powder is weighed by 114 g and dissolved with a 70% ethanol solvent of 450 mL. Extraction is carried out using ultrasonics for 30 minutes at a temperature of 30°C. The filtrate is filtered using filter paper with the help of a Büchner funnel. The resulting filtrate is housed in a beaker glass and covered with aluminum foil. Extraction is carried out 3 times. The liquid extract that has been collected is then concentrated using a rotary evaporator and then in the water bath so that a thick extract is obtained.

Seeds

Bitter melon seeds separated from bitter melon are collected as samples, cleaned of impurities, and washed with clean water. After that, the sample is dried in an oven at 50°C. Then blended until the sample is obtained in powder form. Bitter melon seed powder (up to 75 g) is weighed and dissolved in 300 mL of 70% ethanol. Extraction is performed using ultrasound for 30 minutes at 30°C. The filtrate is filtered through filter paper using a Büchner funnel. The resulting filtrate is housed in a glass beaker and covered with aluminum foil. Extraction is carried out 3 times. The liquid extract that has been collected is then concentrated using a rotary evaporator and then in the water bath, so that a thick extract is obtained.

Preparation of Solution

Phosphate Solution 0.2 M pH 6.6

The solution is prepared by weighing 2 grams of NaOH and dissolving it with CO_2-free aquades to exactly 250 mL in a flask. Then, up to 6.8 grams of KH_2PO_4 was dissolved in 250 mL of CO_2-free water in a measuring flask. Then 16.4 mL of NaOH was added to a measuring flask, mixed with 50 mL of KH_2PO_4, adjusted to pH 6.6, and topped up with CO_2-free aqua to 200 mL.

Potassium Ferricyanide Solution 1%

The solution is prepared by dissolving 1 gram of potassium ferricyanide in aqueous and diluting it in a 100 mL measuring flask.

$FeCl_3$ 0.1% Solution

The solution is prepared by dissolving 0.1 grams of $FeCl_3$ in aqueous and diluting it in a 100 mL measuring flask.

Trichloroacetic acid (TCA) solution, 10%

The solution is prepared by dissolving 10 grams of TCA in aquades and diluted in a 100 mL measuring flask.

Test Antioxidant Activity With the FRAP Method

Standard Antioxidant Activity Measurement of Quercetin

A 50 ppm stock solution is prepared by weighing 2.5 mg of quercetin and dissolving it in 96% ethanol in a 50 ml flask. A series of concentrations (15, 20, 25, 30, and 35 ppm) is then prepared from the 50 ppm solution. Each concentration was piped at 250 µL, and then 250 µL of phosphate, pH 6.6, and 250 µL of potassium ferricyanide [$K_3Fe(CN)_6$] 1% were added. The mixture is vortexed for 5 minutes, then incubated at 50°C for 20 minutes, and then 250 µL of 10% TCA is added. It was then centrifuged at 3000 rpm for 10 minutes; the top layer of a solution of 250 µL was mixed with aquades of 250 µL and 125 µL of $FeCl_3$ 0.1%, after which it was incubated at room temperature for 5 minutes. 200 µL was then pipetted into the microplate and measured at 706 nm.

Measurement of Antioxidant Activity of Ethanol Extract of Bitter Melon Seeds and Fruit (Momordica Charantia L.*)*

A total of 10 mg of the extract was dissolved in 10 mL of 96% ethanol, then 250 µL was pipetted, 250 µL of 0.2 M phosphate (pH 6.6), and 250 µL of 1% $K_3Fe(CN)_6$ were added, and after that, it was vortexed for 5 minutes and then incubated for 20 minutes at 50°C. After incubation, 250 µL of TCA is added and then centrifuged at a speed of 3000 rpm for 10 minutes. After centrifugation, 250 µL of the top layer is added, and then 250 µL of aquades and 125 µL of 0.1% $FeCl_3$ are added. The solution is incubated at room temperature for 5 minutes. Then a 200 µL pipette was printed into a microplate and measured at a 706 nm wavelength.

RESULTS AND DISCUSSION

The sample in this study is an ethanol extract of bitter melon seeds and fruits (*Momordica charantia* L.) obtained in the UAE. Furthermore, the sample extract was used to test the antioxidant capacity using the FRAP method with the mechanism of reducing Fe^{3+} ions to Fe^{2+} in a sample so that the antioxidant strength of a compound is analogous to the ability to reduce the compound. However, this method has limitations that need to be considered. This method cannot detect antioxidants with glutathione-like groups. The extraction results can be seen in Tables 1.

Table 1. Extraction Yield and Percentage Immersion of Ethanol Extract of Bitter Melons (*Momordica Charantia* L.)

Sample	Sample Weight (gram)	Extract Results (gram)	Extract Rendamen (%)
Bitter Melons Seeds	75	14.44	19.20
Bitter Melon Fruits	224	72.12	32.196

The UAE method was chosen for this study because it can increase yields and shorten extraction times. The UAE process is carried out using 70% ethanol solvent. The selection of 70% ethanol as a solvent for extraction is because it has both polar and nonpolar groups, allowing it to attract both polar and nonpolar compounds. A thick extract of bitter melon seeds was obtained (14.44 grams) with a percentage of yield of 19.20%, while a thick extract of bitter melon fruits was obtained (72.12 grams) with a percentage of yield of 32.196%.

In this study, quercetin is used as a comparison because it is one of the flavonols from the group of polyphenol flavonoid compounds found in almost every type of plant, and standard quercetin is a natural antioxidant that has very strong antioxidant activity. To determine the maximum wavelength, a standard solution of quercetin was measured at 15, 20, 25, 30, and 35 ppm over 400-800 nm, with a maximum at 706 nm. The absorbance values of the standard solution at each concentration are shown in Table 2.

Table 2. Results of Measurement of Absorbance of Quercetin Standard Solution at Maximum Wavelength 706 nm

Concentration (ppm)	Absorbance
15	0.331
20	0.371
25	0.440
30	0.510
35	0.573

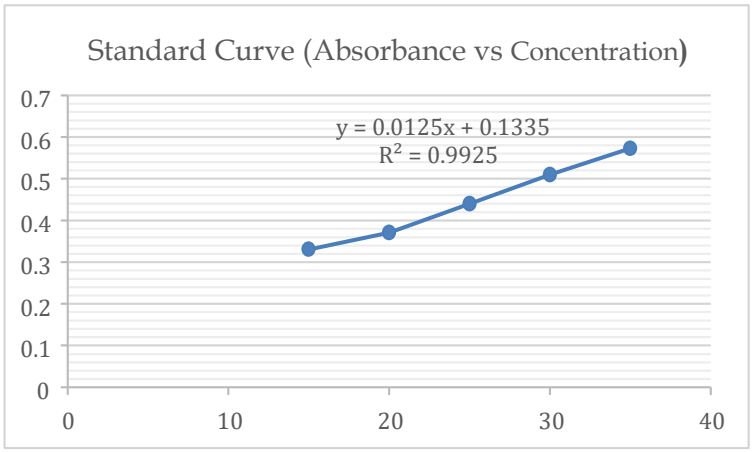

Figure 1. Quercetin Standard Solution Series Raw Curve at 706 nm Wavelength

The absorbance value of the standard quercetin solution obtained from each concentration is then made into a standard curve, so that the linear equation y = 0.0125x + 0.1335 is obtained with the value of the correlation coefficient (r) = 0.9925. The value (r) close to 1 indicates that the regression equation is linear, suggesting that absorbance and concentration have a very strong correlation. This standard curve is used to determine the relationship between solution concentration and absorbance. The regression equation for the relationship between concentration (x) and absorbance (y) of standard quercetin was then used to calculate the antioxidant activity of ethanol extracts from bitter melon seeds and bitter melon fruit using the FRAP method. Extraction measurements were carried out in 3 replications, as shown in Table 3.

Table 3. Results of Measurement of Antioxidant Activity of Ethanol Extract of Bitter Melon (*Momordica Charantia* L.) using the FRAP method

Sample	Replication	Sample Weight (gram)	Absorbance Sample (y)	Antioxidant Activity (mgQE/g extract)	Average Antioxidant Activity (mgQE/g extract)
Bitter Melons Seeds	1	0.01	0.246	9	8.680 ± 1.076
	2	0.01	0.253	9.560	
	3	0.01	0.227	7.480	
Bitter Melon Fruits	1	0.01	0.212	6.280	8.626 ± 2.284
	2	0.01	0.243	8.760	
	3	0.01	0.269	10.840	

The reagents used in this study include 0.2 M phosphate (pH 6.6), which maintains solution pH, as this complex is known to be stable in acidic conditions. Acidic conditions in the FRAP test can generally reduce the ability of antioxidant compounds to be reduced, due to protonation. Potassium ferricyanide 1% is used as an oxidizer that reacts with the reducing sample so that the Fe^{3+} ions of potassium ferricyanide are reduced to Fe^{2+}. The addition of 10% trichloroacetate

precipitates the potassium complex from potassium ferricyanide, while 0.1% $FeCl_3$ is added to form a green-to-blue complex (Berlin blue).

In addition to adding reagents, several treatments are performed: the solution is vortexed for 5 minutes to obtain a homogeneous solution, then incubated at 50 °C for 20 minutes to ensure optimal reaction with the reagent. Next, the solution is centrifuged at 3000 rpm for 10 minutes to obtain a supernatant. The supernatant is then reincubated for 5 minutes before taking measurements, to ensure the solution reacts fully with the reagent.

The antioxidant activity of the ethanol extracts from bitter melon seeds and fruits (*Momordica charantia*) was evaluated using the FRAP method, with triplicate determinations (n = 3) to ensure data reliability and reproducibility. The mean antioxidant activity of the seed extract was 8.680 mg QE/g extract, indicating that each gram of extract contains 8.680 mg quercetin equivalent, while the fruit extract demonstrated a mean value of 8.626 mg QE/g extract. All results were expressed as mean ± standard deviation (SD). Statistical analysis was performed using an independent-samples t-test with a significance level of $p < 0.05$. The analysis revealed no statistically significant difference in antioxidant activity between the seed and fruit extracts ($p > 0.05$), indicating that both extracts exhibit comparable antioxidant capacity as measured by the FRAP assay.

Research on antioxidant activity in seeds and fruits using the FRAP method was also studied by Wardani et al. (2025), but with different samples. This study uses methanol solvents. The results show that the seeds have higher antioxidant activity than the fruit. This difference can be caused by the solvent used: methanol has a higher dielectric constant (33.640) than ethanol (25.16). The higher the dielectric constant value of a solvent, the more polar the solvent. Thus, the methanol solvent is more polar than the ethanol solvent and can attract more flavonoid compounds according to the like dissolves like principle.

CONCLUSIONS

Based on the results of the study, the antioxidant activity of the ethanol extracts of bitter melon seeds and bitter melon fruits showed almost identical average values, namely 8.680 mgQE/g and 8.626 mgQE/g, respectively. It can be concluded that there is no significant difference in antioxidant activity between bitter melon seed extract and bitter melon fruit, indicating comparable antioxidant capacity. These results highlight that the seeds and fruits of Momordica. Bitter melon can serve as a promising natural source of antioxidants for functional and pharmaceutical food applications.

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