Journal of Experimental and Clinical Pharmacy (JECP)
Doi: 10.52365/jecp.v6i1.1629
http://jurnal.poltekkesgorontalo.ac.id/index.php/JECP/
2026, 6(1), 52-61

Research Article

Flavonoid Quantification in Fruits and Seeds of *Momordica charantia* L. by UV-Vis Spectrophotometry

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ARTICLE INFO

Received : 12.Dec.2025
Revised : 03.May.2026
Accepted : 05.May.2026

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ABSTRACT

Momordica charantia L. (bitter melon) is a medicinal plant widely recognized for its bioactive compounds, particularly flavonoids, which exhibit potent antioxidant properties. This study aimed to quantify the total flavonoid content in the fruits and seeds of *M. charantia* using UV-Vis spectrophotometry and quercetin as a standard compound. Samples were extracted with 70% ethanol and analyzed by the aluminum chloride–potassium acetate colorimetric method at the specific absorption wavelength, using quercetin as the standard. The results demonstrated that the fruit's average flavonoid content was 2.261 mg QE/g extract, whereas the seeds contained 2.906 mg QE/g extract. These findings indicate that the seeds possess the same flavonoid concentration as the fruit, highlighting their potential as a valuable natural source of antioxidant compounds.

Keywords: *Momordica charantia* L.; fruit and seed; flavonoid content; UV-Vis spectrophotometry

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eISSN 2775-1368

INTRODUCTION

Bitter melon (*Momordica charantia* L.) is a fruit vegetable crop that is widely cultivated by farmers because of its relatively stable price, easy cultivation methods, and fairly high market demand. This aligns with the growing public interest in vegetable consumption and healthy lifestyles. The *M. charantia* can be processed to meet the nutritional and fiber needs in food, and it can be used as a traditional medicine to treat a wide variety of diseases, such as cardiovascular diseases and diabetes mellitus (Wadhani 2021).

M. charantia has been extensively investigated for its broad spectrum of pharmacological activities, encompassing antioxidant and anti-inflammatory, antimicrobial (antibacterial and antifungal), neuroprotective (anti-dementia), anticancer, and hypocholesterolemic effects (Bortolotti et al. 2019; Joshi et al. 2017; Bai, Ying Zhu, and Dong 2016; Saeed et al. 2018). Mechanistically, these bioactivities are attributed to its rich phytochemical composition, including an insulin-mimetic compound, commonly termed "plant insulin," which has been shown to modulate glucose homeostasis by reducing both blood and urinary glucose levels (Janagal et al. 2018). Furthermore, comprehensive phytochemical investigations have revealed that all parts of the plant, particularly the fruits and seeds, contain over 60 bioactive phytoconstituents with therapeutic potential against more than 30 disease conditions, notably including diabetes mellitus and cancer (Chittaranjan Kole, Matsumura, and Behera 2020).

Metabolites in *M. charantia* are broadly classified into primary and secondary groups. Primary metabolites, such as sugars, proteins, and chlorophyll, mainly contribute to its nutritional value. In contrast, secondary metabolites—including phenolics, carotenoids, cucurbitane-type triterpenoids, alkaloids, and saponins—are primarily responsible for its pharmacological activities. These compounds have been reported to exhibit a wide range of biological effects, including antioxidant, antidiabetic, anti-inflammatory, and antimicrobial activities, thereby contributing to the therapeutic potential of *M. charantia* despite its limited role in basic nutrition (Daniel, Supe, and M.G.Roymon. 2014). Furthermore, approximately 228 compounds have been identified from different parts of *M. charantia*, indicating its extensive phytochemical diversity and pharmacological relevance (Nagarani, Abirami, and Siddhuraju 2014)

M. charantia seeds contain polyphenol compounds, namely flavonoids, phenolic acids, tannins, and lignin, which have antioxidant properties. Phenolic compounds contained in *M. charantia* seeds, fruits, and leaves also act as anti-inflammatories and antioxidants. *M. charantia* seeds are high in flavonoids (Rosnah and Haryoto 2024).

The fruit contains flavonoids, particularly rutin and quercetin derivatives, as important bioactive constituents that contribute to its antioxidant properties and enhance its therapeutic potential. In addition, phenolic compounds such as gallic

acid, catechin, and chlorogenic acid, triterpene glycosides—especially momordicosides A–K—and alkaloids including momordicine I and II have also been identified in various fruit extracts (Tan et al. 2008; George et al. 2025). Furthermore, other key constituents such as charantin, polypeptide-p, and vicine, have been reported to play roles in the regulation of glucose homeostasis through multiple molecular mechanisms.

The fruit of *M. charantia* is widely consumed as a culinary vegetable, whereas its seeds are largely discarded as agro-waste; however, emerging phytochemical evidence indicates that both the fruit and the seeds possess comparable spectra of bioactive constituents, particularly flavonoids, phenolic compounds, and other secondary metabolites with significant biological potential.

Studies comparing the flavonoid content between the fruit and seeds of *M. charantia* remain limited; therefore, the present study aims to quantitatively evaluate and compare the flavonoid levels in both plant parts to elucidate their relative phytochemical potential. Accordingly, the findings of this study are expected to yield a robust baseline dataset that supports the valorization of both the fruit and seed matrices of *M. charantia*, thereby contributing to sustainable development and the expanded utilization of underexplored plant parts as potential sources of bioactive compounds.

RESEARCH METHOD

Instruments

The instruments used in this study were micropipette (DragonLab), UV–Vis spectrophotometer (Genesys 10S), analytical balance (ACIS), ultrasonic-assisted extraction (UAE) system (Elmasonic S), and rotary vacuum evaporator (Heidolph).

Material

Bitter melon (*Momordica charantia* L.), was obtained in Pinrang, aquadest (OneMed), aluminium klorida (Merck KGaA), kalium asetat (Merck KGaA), quersetin (Sigma-Aldrich), etanol 70% (OneMed), metanol pa (Merck)

Procedure

Sample Preparation

Sample Processing

Samples of *M. charantia* that have been taken Sampling is carried out by taking fruit that is young and fresh and not rotten or damaged, washed, cleaned with running water, split the fruit, then separate the pulp and seeds, then the seeds are cleaned of mucus, cut into small pieces, then dried using an oven with a temperature of 50°C about 2 days.

Extraction

Each sample of *M. charantia* was carefully weighed at 6 kg, mixed, and the powder was placed in a 500 ml beaker, then 70% ethanol solvent was added, and the beaker was covered with aluminum foil to prevent evaporation during the extraction process. Place the beaker into the shaking water bath (Ultrasonic-Assisted Extraction) set the temperature to 30°C (do not overheat so that the compound is not damaged) for 60 minutes. This method has the advantage of providing optimal extraction results, so it can save time and solvent. Another advantage of ultrasonic extraction-assisted stirring is that the surface contact between the solid and the liquid is wider and more optimal, due to direct contact between the particles and ultrasonic waves (Buanasari et al. 2019). After completion, the solution was filtered using filter paper to separate the filtrate from the dregs. The filtrate, containing the solvent, was evaporated using a rotary evaporator at 60°C and 60 rpm until a thick extract was obtained. After which it is stored in a desiccator (Yudhantara, Christina, and Hastuti 2024)

Preparation of Reagent Solutions

Preparation of 10% $AlCl_3$ Solution

Weigh 1 g of aluminum chloride ($AlCl_3$) powder and dissolve it in distilled water, then make up the volume to 10 mL in a volumetric flask.

Preparation of Potassium Acetate Solution

Weigh 0.98 g of potassium acetate and dissolve it in distilled water, then make up the volume to 10 mL in a volumetric flask

Preparation of Quercetin Standard Solution

A 250 ppm stock solution was prepared by accurately weighing 2.5 mg of quercetin, dissolving it in methanol p.a., and adjusting the volume to 10 mL. From this stock solution, a series of standard quercetin solutions at 6, 7, 8, 9, and 10 ppm was prepared by appropriate dilution. Subsequently, 1 mL of 10% $AlCl_3$ solution was added to each standard solution, and the mixture was vortex-mixed to ensure homogeneity. After that, 1 mL of potassium acetate solution was added and the mixture was incubated for color development prior to spectrophotometric analysis.

Determination of Maximum Wavelength

A concentration of the standard solution is taken, and its absorbance is measured over 400-800 nm. The wavelength with the highest absorption value is the maximum wavelength.

Preparation of Quercetin Calibration Curve

The calibration curve was obtained by correlating the concentrations of quercetin standard solutions with their corresponding absorbance values measured at the maximum wavelength (λmax) using an ultraviolet–visible (UV–Vis) spectrophotometer. A linear relationship between concentration and absorbance was used to derive a regression equation for quantification.

Determination of Total Flavonoid Levels

The first, 50 mg of *M. charantia* fruit extract sample and 20 mg of *M. charantia* seeds were weighed, 3 replications were made for each sample of *M. charantia* fruit and seed extract, dissolved with methanol p.a then vortexed until mixed, added 0.2 ml of 10% $AICI_3$ then vortexed to make it homogeneous and added 0.2 ml of potassium acetate then vortexed, after homogenization, it was added with methanol p.a to the mark and then incubated for 30 minutes. The treatment was left for 30 minutes before measurement to ensure the reaction ran smoothly, thereby providing maximum color intensity (Budianta, Widyawati, and Haditanojo 2019). Then the absorbance was measured using an ultraviolet-visible (UV-VIS) spectrophotometer at the maximum wavelength.

RESULTS AND DISCUSSION

M. charantia is a horticultural plant with a unique, bitter taste. This plant offers various benefits for body health and can serve as an alternative ingredient in herbal medicine. Chemical compounds contained in *M. charantia* that are beneficial for the body include antioxidants, glycosides, momordicin, and charantin. These compounds can be useful as anticancer, anti-inflammatory, antimicrobial, and antimalarial agents. The antioxidants found in *M. charantia* fruit have the potential to be an antidote to free radicals (Armadianty et al. 2025).

In this study, *M. charantia* was obtained in Pinrang Regency, South Sulawesi. The *M. charantia* fruit was cut into small pieces before extraction. Then the *M. charantia* fruit and seeds were processed into powdered simplicia for further extraction using UAE (Ultrasonic-Assisted Extraction) with 70% ethanol solvent, which was then evaporated in a rotary evaporator to obtain a thick extract of the *M. charantia* fruit and seeds. Ethanol was chosen because it is polar, making it suitable for extraction with polar solvents. From the extraction results, a thick, dark-green extract was obtained. This procedure aims to increase the sample's surface area, enabling more efficient extraction. The results of the extraction of *M. charantia* using UAE (Ultrasonic-Assisted Extraction), weighing 75 grams of *M. charantia* seeds with 70% ethanol solvent produced 14.44 grams of ethanol extract with a percentage of 19.20 % and 224 grams with 70% ethanol solvent produced 72.12 grams of ethanol extract with a percentage of 32.196%.

Table 1. Extraction yield and percentage immersion of ethanol extract of *M. charantia*

Sample	Sample Weight (gram)	Extract Results (gram)	Extract Rendamen (%)
M. charantia seeds	75	14.44	19.200
M. charantia fruits	224	72.12	32.196

Table 2. Results of quercetin standard solution absorbance measurement

Concentration (ppm)	Absorbance Value
6	0.278
7	0.368
8	0.462
9	0.574
10	0.688

After obtaining the results of the absorbance measurement of the quercetin standard solution in Table 3, where the results in this study, the absorbance of each concentration series is appropriate and meets the good absorbance range, also known as the Lambert-Beer law, namely $0.2 \leq A < 0.8$. Then the results are depicted in a standard curve graph of the quercetin standard solution. The maximum wavelength of the standard quercetin solution is determined by scanning from 400 to 800 nm. The measurement of the maximum wavelength aims to determine the absorption area by comparing the absorbance of the standard comparison solution, which is measured using a UV-Vis spectrophotometer in the wavelength range of 400-800 nm (Sukmawati Sukmawati 2018). The results of the running show that the maximum wavelength of the quercetin solution is at a wavelength of 428 nm. The color produced from the quercetin standard solution is yellow. The higher the concentration, the more intense the yellow color.

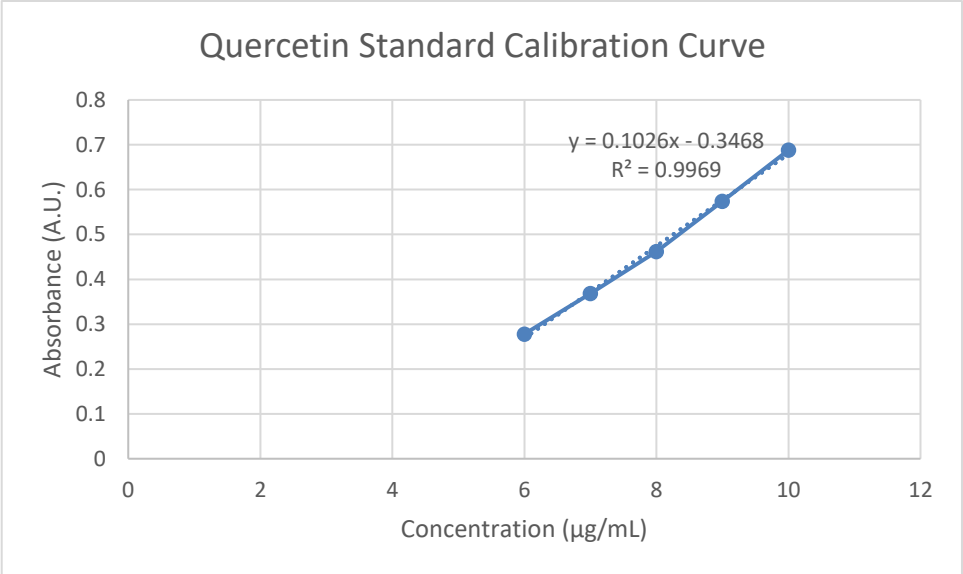

Figure 1. Calibration curve of quercetin standard for the quantification of total flavonoid content using the $AlCl_3$ colorimetric method

Quercetin is commonly used as a reference standard in total flavonoid content (TFC) assays because it is one of the most abundant and widely distributed flavonols in the plant kingdom, occurring in a broad range of fruits, vegetables, and medicinal plants. Its

chemical structure, comprising multiple hydroxyl groups and a conjugated aromatic system, facilitates stable complex formation with aluminum chloride, yielding a strong, reproducible absorbance signal suitable for spectrophotometric quantification. The quercetin standard curve yielded the equation y = 0.1026x – 0.3468, which was used to determine the flavonoid content of *M. charantia* fruit and seeds. The results were then graphed to obtain a correlation coefficient (r) of 0.9969, indicating good linearity and an acceptable value of ≥ 0.995. After measuring the quercetin standard, measurements were performed on *M. charantia* fruit and seeds samples three times. The absorbance measurements were then substituted into the regression line equation of the quercetin standard curve to determine the flavonoid content of *M. charantia* fruit and seeds, as shown in Table 3.

Table 3. Determination of flavonoid levels of *M. charantia* fruit and seeds

Sample	Replication	Absorb	Flavonoid Content (mg QE/g extract)	Average flavonoid content (mg QE/g extract)
M. charantia seeds	1	0.836	2.305	2.261
	2	0.802	2.239	
	3	0.803	2.241	
M. charantia fruits	1	0.249	2.903	2.906
	2	0.258	2.947	
	3	0.242	2.869	

Based on the Table 3, the flavonoid content of *M. charantia* fruit was 2.261 mg QE/g extract, while the flavonoid content of *M. charantia* seeds was 2.906 mg QE/g extract. The flavonoid content of *M. charantia* fruit was lower than that of the seeds.

From the test results, it can be concluded that *M. charantia* seeds have a higher flavonoid content than the fruit. This may be due to differences in the plant parts used, with seeds containing more secondary metabolites. This aligns with Hawari, Pujiasmanto, and Triharyanto (2022), which found that flavonoid content can vary across plant species, organs, growth stage, and development. It also varies depending on altitude. Another study Mahmud, Maryam, and Suhaenah (2024), found that flavonoid levels vary across plants. Both internal and external factors influence this. Internal factors include genes, while external factors include light, temperature, humidity, pH, soil nutrient content, and altitude. Temperature differences at different altitudes alter metabolic processes in plants, leading to distinct secondary metabolites.

CONCLUSIONS

Based on the results, the flavonoid content in the *M. charantia* fruit sample is 2.26 mg QE/g extract, while in *M. charantia* seeds it is 2.906 mg QE/g extract. These findings indicate that the seed and fruit have the same flavonoid content, highlighting their potential as a valuable natural source of antioxidant compounds.

ACKNOWLEDGMENTS

This research was funded by the Ministry of Higher Education, Science, and Technology (Kemdiktisaintek), Republic of Indonesia, supported by the Research and Community Service Institute (LP2S), Universitas Muslim Indonesia, and the Faculty of Pharmacy, Universitas Muslim Indonesia (UMI).

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