Pharmacogn. Res., 2024; 16(1):26-33.
https://www.phcogres.com

Original Article

The Potential of Bitter Melon Residues Concerning its Physico-chemical Characterization, Bioactive Compounds, and Antioxidant Effects

Julianna Karla Santana Andrade[1], Romy Gleyse Chagas Barros[1], Juliete Pedreira Nogueira[1], Christean Santos de Oliveira[1], George Ricardo Santana Andrade[2], Silvânio Silvério Lopes da Costa[3], Narendra Narain[1], Murugan Rajan[4,*]

[1]Laboratory of Flavor and Chromatographic Analysis, PROCTA, Federal University of Sergipe, São Cristóvão – SE, BRAZIL.
[2]Postgraduate Program in Energy, Federal University of Espírito Santo, Rod. Gov. Mário Covas, BR-101 Litorâneo, São Mateus - ES, 29932-540, BRAZIL.
[3]Laboratory of Technology and Environmental Monitoring, Oil and Gas Competence Center, Federal University of Sergipe, São Cristóvão–SE, BRAZIL.
[4]Centre for Research and Postgraduate Studies in Botany, Ayya Nadar Janaki Ammal College (Autonomous), Sivakasi, Tamil Nadu, INDIA.

ABSTRACT

Background: Bioactive compounds from vegetables have played a substantial role as therapeutic agents in drug discovery since it contains nutraceutical and nutraceutical properties. Cucurbitaceae family encompasses several edible plants that contain valuable natural compounds. **Objectives:** To evaluate the physicochemical and mineral composition on *Momordica charantia* fruit residues. **Materials and Methods:** The physiochemical composition and mineral composition were characterized by FTIR and optical emission spectrophotometer, respectively. The phenolics, flavonoids and total antioxidant activity were employed by spectrophotometrically. The green ultrasound assisted extraction was used for aqueous and 12% ethanolic extraction for evaluation of phenolic compounds by UPLC/MS. **Results:** The results revealed the minerals such as K, P, Ca, Na, Mg, Fe, Zn, Sr, Cu and Mn found in bitter melon. High contents of total phenolics content (683.820 to 1753.345 galic acid: quercetin (2:1)/100 g of sample) and total flavonoids (106.206 to 311.423 mg of quercetin/100 g of sample) were observed in peel aqueous extract. The aqueous extract of peel exhibited maximum inhibitory potential against DPPH radical (93% of inhibition) than ethanolic extract. This aqueous extract of peel also contains naringenin, catechin, epicatechin, *p*-coumaric, ferulic and chlorogenic acids which were reveled from UPLC/MS analysis. **Conclusion:** This study suggests that *M. charantia* residues are good sources of functional proteins, lipids, fibers and minerals, resulting in greater nutritional value. In addition, *M. charantia* is of great interest in its promising benefits for human health, due to its antioxidant capacity and bioactive compounds.

Keywords: Antioxidant activity, Green extraction, Minerals, *Momordica charantia*, Phenolic compounds, UPLC/MS.

Correspondence:
Dr. Murugan Rajan
Centre for Research and Postgraduate Studies in Botany, Ayya Nadar Janaki Ammal College (Autonomous), Sivakasi, Tamil Nadu, INDIA.
Email: murugan.rajanufs@gmail.com

Received: 20-08-2023;
Revised: 12-09-2023;
Accepted: 05-10-2023.

INTRODUCTION

Bitter melon (*M. charantia* L.) is traditionally used as a medicinal plant and consumed daily as food. The fruits of *M. charantia* are distributed worldwide including in Turkey, Brazil, Africa, China, India, Malaysia, Australia, Thailand, and USA and showed superior antioxidant potential.[1] *M. charantia* fruit is rich in minerals including iron, zinc, phosphorus, magnesium, calcium, and potassium.[2] Studies have determined that the *M. charantia* plant and its parts contain a great diversity of phytoconstituents with therapeutic properties such as being anti-microbial, larvicidal, anti-diabetic, anti-malarial, hypo-cholesterolemic, anti-tumor, anti-viral, anti-genotoxic, anti-helminthic and wound healing.[3-5] Phytochemical analysis of *M. charantia* revealed the presence of saponins, flavonoids, terpenoids, coumarins, emodins, and alkaloids.[6]

Conventional techniques of phenolic extraction are generally associated with high consumption of organic solvent and long extraction times. With increasing environmental pollution caused by the larger use of volatile and harmful organic solvents, recent techniques on extraction focusing on minimum usage of solvents or use alternative bio-rational solvents. Water can be considered as a potentially green solvent since it is not toxic and

DOI: 10.5530/pres.16.1.4

it´s environment friendly.[7] A sustainable alternate technique that requires a moderate use of solvent and energy is Ultrasonic Assisted Extraction, which is most common and reproducible at room temperature. This technique is working on the basis of acoustic cavitation, and it has the capability of breaking the plant cell wall matrix and thus releasing the bioactive compounds.[8]

The phytochemical profiles of seed+pulp and peel of the *M. charantia* are limited in phytochemical aspects. Thus, the current research was undertaken to study the physicochemical parameters and chemical composition of seed+pulp and peel of *M. charantia* and the application of an ultrasound-assisted extraction technique for phenolic compounds, along with the identification and quantification of bioactive compounds. Furthermore, the best solvent conditions for extraction of phenolics/antioxidant compounds from seed+pulp and peel were established.

MATERIALS AND METHODS

Sample preparation

Ripe mature bitter melon fruit was collected in São Cristóvão, Sergipe, Brazil, at 10°55'29.6"S, 37°13'07.9"W. The peel was separated from the seed while the seed and pulp remained together. The samples of bitter melon fruit residues were dried in an oven (Marconi, Model - MA 035-5, Piracicaba, SP, Brazil) at 40°C and ground using a mill (Basic IKA A11, IKA, Campinas, SP, Brazil). The particle sizes of powdered samples were measured and it was about 2 mm. Later, the powdered bitter melon fruit residues were packed in an amber glass vial and stored in a glass desiccator at 29±2°C.

Determination of physicochemical composition of fruit

The pH, soluble solids, titratable acidity, moisture, water activity, ash, protein, lipid, fiber contents, and reducing/ non-reducing sugars were determined according to established methodology.[9]

Estimation of minerals composition

The minerals such as Ba, Ca, Cd, Cr, Cu, Fe, K, Mg, Mn, Mo, Na, Ni, P, S, Se, Sr, V and Zn were estimated by using optical emission spectroscopy (Varian Vista-Pro, Australia) according to the methodology of Correia *et al.*[10]

FTIR and UV/vis spectrometry analysis

The FTIR and UV/vis spectrometry of solid samples and solvent (aqueous and ethanol) extracts of bitter melon fruit residues were measured based on the method prescribed by Andrade *et al.*[11]

Determination of condensed and hydrolyzable tannins contents

Condensed and hydrolyzable tannins contents in the bitter melon fruit residues were measured using a spectrophotometer (SpectraMax M2, Molecular Devices, Sunnyvale, CA) at 500 nm for condensed tannins and 550 nm for hydrolyzable tannin.[12]

Preparation of crude extracts

The crude extracts from bitter melon fruit residues were extracted using aqueous and ethanol (12% v/v) solvents by ultrasonic bath with a frequency of 40 KHz. About 1.5 g samples of peel and seed+pulp was extracted with 15 mL of distilled water at 60°C (aqueous extraction) and ethanol: water (12:88 v/v) (ethanolic extraction) in an ultrasound apparatus (UNIQUE, USC-1400A, Brazil) for 60 min. Then, the extracts were centrifuged (Eppendorf, Centrifuge 5810R, Hamburg, Germany) at 17418*g* for 10 min. Later, the supernatants were collected in a glass vial and stored at 18°C. For the quantitation of phenolic compounds using UPLC/MS, the extracts were filtered through 0.2 µm cellulose filters (Millipore, Bedford, MA).

Determination of total phenolics and flavonoids content

The total phenolics and total flavonoid contents in the Bitter melon fruit residues were determined by the methodology prescribed by Andrade *et al.*,[11] with slight modification. The absorbance of samples was measured using a spectrophotometer at 760 nm for the total phenolics. While, absorbance of the samples was measured at 415 nm for the total flavonoid contents.

Determination of antioxidant activity by DPPH assay

The DPPH radical scavenging activity of bitter melon fruit residues were employed according to Andrade *et al.*[11] method. The decrease in absorbance by plant extracts/antioxidants were monitored every 5 min for 120 min at 515 nm using a spectrophotometer.

Quantification of phenolic and flavonoid compounds using UPLC-MS system

Individual phenolic and flavonoid compounds present in the bitter melon fruit residues were identified and quantified by UPLC-MS analysis which were performed according to Andrade *et al.*[11] method with some modifications. An Ultra Performance Liquid Chromatography (UPLC) Acquity Class H ((Waters, Milford, Massachusetts, USA) was used coupled with a PDA detector and simple quadrupole (QDa) mass spectrometer, equipped with electrospray ionization in negative mode and Selected Ion Monitoring (SIM) provisions. The column used was Ascentis Phenyl (15 cm × 4.6 mm, 5 µm; Supelco analytical) operating at a flow rate of 0.35 mL/min and temperature of 40°C. The mobile phases of ultrapure water+0.1% formic acid (Phase A) and acetonitrile+0.1% formic acid (Phase B) was used. The injection volume for standards and samples were 5 µL. Elution was in the gradient mode, according to the following events: 0-15 min, 100% A; 15-25 min, 75% A; 25-35 min, 60% A; 35-45 min, 50% A; 45-55, 30% A; 55-60 min, 0% A. A standard calibration

curve was prepared, covering the concentration range (0.02 to 1 mg/mL). The data on analytical parameters of standard phenolics and flavonoids are presented in Table S1.

Statistical analysis

The mean values comparison was evaluated using the Tukey test by Statistical Analysis System (SAS) software (ver. 9.0, SAS Institute, Cary, NC). The Pearson correlation coefficient was performed by SPSS software, version 25.0 (IBM SPSS, IBM Corp., Armonk, New York).

RESULTS

Physicochemical and mineral composition

physicochemical parameters such as water activity, °Brix, and titratable acidity values were shown in Figure 1a. The values of water activity and pH were not statistically different between the *M. charantia* residues. However, the Brix and titrable acidity values were higher in *M. charantia* peel compared to that of seed+pulp. The reducing and non-reducing sugar contents were higher in peel compared to seed+pulp (Figure 1b). Similarly, moisture and ash contents also showed higher values in the peel compared to seed+pulp. The protein, fiber, and lipid contents varied among the samples, and the highest concentrations were observed in seed+pulp samples (Figure 1c).

The main minerals such as K, P, Ca, Na, Fe, Mg, Zn, Sr, Cu and Mn were found in the peel and seed+pulp (Table 1). The Fe content was maximum in both peel and seed+pulp compared to other minerals. The contents of K, Ca, Na, and Sr were higher in peel compared to that in seed+pulp. The minerals P and Mg had higher contents in seed+pulp which also contained Fe, Zn, Mn, and Cu.

FTIR and UV/vis absorption spectra of Momordica charantia

The FTIR and UV-vis absorption spectra of bitter melon fruit residues were differed in the peel and seed+pulp, indicating the tissues have different functional groups and characteristics (Figure 1S). UV-vis region in absorption spectra of the aqueous and ethanolic extracts of the peel and seed+pulp presented bands in the range of 200-600 nm (Figure 1S).

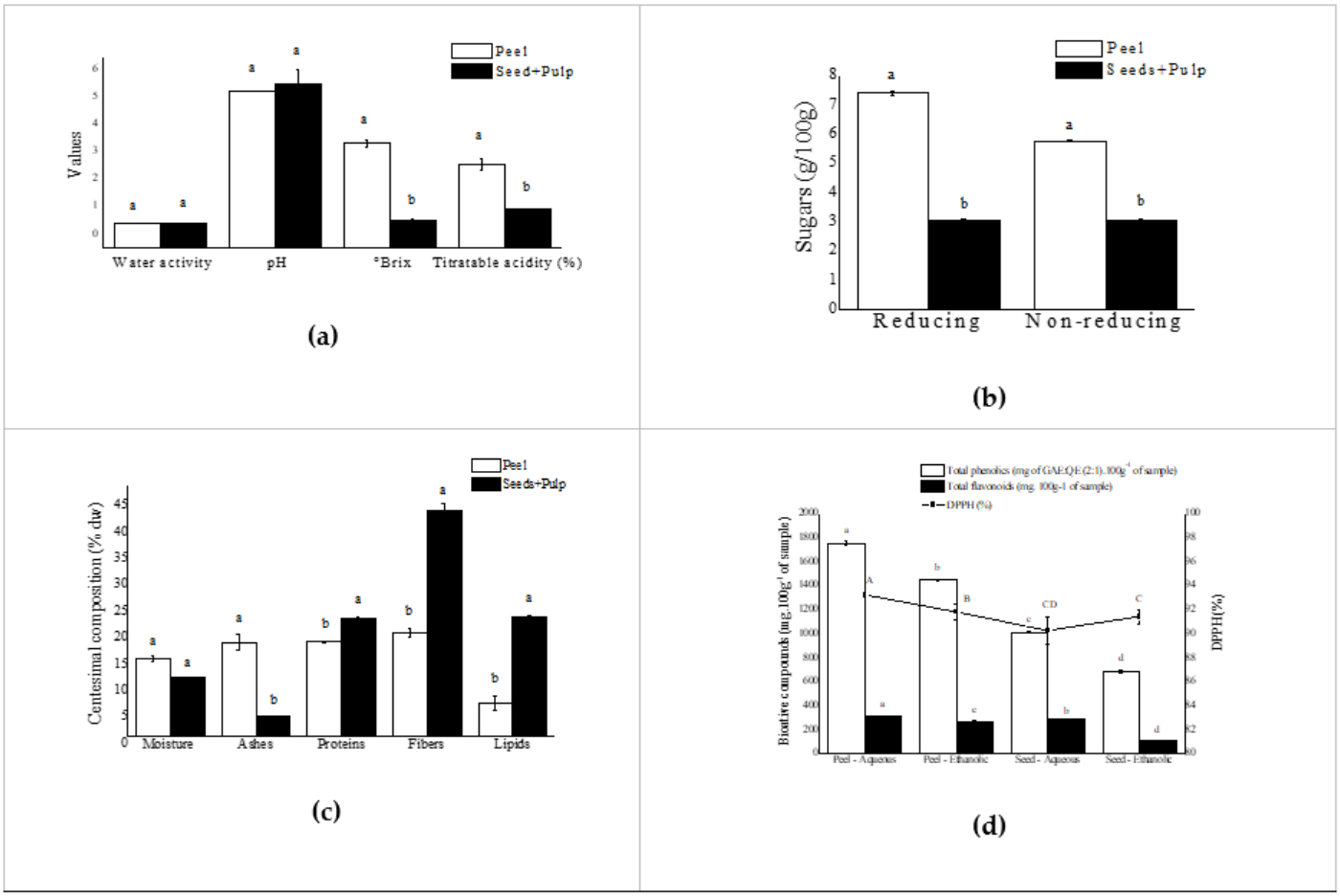

Figure 1: Physico-chemical composition (a), sugars contents (b), centesimal composition (c), total phenolics and flavonoids contents as well as antioxidant activity (d) of peel and seed+pulp of *M. charantia* residues. Values expressed as mean±standard deviation; *n*=3. Letters associated with columns for the same analysis do not differ statistically, $p<0.05$, Tukey test.

Table 1: Minerals composition of peel and seed+pulp of *M. charantia*.

Minerals	Peel	Seed+pulp	LD (µg/g)	LQ (µg/g)
Ba (µg/g)	2.15±0.030[a]	0.68±0.056[b]	0.01	0.018
Ca %	0.45±0.0046[a]	0.07±0.0003[b]	0.03	0.11
Cd (µg/g)	< 0.001[a]	< 0.001[a]	0.001	0.0020
Cr (µg/g)	< 0.04[a]	< 0.04[a]	0.04	0.13
Cu (µg/g)	6.43±0.084[b]	9.16±0.085[a]	0.01	0.019
Fe (µg/g)	50.9±1.61[b]	59.7±0.39[a]	0.04	0.13
K %	8.36±0.15[a]	0.95±0.017[b]	5.9	19.3
Mg %	0.16±0.0019[b]	0.23±0.0041[a]	0.002	0.0067
Mn (µg/g)	6.28±0.084[b]	10.1±0.13[a]	0.003	0.010
Mo (µg/g)	1.61±0.076[a]	1.55±0.18[a]	0.006	0.019
Na (µg/g)	437±37.5[a]	66.8±29.5[b]	6.2	20.4
Ni (µg/g)	0.70±0.20[a]	0.29±0.19[b]	0.08	0.27
P %	0.30±0.0064[b]	0.50±0.016[a]	0.1	0.43
S (µg/g)	0.21±0.0023[b]	0.26±0.0074[a]	3.1	10.1
Se (µg/g)	< 0.4[b]	2.14±0.52[a]	0.4	1.2
Sr (µg/g)	15.2±0.37[a]	1.60±0.10[b]	0.004	0.012
V (µg/g)	0.055±0.0045[a]	0.018±0.0045[b]	0.00004	0.00013
Zn (µg/g)	35.1±0.29[b]	43.7±0.99[a]	0.001	0.0027

Means followed by same letters ([a > b]) in the same row do not differ statistically at $p<0.05$, Tukey test. Values expressed as mean±standard deviation ($n=3$); LD = Limit of detection; LQ = Limit of quantification.

Bioactive compounds and antioxidant activity

Condensed tannins content was found lower in the peel and not found in the seed+pulp sample (Figure 2S). Hydrolyzed tannins content was higher in seed+pulp than in the peel with differences between the 2 materials. Total phenolic content varied in samples as did total flavonoid content (Figure 1d) which were different between samples. The aqueous extraction obtained better results for the analysis. The percent inhibition of the DPPH radical varied among the samples and highest percentage was observed in the aqueous extract of the peel.

Identification and quantification of phenolic compounds

From the UPLC-MS analysis of bitter melon fruit residues, eighteen phenolic compounds were identified, of which naringenin, epicatechin, ferulic acid, and kaempferol were in higher concentrations in both peel and seed+pulp (Table 2). Among them, naringenin is the most common compound in the ethanolic and aqueous extractions of peels and seed+pulp. The catechin and chlorogenic acid compounds were only in seed+pulp, with no difference between the 2 extractions. Moreover, the compounds of *p*-coumaric acid, vanillic acid and gallic acid were detected only in the peel.

DISCUSSION

Physicochemical and mineral composition

The results of physicochemical parameters of water activity, pH, total soluble solids, titrable acidity, moisture, ash, proteins, fibers, lipids, reducing, and non-reducing sugar contents were found in the peel and seed+pulp which were similar to earlier studies. Hamissou *et al.*[13] reported that the protein value of *M. charantia* fruit is 4.11 mg/g fresh weight. The water content in the fruit residues of bitter melon is about 93.2%, while protein and lipids contents ranged between 18.02 and 0.76%.[14] Moreover, Horax *et al.*[15] reported that oven-dried mature bitter melon pericarp showed a moisture content of 14.3%, while seeds obtained 7.73%. The *M. charantia* fruit peel contained moisture (95.30%), ash (3.07%), protein (0.89%), fiber (1.08%), total soluble solids (4.50 ºBrix), titrable acidity (0.19 mg citric acid/100 g), pH (5.67), and presence of sugars such as fructose, glucose, sucrose, raffinose, estequiose.[16]

M. charantia peel and seed+pulp were enriched with minerals like K, Ca, Zn, Mg, P, Se, and Fe. Likewise, bitter melon residues are rich in micronutrients such as Ca, Fe, P, Mn, K, Mg, and Zn reported by Mukherjee and Karati,[17] Horax *et al.*[15] reported the mineral contents (K, Ca, Zn, Mg, P, Fe, S, Na, Mn, and Cu) of pericarp and seed of bitter melon, of which, K, P, and Mg were the major minerals with values of the concentrations of K (42.7 and 8.6 mg/g for peel and seed, respectively) and S (1.1 and 2 mg/g

Table 2: Identification and quantification of phenolic and flavonoid compounds in peel and seed+pulp extracts of *M. charantia* by LC-MS system.

Compounds	Contents (µg/g of sample)			
	Peel		Seed+pulp	
	Aqueous extract	Ethanolic extract	Aqueous extract	Ethanolic extract
Acacetin	tr	tr	tr	tr
Apigenin	tr	tr	tr	tr
Biochanin A	tr	tr	tr	tr
Caffeic acid	nd	nd	nd	nd
Catechin	nd	nd	10.51 ± 0.08^{bA}	10.85 ± 0.25^{bA}
Chlorogenic acid	nd	nd	14.58 ± 0.12^{bA}	14.14 ± 0.06^{bA}
Chrysin	tr	tr	tr	tr
p-Coumaric acid	37.27 ± 0.35^{bA}	32.64 ± 0.20^{bA}	nd	nd
Daidzein	tr	tr	tr	tr
Epicatechin	14.43 ± 0.30^{bA}	13.41 ± 0.04^{bA}	10.20 ± 0.13^{bA}	10.07 ± 0.21^{bA}
Ethyl gallate	tr	tr	tr	tr
Ferulic acid	29.58 ± 0.25^{bA}	29.55 ± 1.09^{bA}	30.52 ± 0.08^{bA}	28.90 ± 1.30^{bA}
Galic acid	tr	tr	nd	nd
Kaempferol	9.47 ± 0.10^{bA}	9.15 ± 0.04^{bA}	9.37 ± 0.08^{bA}	9.14 ± 0.00^{bA}
Luteolin	tr	tr	tr	tr
Naringenin	2302.92 ± 96.28^{aA}	1876.91 ± 1.36^{aB}	2306.38 ± 9.38^{aA}	2072.33 ± 88.33^{aB}
Protocatechuic acid	tr	tr	tr	tr
Rutin	tr	tr	tr	tr
Vanillic acid	nd	0.034±0.000	nd	nd
Vanillin	nd	nd	nd	nd

Values expressed as mean±standard deviation (n=3); tr = <Traces-Below limit of quantification (2 µg/mL); nd = Not detected. **Means followed by the same capital letters ($^{A>B}$) between aqueous and ethanolic extracts for each type of extract (peel or seed+pulp) did not differ statistically, at $p<0.05$, Tukey's test. ***Means followed by the same lowercase letters ($^{a>b}$) in the columns did not differ statistically in relation to the content of compounds in each extract, at $p<0.05$, Tukey's test.

for the peel and seeds, respectively) and the minerals of Ca (2.7 mg/g), Na (264 µg/g) and Fe (45 µg/g) were rich in peel.

Research studies highlighted that potassium element is involved in the regulation of function in the neurons and muscles. Earlier studies also reported that potassium, sodium, calcium, phosphorus, and magnesium are considered essential dietary minerals that should be supplied in the diet which regulate and maintain the metabolic functions.[18] Selenium is a mineral of fundamental importance for the human diet, making it antagonistic and resistant to oxidative stress related disorders including tumors, arthritis, brain and heart diseases. Trace minerals such as Se, Cu, Zn, and Mn alleviate inflammation and oxidative stress in the human body.[19]

FTIR and UV-vis absorption spectra of *Momordica charantia*

Infrared spectroscopy is considered to be more suitable for the indetification of functional groups which are responsible for biological activity. The peaks from 3220 cm^{-1} to 3540 cm^{-1} correspond to O-H bonds representing alcohols, phenols, and carboxylic acid groups.[20] The band at approximately 2920 cm^{-1} in the seed+pulp sample was assigned to the C–H stretching vibration of alkanes, alkenes, aromatics, and aldehydes.[20] The absorption peak at 1723 cm^{-1} was identified as esterified carboxylic groups (–COOR).[20] This region was due to carbonyl groups representing aldehydes, carboxylic acids, esters, and ketones.[20] Moreover, absorption peaks at 1550 cm^{-1} to 1640 cm^{-1}, 1619 cm^{-1}, 1422 $cm^{-1,}$ and 1350 cm^{-1} correspond to the asymmetric and symmetric stretching of the carboxylate anion groups (–COO–), referred to as nitro groups.[21] The absorption peak at 1020 cm^{-1} possessed the pyranose ring skeleton.[22] In addition, peak ranges from 770 to 920 cm^{-1} represent the C-H out-of-plane vibrations from alkanes and aromatics.[20]

The UV-vis absorption spectra of the aqueous and 12% ethanolic extracts of the peel and seed+pulp presented bands in the range of 200-600 nm. However, the intensity of absorption varied depending on the fruit sample and type of solvents used for extraction. The results showed an absorbance range of approximately 250 to 280 nm for all samples. The intensity of the peel was higher concerning the seed+pulp, although being independent of the type of extraction. The absorption

spectra of the extracts of bitter melon fruit residues are similar to polyphenol spectra. The findings from the FTIR and UV-vis absorption spectrum revealed the presence of alkyl halides, alkenes, cellulose, aromatic ring, ketone, carbonyls, alcohol, and amines in the bitter melon fruit residues.

Bioactive compounds and antioxidant activity

The tannins are classified as hydrolyzable or condensed tannins and found in maximum amounts in seed+pulp of *M. charantia*. The content of hydrolysable tannins in the aryl of the seed+pulp of *M. subangulata* varied from 374.3 to 495.4 mg tannic acid/100 g and of peel was 360.4 to 492.4 mg tannic acid/100 g, depending on the maturation stage of bitter melon.[23] Tannins can reduce the risk of cardiovascular disease, cancer, and Alzheimer's and can be used as nutraceuticals to maintain good intestinal microbiota.[24]

The phenolic compounds are cited as polyphenols and these compounds were major contributors to antioxidant activity. The present study demonstrated that antioxidant activity in the aqueous extract of bitter melon fruit peel is due to the higher total phenolic contents found in them compared to that of the ethanolic extract. The aqueous extract of bitter melon fruit peel exhibited good total phenolic content (13.28 GAE/g fresh weight) and DPPH radical scavenging activity at 82.05%.

Nehra and Deen,[25] reported that dried fruit of *M. charantia* has higher amounts of total phenolics (3.18 mg GAE/g DW), flavonoids (2.64 mg CAE/g), and DPPH· scavenging activity (86.21%) in ethanolic extract than in the aqueous extract. Earlier study by Vikneswari *et al.*[26] compared the antioxidant property in the *M. charantia* fruit with ethanol and aqueous extract. The results demonstrated that ethanolic extract showed higher DPPH· scavenging activity (IC_{50}=0.53 mg/mL) than aqueous extract. Khalid *et al.*[27] reported that ethanolic (80%) extract of *M. charantia* fruit exhibited maximum amount of total phenolics (34.3 mg GAE/g DW) and flavonoids (63.3 mg CE/g DW) and DPPH· scavenging activity (60.3%) than 100% ethanol. Saha and Chatterjee,[28] demonstrated that bitter gourd seed obtained the maximum amount of total phenolics (47.14 mg GAE/g), flavonoids (16.82 mg QE/g of dry tissue), and tannin (23.24 mg TA/g of dry tissue) contents in alcoholic extract than aqueous extract. The present study confirmed that the 12% ethanolic extract of seed+pulp exhibited higher DPPH· scavenging activity compared to the aqueous extract of seed+pulp. However, the total phenolic contents and total flavonoid contents were higher in the aqueous extract of seed+pulp compared to ethanolic extract. Previous study also demonstrated that 80% ethanolic extract of *M. charantia* peel obtained higher DPPH radical scavenging activity compared to its values in pulp and seed. Although the phenolic compounds concentration was very low. Tan *et al.*[29] reported that 50% ethanolic extract of *M. charantia* fruit exhibited 2.29 g gallic acid equivalent/100 g dried fruit of total phenolic content and 51.1% of inhibition against DPPH radical.

Khan *et al.*[30] also demonstrated that ethanolic extract using the maceration technique showed good DPPH radical scavenging activity compared to soxhlet extraction. Recent research findings were done by Sharma *et al.*,[31] Mbaiogaou *et al.*[32] and Singh *et al.*[33] also supported that *M. charantia* fruit residues showed good total phenolic contents and DPPH radical scavenging activity. Thus, these findings suggested that optimization of solvent extraction for phenolic or antioxidant compounds from *M. charantia* fruit residues is needed for a good recovery.

Identification and quantification of phenolic compounds

Studies reported that dietary extract of *M. charantia* can prevent diabetes and metabolic abnormalities.[34] From the HPLC analysis, an aqueous extract obtained higher phenolic compounds in seed+pulp of *M. charantia* compared to the ethanolic extract. Similarly, Kubola and Siriamornpun[35] reported that aqueous extraction of *M. charantia* (green fruit and ripe fruit) contains tannic acid, gallic acid, catechin, caffeic acid, benzoic acid, *p*-coumaric acid and ferulic acid which were responsible for DPPH radical scavenging activity. However, HPLC analysis of aerial parts of *M. charantia* showed a good amount of phenolic compounds in 80% ethanolic extract and exhibited good DPPH radical scavenging activity.[36] The phenolic compounds concentrations were varied in the bitter melon depends on the maturation stage and parts of the tissues of bitter melon fruit.[37] Previous studies reported the presence of vanillic, ferulic, and gallic acids in the pericarp of bitter melon[38] and values of chlorogenic acid increased from 12.26 to 17.35 µg/g of dry material, with maturation. The contents of vanillic acid (0.87 µg/mg), catechin (0.30 µug/mg), and coumaric acid (0.01 µg/mg) were also found in aqueous extracts of *M. charantia* fruit residues.[39] Akyüz *et al.*[40] also proved that the presence of phenolic compounds of catechin, rutin, gallic acid, quercetin, vanillic acid, gentisic acid, chlorogenic acid, epicatechin, syringic acid, *p*-coumaric acid and benzoic acid in the *M. charantia* fruit are the major contributors for the antioxidant property. Vikneswari *et al.*[26] reported that margaric acid, ascorbic acid, quercetin-3-glycosides, brevifolincarboxylic acid, Augustin H, goya glycoside G and cucurbitacin *E* 3-Malonylmomordicin I were found in the *M. charantia* fruit which was analyzed by LC-MS-QTOF. A previous study by Khalid *et al.*[27] identified and quantified the bioactive compounds using HPLC. The results showed that the fruit peel contains 4-hydroxy, 3-methoxy benzoic acid (403.7 ppm), *m*-coumaric acid (17.21 ppm), whereas the seed contained syringic acid (56.34 ppm) and vanillic acid (301.9 ppm). Among the phenolic compounds obtained in the fruit residues, Naringenin was found in higher concentration in the aqueous extract of bitter melon frui seed+pulp compared to peel. Naringenin is a bioflavonoid that possesses antioxidant and anti-inflammatory properties, such as sepsis, fulminant hepatitis, fibrosis, cancer, and treatment of Alzheimer's disease.[21]

Thus, HPLC analysis proved that aqueous extraction is good for obtaining naringenin from *M. charantia* fruit seed+pulp.

CONCLUSION

The present study concluded that *M. charantia* residues are good sources of natural antioxidants which have a large potential for human health benefits and can contribute to the development of nutraceuticals or functional foods.

ACKNOWLEDGEMENT

This study was funded in part by the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) vide research Project Instituto Nacional de Ciência e Tecnologia de Frutos Tropicais (Process 57573781/2008-7). Authors gratefully acknowledge the financial support of Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brazil under Programa Nacional de Pós-doutorado (PNPD/CAPES- 086/2013 – Financial code 001).

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

FTIR: Fourier Transform Infrared; **UV/vis:** Ultraviolet-visible Spectrometry; **UV-vis:** Spectroscopy; **UPLC/MS:** Ultra Performance Liquid Chromatography/Mass spectroscopy; **DPPH:** 2,2-diphenyl-1-picrylhydrazyl; **SIM:** Selected Ion Monitoring; **SAS:** Statistical Analysis System; **PDA detector:** Photodiode Array Detector; **ANOVA:** Analysis of Variance; **K:** Potassium; **P:** Phosphorous; **Ca:** Calcium; **Mg:** Magnesium; **Na:** Sodium; **Fe:** Iron; **Zn:** Zinc; **Sr:** Strontium; **Cu:** Copper, **Mn:** Mangenese; **GAE:** Gallic acid equivalents; **CE:** Catechin equivalents.

AUTHOR CONTRIBUTIONS

Project administration: Narendra Narain; Funding acquisition: Narendra Narain; Concept and Design: Julianna Karla Santana Andrade; Experimental Studies: Romy Gleyse Chagas Barros; Juliete Pedreira Nogueira; Christean Santos de Oliveira; George Ricardo Santana Andrade; Silvânio Silvério Lopes da Costa and Murugan Rajan; Literautre Search: Murugan Rajan; Statistical analysis: Julianna Karla Santana Andrade and Juliete Pedreira Nogueira; Roles/Writing - original draft: Julianna Karla Santana Andrade, Murugan Rajan, and Narendra Narain; Writing - Review and editing: Julianna Karla Santana Andrade, Murugan Rajan, and Narendra Narain.

SUMMARY

Momordica charantia fruit is a rich source of nutraceuticals and polyphenolic compounds with good antioxidant activity. Naringenin is a bioflavonoid found in peel which could be used as the nutraceutical supplement. The effectiveness of *M. charantia* fruit peel bioactive compounds would prevent the intestinal related disorders.

REFERENCES

1. Krishnaiah D, Sarbatly R, Nithyanandam R. A review of the antioxidant potential of medicinal plant species. Food Bioprod Process. 2011;89(3):217-33. doi: 10.1016/j.fbp.2010.04.008.
2. Horax R, Hettiarachchy N, Kannan A, Chen P. Proximate composition and amino acid and mineral contents of *Mormordica charantia* L. pericarp and seed at different maturity stages. Food Chem. 2010;122(4):1111-5. doi: 10.1016/j.foodchem.2010.03.093.
3. Yue J, Sun Y, Xu J, Cao J, Chen G, Zhang H, *et al.* Cucurbitane triterpenoids from the fruit of *Momordica charantia* L. and their anti-hepatic fibrosis and anti-hepatoma activities. Phytochemistry. 2019;157:21-7. doi: 10.1016/j.phytochem.2018.10.009, PMID 30352327.
4. Chekka SV, Mantipelly NK. *Momordica charantia*: A natural medicinal plant. GSC Biol. Pharm Sci. 2020;12(02):129-35.
5. Villarreal-La Torre VEV, Guarniz WS, Silva-Correa C, Cruzado-Razco L, Siche R. Antimicrobial activity and chemical composition of *Momordica charantia*: a review. Pharmacogn J. 2020;12(1):213-22. doi: 10.5530/pj.2020.12.32.
6. Supe U, Daniel P. HPLC method for analysis of bioactive compound from *Momordica charantia*. Res J Pharm Technol. 2015;8(12):1649. doi: 10.5958/0974-360X.2015.00297.8.
7. Castro-Puyana M, Marina ML, Plaza M. Water as green extraction solvent: principles and reasons for its use. Curr. Op. green Sus. Chem. 2017;5:31-6.
8. McDonnell C, Tiwari BK. Ultrasound. Compr Anal Chem. 2017;76:111-29. doi: 10.1016/bs.coac.2017.03.005.
9. AOAC official method of analysis. 19th ed. Washington, DC: Association of Analytical Chemists; 2012:121-30.
10. Correia FO, Silva DS, Costa SSL, Silva IKV, da Silva DR, Alves JPH, *et al.* Optimization of microwave digestion and inductively coupled plasma-based methods to characterize cassava, corn, and wheat flours using chemometrics. Microchem J. 2017;135:190-8. doi: 10.1016/j.microc.2017.09.007.
11. Andrade JKS, Denadai M, Andrade GRS, da Cunha Nascimento CC, Barbosa PF, Jesus MS, *et al.* Development and characterization of microencapsulated containing spray dried powder obtained from Brazilian brown, green and red propolis. Food Res Int. 2018;109:278-87. doi: 10.1016/j.foodres.2018.04.048, PMID 29803451.
12. Rhazi N, Hannache H, Oumam M, Sesbou A, Charrier B, Pizzi A, *et al.* Green extraction process of tannins obtained from Moroccan *Acacia mollissima* barks by microwave: modeling and optimization of the process using the response surface methodology RSM. Arab J Chem. 2019;12(8):2668-84. doi: 10.1016/j.arabjc.2015.04.032.
13. Hamissou M, Smith AC, Carter RE, Triplett JK. Antioxidative properties of bitter gourd (*Momordica charantia*) and zucchini (*Cucurbita pepo*). Emir. J Food Agric. 2013;25(9):641-7.
14. Saad DY, Soliman MM, Baiomy AA, Yassin MH, El-Sawy HB. Effects of karela (Bitter Melon; *Momordica charantia*) on genes of lipids and carbohydrates metabolism in experimental hypercholesterolemia: a biochemical, molecular and histopathological study. BMC Complement Altern Med. 2017;17(1):319. doi: 10.1186/s12906-017-1833-x, PMID 28623919.
15. Horax R, Hettiarachchy N, Chen P. Extraction, quantification, and antioxidant activities of phenolics from pericarp and seeds of Bitter Melons (*Momordica charantia*) harvested at three maturity stages (Immature, Mature, and Ripe). J Agric Food Chem. 2010;58(7):4428-33. doi: 10.1021/jf9029578, PMID 20225855.
16. Otero DM, Jansen-Alves C, Fernandes K, Zambiazi RC. Physicochemical characterization and bioactive potential of *Momordica charantia* L., Int. J. Dev Res. 2020;10(06):36461-7.
17. Mukherjee S, Karati D. Exploring the phytochemistry, pharmacognostic properties, and pharmacological activities of medically important plant *Momordica charantia*. Pharmacol. Res. Mod Chin Med. 2023;6:100226.
18. Katarzyna J, Karolina J, Patrycja K, Mateusz B, Izabela G. Mineral composition and antioxidant potential in the common poppy (*Papaver rhoeas* L.) petal infusions. Biol Trace Elem Res. 2021;199(1):371-81. doi: 10.1007/s12011-020-02134-7, PMID 32306285.
19. Pate RT, Cardoso FC. Injectable trace minerals (selenium, copper, zinc, and manganese) alleviate inflammation and oxidative stress during an aflatoxin challenge in lactating multiparous Holstein cows. J Dairy Sci. 2018;101(9):8532-43. doi: 10.3168/jds.2018-14447, PMID 29935830.

20. Khatib A, Perumal V, Ahmed QU, Uzir BF, Abas F, Murugesu S. Characterization of antioxidant activity of *Momordica charantia* fruit by infrared-based fingerprinting. Anal Lett. 2017;50(12):1977-91. doi: 10.1080/00032719.2016.1261877.
21. Md S, Gan SY, Haw YH, Ho CL, Wong S, Choudhury H. *In vitro* neuroprotective effects of naringenin nanoemulsion against β-amyloid toxicity through the regulation of amyloidogenesis and tau phosphorylation. Int J Biol Macromol. 2018;118(A):1211-9. doi: 10.1016/j.ijbiomac.2018.06.190, PMID 30001606.
22. Yan JK, Wu LX, Qiao ZR, Cai WD, Ma H. Effect of different drying methods on the product quality and bioactive polysaccharides of bitter gourd (*Momordica charantia* L.) slices. Food Chem. 2019;271:588-96. doi: 10.1016/j.foodchem.2018.08.012, PMID 30236720.
23. Singh S, Swain S, Nisha M, Banu VS, Singh DR, Roy SD. Changes in lycopene, total carotenoid, and anti-radical activity in teasel gourd [*Momordica subangulata* ssp. renigera (G. Don) de Wilde]. Ind Crops Prod. 2015;73:154-63. doi: 10.1016/j.indcrop .2015.04.016.
24. Melo LFM, Aquino-Martins VGQ, Silva APD, Oliveira Rocha HAO, Scortecci KC. Biological and pharmacological aspects of tannins and potential biotechnological applications. Food Chem. 2023;414:135645. doi: 10.1016/j.foodchem.2023.135645, PMID 36821920.
25. Nehra S, Deen MK. Estimation of the Effect of drying on the chemical composition and antioxidant activity of the fruits of *Momordica charantia* (karela). Int J Pharm Sci Rev Res. 2017;43(2):90-6.
26. Vikneswari P, Khatib A, Ahmed QU, Uzir BF, Abas F, Murugesu S, *et al.* Antioxidants profile of *Momordica charantia* fruit extract analyzed using LC-MS-QTOF-based metabolomics. Food Chem Mol Sci. 2021;2:100012.
27. Khalid Z, Hassan SM, Mughal SS, Hassan SK, Hassan H. Phenolic profile and biological properties of *Momordica charantia*. Chem. Biomol Eng. 2021;6(1):17-29.
28. Saha M, Chatterjee DS. Biochemical analysis and evaluation of the free radical scavenging activity of bitter gourd seeds' aqueous and ethanolic extracts: comparative study. J Med Plants Stud. 2022;10(1):111-7. doi: 10.22271/plants.2022 .v10.i1b.1369.
29. Tan ES, Abdullah A, Kassimc NK. Antioxidant and antidiabetic properties of Bitter Gourd (*Momordica charantia*) fruit. Int J Sci Basi App Res. 2019;48(5):76-85.
30. Khan MF, Abutaha N, Nasr FA, Alqahtani AS, Noman OM, Wadaan MAM. Bitter gourd (*Momordica charantia*) possesses developmental toxicity as revealed by screening the seeds and fruit extracts in zebrafish embryos. BMC Complement Altern Med. 2019;19(1):184. doi: 10.1186/s12906-019-2599-0, PMID 31340810.
31. Sharma GK, Sharma S, Chasta P, Joshi RK, Tiwari A, Chandrul KK. Assessment of pharmacognostic parameters and antioxidant potential of Bitter melon or karela (*Momordica charantia* L.) fruits by DPPH Method. Res J Pharm Technol. 2021;14(1):437-41. doi: 10.5958/0974-360X.2021.00079.2.
32. Mbaiogaou A, Madjitoloum Betoloum SM, Mbaihougadobe S, Naitormbaide M, Sale S, Mahmout Y. Determination of phenolic compound contents in fruits of *Momordica charantia* L. (Cucurbitaceae) from Chad. Int J Bio Chem Sci. 2022;16(4):1475-83. doi: 10.4314/ijbcs.v16i4.10.
33. Singh A, Gupta A, Srivastava S, Choudhury B, Jain S, Bajpai A. Assessment of the antioxidant, antimicrobial, and anti-inflammatory potential of *Momordica charantia*. Biomed Pharmacol J. 2023;16(2):899-906. doi: 10.13005/bpj/2672.
34. Sun K, Ding M, Fu C, Li P, Li T, Fang L, *et al.* Effects of dietary wild bitter melon (*Momordica charantia* var. abbreviate Ser.) extract on glucose and lipid metabolism in HFD/STZ-induced type 2 diabetic rats. J Ethnopharmacol. 2023;306(24):116154. doi: 10.1016/j.jep.2023.116154, PMID 36634725.
35. Kubola J, Siriamornpun S. Phenolic contents and antioxidant activities of bitter gourd (*Momordica charantia* L.) leaf, stem and fruit extracts *in vitro*. Food Chem. 2008;110(4):881-90. doi: 10.1016/j.foodchem.2008.02.076, PMID 26047274.
36. Svobodova B, Barros L, Calhelha RC, Heleno S, Alves MJ, Walcott S, *et al.* Bioactive properties and phenolic profile of *Momordica charantia* L. medicinal plant growing wild in Trinidad and Tobago. Ind Crops Prod. 2017;95:365-73. doi: 10.1016/j.indcrop .2016.10.046.
37. Lee SH, Jeong YS, Song J, Hwang K, Noh GM, Hwang IG. Phenolic acid, carotenoid composition, and antioxidant activity of bitter melon (*Momordica charantia* L.) at different maturation stages. Int J Food Prop. 2017;20;sup3:S3078-87. doi: 10.1080/ 10942912.2016.1237961.
38. Perez JL, Jayaprakasha GK, Crosby K, Patil BS. Evaluation of bitter melon (*Momordica charantia*) cultivars grown in Texas and levels of various phytonutrients. J Sci Food Agric. 2019;99(1):379-90. doi: 10.1002/jsfa.9199, PMID 29888551.
39. Atta S, Waseem D, Naz I, Rasheed F, Phull AR, Ur-Rehman T, *et al.* Polyphenolic characterization and evaluation of multimode antioxidant, cytotoxic, biocompatibility and antimicrobial potential of selected ethnomedicinal plant extracts. Arab J Chem. 2023;16(2):104474. doi: 10.1016/j.arabjc.2022.104474.
40. Akyüz E, Sercan T, Sözgen BK, Esma T, Reşat AM. Comparison of antioxidant capacities and antioxidant components of commercial bitter melon (*Momordica charantia* L.) products. Turk J Chem. 2020;44(6):1-10.

Cite this article: Andrade JKS, Barros RGC, Nogueira JP, Oliveira CS, Andrade GRS, Cost SSL, *et al*. The Potential of Bitter Melon Residues Concerning its Physicochemical Characterization, Bioactive Compounds, and Antioxidant Effects. Pharmacog Res. 2024;16(1):26-33.

Supplementary

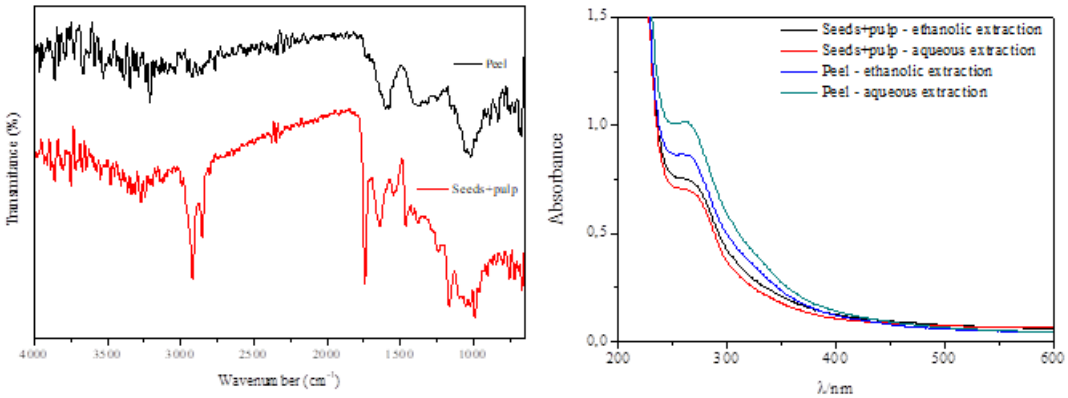

Figure 1S: FTIR spectra of solid samples (a) and absorption spectra in the UV/vis region of the aqueous and ethanolic extracts (b) of the peel and seed+pulp of *M. charantia* residues.

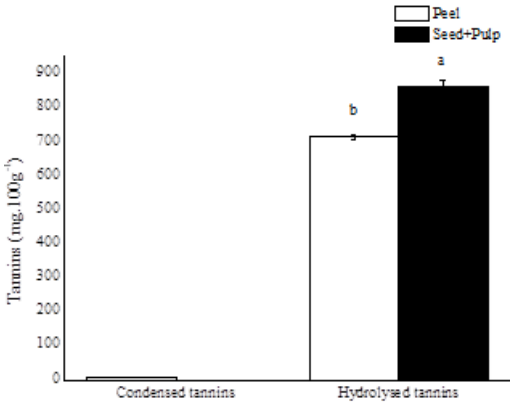

Figure 2S: Condensed and hydrolyzed tannins contents in peel and seed+pulp of samples of *M. charantia* residues.

Values expressed as mean±standard deviation; *n*=3. The same lower-case letters associated with columns for the same analysis do not differ statistically, $p<0.05$, Tukey test.

Table 1S: Analytical MS parameters: Retention time, linearity parameters, correlation coefficients and detection limits for phenolic and flavonoid compounds.

Compound	Molecular formula	ESI Polarity	Precursor ion	Collision energy (eV)	Retention time (min)	Calibration equations[a]	Correlation coefficients (r^2)	MDL[b] (mg/mL)
Acacetin	$C_{16}H_{12}O_5$	-	285	15	42.9	Y = 1.45e+007 X + 1.66e+004	0.9975	0.002
Apigenin	$C_{15}H_{10}O_5$	-	271	15	34.8	Y = 3.50e+007 X + 1.77e+005	0.9907	0.002
Biochanin A	$C_{16}H_{12}O_5$	-	285	15	44.2	Y = 4.10e+007 X + 1.48e+005	0.9931	0.002
Caffeic acid	$C_9H_8O_4$	-	181	15	20.9	Y = 2.30e+007 X + 3.17e+004	0.9957	0.002
(+)-catechin	$C_{15}H_{14}O_6$	-	291	15	19.4	Y = 2.80e+007 X - 2.71e+004	0.9924	0.002
Chrologenic acid	$C_{16}H_{18}O_9$	-	355	15	18.5	Y = 2.29e+007 X - 3.14e+004	0.9926	0.002
Chrysin	$C_{15}H_{10}O_4$	-	255	15	42.6	Y = 3.22e+007 X + 1.44e+005	0.9918	0.002
p-coumaric acid	$C_9H_8O_3$	-	165	15	24.5	Y = 1.04e+007 X - 2.64e+004	0.9973	0.002
Daidzein	$C_{15}H_{10}O_4$	-	255	15	30.4	Y = 3.47e+007 X +1.47e+005	0.9912	0.002
Epicatechin	$C_{15}H_{14}O_6$	-	291	15	20.6	Y = 2.38e+007 X - 2.10e+004	0.9949	0.002
Ethyl gallate	$C_9H_{10}O_5$	-	199	15	23.5	Y = 2.68e+007 X + 1.74e+005	0.9935	0.002
Ferulic acid	$C_{10}H_{10}O_4$	-	195	15	25.5	Y = 3.26e+006 X - 8.66e+003	0.9935	0.002
Gallic acid	$C_7H_6O_5$	-	171	15	12.4	Y = 8.28e+006 X + 1.66e+004	0.9926	0.002
Kaempferol	$C_{15}H_{10}O_6$	-	287	15	35.2	Y = 5.14e+007 X - 4.66e+004	0.9943	0.002
Luteolin	$C_{15}H_{10}O_6$	-	287	15	31.2	Y = 3.01e+007 X + 2.03e+005	0.9921	0.002
Narigenin	$C_{15}H_{12}O_5$	-	273	15	10.8	Y = 4.66e+004 X + 3.41e+002	0.9999	0.002
Protocatechuic acid	$C_7H_6O_4$	-	155	15	16.6	Y = 1.34e+007 X + 4.09e+004	0.9967	0.002
Rutin	$C_{27}H_{30}O_{16}$	-	611	15	21.8	Y = 3.58e+007 X + 1.69e+005	0.9903	0.002
Vanillic acid	$C_8H_8O_4$	-	169	15	21.4	Y = 4.61e+005 X - 1.12e+003	0.9921	0.002
Vanilin	$C_8H_8O_3$	-	153	15	25.1	Y = 1.47e+006 X - 2.61e+003	0.9950	0.002

ESI - Electrospray ionization; [a]Y is the value of the peak area. X is the concentration of the standard compound. [b]MDL - Method Detection Limit