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Bioactive polyphenolic compounds and antioxidant potentials of two leafy vegetables in Bangladesh: the *Momordica charantia* and the *Ipomoea aquatica*

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Abstract

Momordica charantia and Ipomoea aquatica leaves are two green leafy vegetables in Bangladesh that are commonly consumed considering their characteristic taste and abundant availability in nature. The aim of this study was to determine the available bioactive phenolic constituents as well as total flavonoid content (TFC), tannin content (TTC), phenolic content (TPC), antioxidant activity (TAA) and DPPH radical scavenging activity (IC_{50}) of the ethanolic extracts of *M. charantia* (MCE) *and I. aquatica* (IAE). HPLC–DAD and UV–visible spectrophotometer were used to determine the phenolic compounds and antioxidant properties, respectively. In this study, TFC, TTC, TPC, TAA and IC_{50} values were in the order of IAE (40.73 ± 1.0 mg QE/g) > MCE (34.60 ± 0.46 mg QE/g); MCE (40.93 ± 0.70 mg TAE/g) > IAE (31.13 ± 0.42 mg TAE/g); MCE (27.76 ± 0.58 mg GAE/g) > IAE (21.29 ± 0.43 mg GAE/g); MCE (52.03 ± 0.21 mg AAE/g) > IAE (40.77 ± 0.15 mg AAE/g) and MCE *(*333.22 ± 67.37 µg/mL*)* > IAE *(*560.74 ± 10.25 µg/mL*)*. *M. charantia* ethanolic extracts contained five hydroxycinnamic acid: ferulic acid, chlorogenic acid, *p*-coumaric acid, rosmarinic acid and cinnamic acid; five flavonoids: epicatechin, quercetin, catechin, rutin hydrate and myricetin; two hydroxybenzoic acid: gallic acid and vanillic acid; and one phenolic aldehyde: vanillin. Whereas, *I. aquatica* extracts possessed four hydroxycinnamic acid: chlorogenic acid, *p*-coumaric acid, *trans*-ferulic acid and *trans*-cinnamic acid; four flavonoids: epicatechin, quercetin, catechin, and rutin hydrate; two hydroxybenzoic acid: gallic acid and vanillic acid; and one phenolic aldehyde:vanillin. These underutilised sources of leafy vegetables may be used to develop functional foods by emphasising their remarkable bioactive components.

Keywords *Momordica charantia*, *Ipomoea aquatica*, HPLC–DAD, Antioxidant activity, Polyphenolic compound

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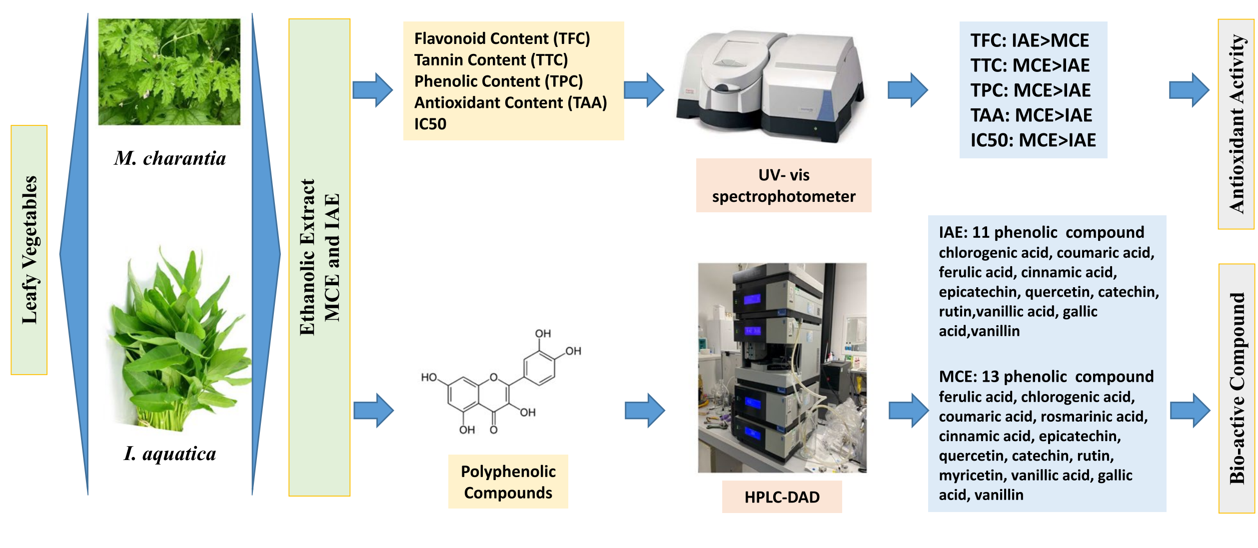
Graphical Abstract



Introduction

M. charantia and *I. aquatica* leaves are two indigenous sources of green leafy vegetables, of which one is a terrestrial and the other is a semi-aquatic plant. *M. charantia* leaves (English: bitter gourd leaves, Bengali: korolla shak) are consumed by the tribal people of the Chattogram hill tracts of Bangladesh as an unconventional source of leafy vegetables, whereas *I. aquatica* leaves (English: water spinach leaves, Bengali: kolmi shak) are consumed as a cheap source of green leafy vegetables for inhabitants of low land, for their distinctive taste as well as medicinal properties.

The bitter gourd (*M. charantia* L.) is an annual climbing common herb, and its fruit is usually consumed as a vegetable (Nagarani et al. 2014). As an indigenous herb, its leaves are consumed by the tribal people of Bangladesh as green leafy vegetables as well as for folk medicinal purposes for several ailments like malaria, parasitic infestation, inflammation, abdominal and digestive problems, menstrual disorders, hypoglycemia, jaundice, wounds, hepatitis, and tumours (Taylor 2002; Ganesan et al. 2008; Bakare et al. 2011; Annapoorani & Manimegalai 2013).

Water spinach (*I. aquatica* L.) is a vascular semi-aquatic plant that abundantly grows wild near waterways in tropical and subtropical ecologies. The kolmi shak is widely and commercially grown in Bangladesh in the dry land as a leafy vegetable. Moreover, a remarkable amount of kolmi shak is grown in the wetland of the country which remain underutilised and of which few are consumed by the local inhabitants. *I. aquatica* is used as a leafy vegetable in rural areas of the Indian subcontinent and is also considered effective against various health ailments, such as diabetes, liver malfunction, and constipation, as well as in the treatment of arsenic and heavy metal poisoning (Dua et al. 2015; El-Sawi et al. 2017).

Phenolic compounds are secondary metabolites of plants and they contain benzene rings, with one or more hydroxyl substituents, and range from simple phenolic molecules to highly polymerized compounds (Lin et al. 2016). Polyphenols are widely found in leafy vegetables and these phenolic substances, or polyphenols, contain numerous varieties of compounds: as simple phenols (Tarafder et al. 2023), numerous phenolic acids, such as hydroxybenzoic acids (Sarker & Ercisli 2022) and hydroxycinnamic acids (Sarker & Oba 2020a) and flavonoids, such as flavone (Sarker et al. 2023), flavanones (Sarker et al. 2020a; Sarker & Oba 2018a), flavonols (Sarker & Oba 2020b) and flavanols (Sarker & Oba 2020c; Sarker & Oba 2018a). These phenolic compounds are usually related to defense responses in the plant. Plant polyphenols as dietary antioxidants in human health and disease might protect against oxidative damage. Different groups of phenolic compounds have different biological characteristics, and very little is known about the mechanisms by which they could contribute to the prevention of disease; there still is the need for further studies.

Research is now focusing on the untapped potential of phytochemicals like phenols, steroids, alkaloids, and flavonoids found in many green leafy vegetables. But not much is known about the wild varieties of *M. charantia* and *I. aquatica* in Bangladesh, especially about how they might be related to their phenolic content. As wild plant varieties are often higher in bioactive phenolic compounds than their cultivated counterparts (Braca et al. 2003; Svobodova et al. 2017), we decided to take a close look at this plant's biological activity and figure out which polyphenolic compounds are most abundant in the ethanolic extract of these two plants. We have put the plants in the nutraceutical landscape based on their health benefits and uses, even though we don't know all of the bioactive compounds in them yet. The goal of this work was to get reference data for using the crude extract of wild *M. charantia* and *I. aquatica* as a natural agent with multiple biological activities for making new nutraceuticals and developing functional food.

Material and method

Sample collection

M. charantia leaves were collected from three different weekly markets in Bandarban district in Chattogram Hill Tract of Bangladesh (Islam et al. 2010). Whereas, wild *I. aquatica* leaves were collected from three different markets around Dhaka city of Bangladesh. Samples were collected during the period of January- March, 2022. Approximately 1.0 kg of each vegetable was purchased, water sprayed, and kept in a ziplock plastic bag at a chilling temperature of 4 °C for maximum 24 h prior to freeze drying (Islam et al. 2010).

Sample extraction

The edible part of the vegetables were taken and cleaned properly. Vegetables were freeze dried at -40 ℃ (Thermo Fisher, Modulyod-230, USA) and the dried samples were powdered by a grinder (Panasonic, MX-AC400). A single composite sample of a homogeneous mix of the same type and source of plant was prepared and stored at 4 ℃ (Shaheen et al. 2013; Alam et al. 2020). Samples (20 g) were soaked in 200 mL ethanol and shaken for 48 h in a shaker (GFL orbital shaker 3005). The mixer was centrifuged and filtered followed by the evaporation of solvent by vacuum rotary evaporator (IKA RV10 D S99). Ethanolic extracts of *M. charantia* (MCE) and *I. aquatica* (IAE) were stored at -20 ℃. The unit of measurement was considered for the dry weight (DW) of extract in each determination.

Solvents and reagents

Acetonitrile, acetic acid, ethanol and methanol were used as solvent in the study. Aluminium chloride, sodium acetate, folin-ciocalteu phenol reagent, sodium carbonate, tannic acid, sulphuric acid, sodium phosphate, ammonium molybdate, ascorbic acid and 1, 1-diphenyl-2-picrylhydrazyl were the major reagent. Gallic acid, catechol, chlorogenic acid, catechin hydrate, vanillic acid, caffeic acid, syringic acid, epicatechin, vanillin, *p*-coumaric acid, *trans*-ferulic acid, rutin hydrate, rosmarinic acid, myricetin, quercetin, *trans*-cinnamic acid, naringenin, kaempferol, and apigenin were used as standard of phenolic compound. All chemicals and standards were collected from Sigma–Aldrich (St. Louis, MO, USA). De-ionized (DI) water was prepared by Milli-Q systems (Millipore, Bedford, MA, USA).

Sample preparation

The MCE and IAE were thawed and stock solutions (10,000 µg extract/mL) were prepared by dissolving 0.1 g of extract into 10 mL of ethanol and were stored at 4 ℃ for analytical procedure.

Yield determination

The yield percentage was calculated to track how the solvent system affected the extraction. Yield (percent) = 100*(A-B)/W, where A is the weight of the extract-containing flask after evaporation, B is the weight of the dry empty flask, and W is the weight of the dry sample.

Determination of Total Flavonoid Content (TFC)

Reagent for TFC was prepared by dissolving 0.3325 g $AlCl_3$ and 1 g CH_3COONa in 100 mL DI water. Stock solution (0.2 mL) was taken in a test tube followed by the addition of DI water (4.8 mL) and $AlCl_3$ reagent (2.5 mL) and was incubated for 5 min. Standard solution (20—100 µg/mL) of quercetin was prepared to construct a calibration curve and absorbance was taken at 430 nm using a UV–VIS spectrophotometer (Thermo Scientific, Model: Evolution 300). Total Flavonoid Content was expressed as mg of Quercetin equivalent (QE) /g of dry extract (Chang et al. 2001).

Determination of Total Tannin Content (TTC)

A stock solution (0.5 mL), DI water (8.5 mL) and Folin-Ciocalteu phenol reagent (0.5 mL) were sequentially added to a 15 mL test tube and kept at room temperature for 5 min. Then 1 mL sodium carbonate solution (35%) was added followed by a 20 min incubation period. A set of standard solution (20—100 µg/mL) of tannic acid were used for the calibration curve (r^2 = 0.995) and the absorbance was measured at 725 nm. The TTC was expressed as mg of Tannic acid equivalent (TAE) /g of dry extract (Haile & Kang 2019; Rahman et al. 2022).

Determination of Total Phenolic Content (TPC)

The stock solution (0.5 mL) and DI water (8.5 mL) were taken in a test tube followed by the addition of Folin-Ciocalteu phenol reagent (0.5 mL) and kept at room temperature for 30 min. Then 1 mL of sodium carbonate solution (35%) was added and incubated for 20 min. In this experiment, gallic acid was used as standard to prepare a calibration curve and the detection range was 20—100 μg/mL. The absorbance was recorded at 765 nm and the results of the TPC were expressed as mg of gallic acid equivalents (GAE) /g of dry extract (Cl & Indira 2016; Haile & Kang 2019).

Determination of Total Antioxidant Activity (TAA)

The stock solution (0.5 mL) was mixed with 3.0 mL of reagent solution (0.6 M H_2SO_4, 28 mM Na_3PO_4, 4 mM ammonium molybdate) and incubated for 90 min at 95 ^{0}C. The absorbance of the solution was measured at 695 nm. Ascorbic acid was used as standard, where 20—100 μg/mL concentration range was selected for the calibration curve construction. TAA was mentioned as mg equivalent of ascorbic acid (AAE) /g of dry extract (Prieto et al. 1999; Rahman et al. 2022).

Determination of DPPH (1, 1-diphenyl-2-picrylhydrazyl) radical scavenging activity

In this assay, 2 mL of 0.2 mM ethanolic DPPH solution was added to 2 mL of the extract solutions, which were prepared at different concentrations and were incubated for 10 min in a dark place (Chang et al. 2001; Erkan et al. 2008). The absorbance was measured against blank at 517 nm and DPPH radical-scavenging activity (%) was determined following the equitation, $\{(A_0\text{-}A)/A_0\} \times 100$; Where, A_0 was the absorbance of the blank solution containing all reagents except plant extracts; A was the absorbance of the DPPH solution containing plant extract. The inhibition concentration (IC_{50}) was determined by plotting DPPH radical-scavenging activity (%) against the extract concentration to determine. Logarithmic nonlinear regression was used to find out the slope for the determination of IC_{50} values (Tanvir et al. 2017).

Identification of bioactive phenolic compound

Sample and standard preparation

Stock solution (2.0 mL) of MCE and IAE extracts were filtered by nylon 0.45 μm syringe filter into a septum vial. A mixed standard solution was prepared in methanol by diluting the stock standard solutions (100 μg/mL) to give a concentration of 5 μg/mL for each compound. The calibration curve of the mixed standard (1.0—5.0 μg/mL) was prepared from chromatograms as peak area vs. concentration of standard (Khan et al. 2020).

Chromatographic system

Chromatographic analyses were carried out on HPLC system (Thermo Fisher Scientific Inc., Dionex UltiMate 3000, USA), which was coupled to a diode array detector (DAD-3000RS), a quaternary pump (LPG-3400RS) and an autosampler (WPS-3000). The phenolic compounds were separated through Acclaim® C18 (4.6 × 250 mm; 5 μm; 120 A°) column (Dionix, USA) at 30 ℃ using a column oven (TCC-3000). Data acquisition, peak integration, and calibration were performed with Dionix Chromeleon software (Version 6.80 RS 10).

The mobile phase consisted of solvent A: acetonitrile; solvent B: deionized water of pH 3.0 adjusted with glacial acetic acid, solvent C: methanol; the gradient elution program was used with variable flow rate shown in Table 1 and the injection volume was 20 μL, which was the slight modification of previous method (Jahan et al 2014; Rahman et al. 2022; Saffoon et al. 2014). For PDA detection, the wavelength program was to monitor phenolic compounds at 280 nm. Data were reported for triple independent analyses where linearity of calibration curves for standards was $r^2 > 0.995$.

Statistical analysis

The mean and standard deviations (SD) were used to express all experimental outcomes. Data was analyzed by using IBM SPSS Statistics (version 26). The Pearson correlation analysis was done to determine the relationship between test parameters. The graphs were created using RStudio 2022.12.0 for Windows and Past 4.11.

Table 1 Chromatographic conditions

Sl no	Retention time [min]	Flow rate [mL/min]	Solvent A (%)	Solvent B (%)	Solvent C (%)
1	0.000	1.000	0.0	100.0	0.0
2	4.000	1.000	3.0	95.0	2.0
3	10.000	1.000	6.0	92.0	2.0
4	14.000	0.800	6.0	90.0	4.0
5	20.000	0.800	10.0	85.0	5.0
6	24.000	0.750	14.0	80.0	6.0
7	30.000	0.750	15.0	75.0	10.0
8	39.000	0.750	20.0	65.0	15.0
9	45.000	0.750	25.0	55.0	20.0

Table 2 Yield and DPPH radical-scavenging activities of *M. charantia* and *I. aquatica* extracts

Samples	Yield (%)	DPPH scavenging activity (IC_{50}, μg/mL)	Regression equation of % Inhibition (r^2)
MCE	16.59 ± 0.42	333.22 ± 67 .37[a]	Y = 14.75ln(x)-35.68 r^2 = 0.976
IAE	14.16 ± 0.27	560.74 ± 10.25[b]	Y = 12.27ln(x)-27.66 r^2 = 0.987
Ascorbic acid	-	16.08 ± 0.96[c]	Y = 14.55ln(x) + 9.58 r^2 = 0.883

The same letter (s) on the top of the IC_{50} of the same experiment did not differ significantly at the 5% level of significance

Result

Yield of the extract

The solvent system affects the yield percentage of the extract. The yield percentage follows the order: MCE > IAE (Table 2). The highest yield percentage was observed for *M. charantia* (16.59%), whereas *I. aquatica was* 15.29%.

TFC, TTC, TPC, TAA and IC50 Value of extracts

In this study, TFC, TTC, TPC, TAA and IC_{50} values of ethanolic extract of *M. charantia* were 34.60 ± 0.46 mg QE/g; 40.93 ± 0.70 mg TAE/g; 27.76 ± 0.58 mg GAE/g; 52.03 ± 0.21 mg AAE/g and 333.22 ± 67.37 μg/mL respectively (Figs. 1 and 2 and Table 2). On the other hand, TFC, TTC, TPC, TAA and IC_{50} values of *I. aquatica* ethanolic extracts were 40.73 ± 1.0 mg QE/g; 31.13 ± 0.42 mg TAE/g; 21.29 ± 0.43 mg GAE/g; 40.77 ± 0.15 mg AAE/g and 560.74 ± 10.25 μg/mL respectively (Figs. 1 and 2 and Table 2).

Polyphenolic bioactive compounds

The bioactive polyphenols of *M. charantia* ethanolic extracts were analyzed by HPLC–DAD and revealed the presence of 13 phenolic compounds (Fig. 3b) considering the chromatogram of 19 standard polyphenolic compound (Fig. 3a). epicatechin > ferulic acid > quercetin > chlorogenic Acid > catechin were the major phenolic compounds in MCE observed in the study (Table 3). Besides that, gallic acid, vanillic acid, vanillin, *p*-coumaric acid, rutin hydrate, rosmarinic acid, myricetin, and cinnamic acid were also found in the MCE.

Whereas the liquid chromatographic fingerprint of *I. aquatica* ethanolic extracts possessed 11 polyphenols in the same chromatographic conditions analyzed by HPLC–DAD (Fig. 3c). As presented in Table 3, quercetin > epicatechin > catechin were the predominant phenolic compounds, whereas gallic acid, chlorogenic acid, vanillic acid, *p*-coumaric acid, *trans*-ferulic acid, *trans*-cinnamic acid, vanillin and rutin hydrate were also found in the IAE.

Discussion

Solubility, extraction duration, temperature, kind of plant material, and concentration of the solvent are just some of the variables that can affect the yield of solvent extraction of plant extracts (Do et al. 2014; Rahman et al. 2022). When compared to Nagarani et al., the results of this experiment showed a higher level of extraction (Nagarani et al. 2014).

The TPC results of both *M. charantia* and *I. aquatica* were much higher than various leafy vegetable amaranth of Bangladesh, such as dantashak (Sarker et al. 2022a), Slim amaranth (Sarker et al. 2022b), *Amaranthus tricolor* (Sarker et al. 2022c), *Amaranthus lividus* (Hossain et al. 2022), leafy Amaranths (Sarker et al. 2022d, e). Besides, Nagarani et al. found that the flavonoids, tannins and phenolic content of ethanolic extract were 72.83 ± 0.44 mg RUE/g extract, 72.83 ± 0.44 mg RUE/g extract and 33.90 ± 1.99 mg TAE/g extract, which was similar to the current study (Nagarani et al. 2014). In another research finding, Shodehinde et al. claimed that the bioactive component of methanol extract was significantly higher than that of aqueous extract (Shodehinde et al. 2016). In water extract, Kubola et al. found that TPC, TAA and IC_{50} of *M. charantia* were 474 ± 0.71 mg GAE/g dry sample, 61 mg/g of bitter gourd, and 9.72 ± 0.25 mg/mL respectively (Kubola et al. 2008). Svobodova et al. also stated similar agreement in the case of the IC_{50} value of ethanolic extract (Svobodova et al. 2017). According to the study by Mariani et al., the total flavanoids and phenolic values of *I. aquatica* ethanolic extracts were 24 mg QE/g and 76.96 mg GAE/g respectively (Mariani et al. 2019).

The TFC results of both *M. charantia* and *I. aquatica* were much higher than different leafy vegetable of Amaranthaceae family grown in Bangladesh like *A. hypochondriacus* (Sarker & Oba 2020d), *A. blitum* (Sarker & Oba 2020e), weedy species (Sarker & Oba 2019), green morph amaranth (Sarker et al. 2020b), stem amaranth (Sarker et al. 2020c). On the other hand, TAA results of both *M. charantia* and *I. aquatica* were much higher than

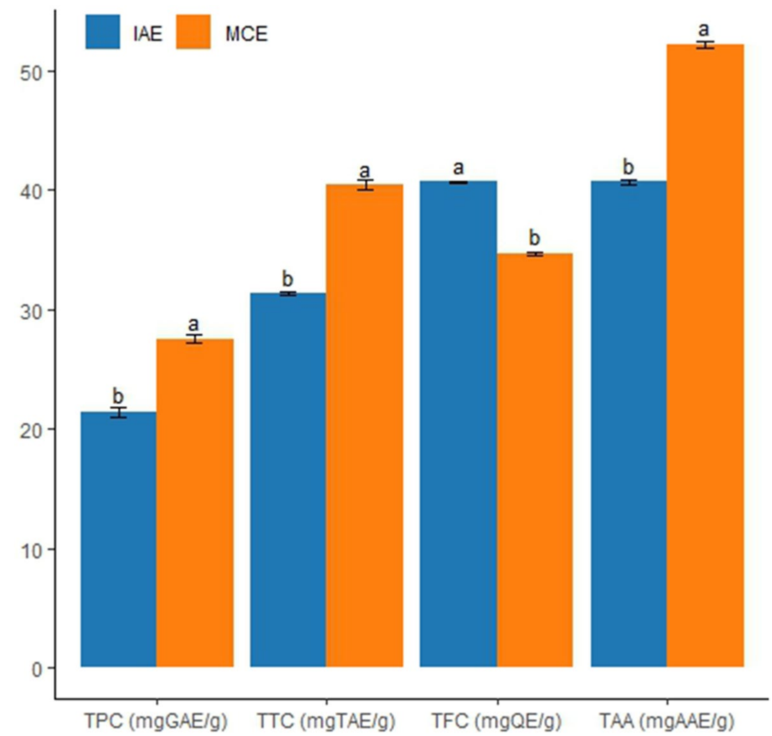


Fig. 1 Total flavonoid content (TFC), total tannin content (TTC), total phenolic content (TPC) and total antioxidant activity (TAA) of ethanolic extract of *M. charantia* and *I. aquatica*. The same letter (s) on the top of the bar of the same experiment did not differ significantly at the 5% level of significance

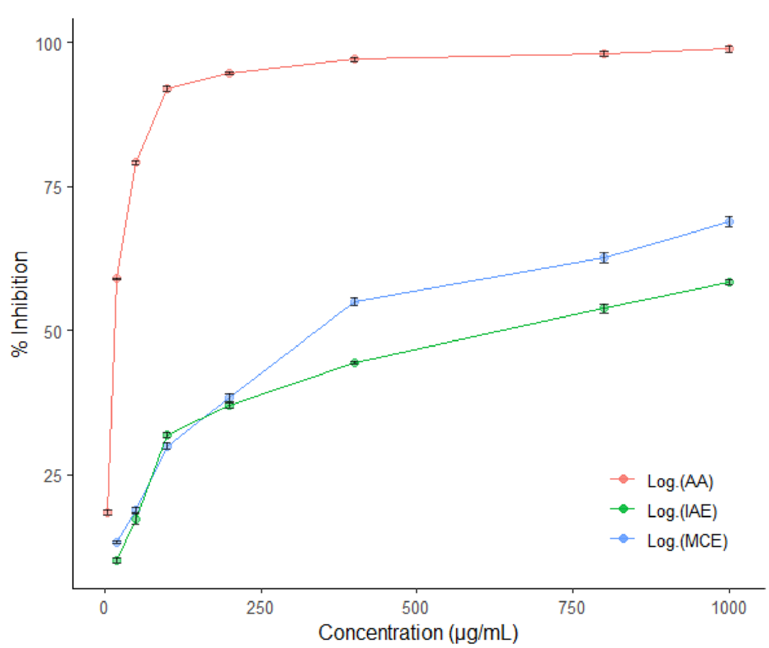


Fig. 2 Logarithmic trendline for DPPH radical- scavenging activity (inhibition concentration) of *M. charantia* (MCE) and *I. aquatica* (IAE) extracts; and ascorbic acid (AA)

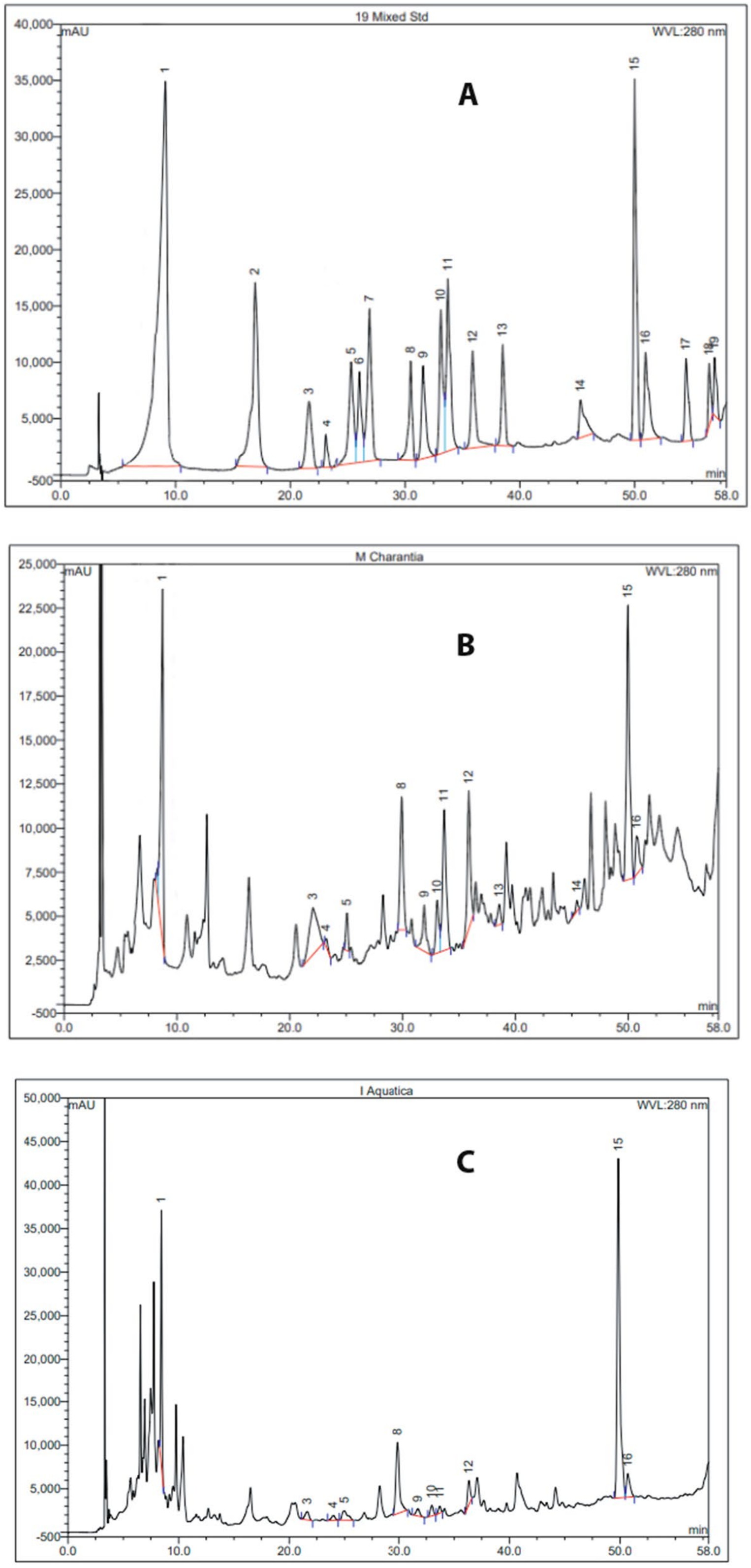


Fig. 3 HPLC–DAD chromatogram of bioactive phenolic compound: **A** Chromatogram of 19 standard phenolic compound, **B** Chromatogram of MCE and **C**) Chromatogram of IAE. Where peak: 1 = gallic acid, 2 = catechol, 3 = chlorogenic acid, 4 = catechin hydrate, 5 = vanillic acid, 6 = caffeic acid, 7 = syringic acid, 8 = (-) epicatechin, 9 = vanillin, 10 = p-coumaric acid, 11 = trans-ferulic acid, 12 = rutin hydrate, 13 = rosmarinic acid, 14 = myricetin, 15 = quercetin, 16 = trans-cinnamic acid, 17 = naringenin, 18 = kaempferol, 19 = apigenin

Table 3 Composition of the polyphenolic compounds in MCE and IAE determined by HPLC–DAD system

Compound	MCE (μg/g dry extract)	IAE (μg/g dry extract)
Gallic acid (GA)	63.75 ± 4.19[a] ± 1.63	69.2 ± 1.15[a]
Catechol (Cat)	nd	nd
Chlorogenic acid (CA)	4018.66 ± 125.8[a]	717.5 ± 13.17[b]
Catechin hydrate (CH)	3298 ± 471.64[a]	4925.75 ± 831.52[b]
Vanillic acid (VA)	202.25 ± 12.03[a]	193.25 ± 72.32[a]
Caffeic acid (CafA)	nd	nd
Syringic acid (SA)	nd	nd
(-) Epicatechin (EC)	4417 ± 550.49[a]	5245.75 ± 855.36[b]
Vanillin (Val)	232.75 ± 75.48[a]	94 ± 10.92[b]
p-Coumaric acid (PCA)	186.5 ± 47.27[a]	88.25 ± 4.99[b]
trans-Ferulic acid (TFA)	4377.5 ± 544.04[a]	371.25 ± 63.41[b]
Rutin Hydrate (RH)	637 ± 17.56[a]	206.63 ± 7.36[b]
Rosmarinic acid (RA)	470.25 ± 61.81	nd
Myricetin (Myr)	47.25 ± 9.91	nd
Quercetin (QH)	4207.5 ± 91.74[a]	10,541.75 ± 957.25[b]
trans-Cinnamic acid (TCA)	493.75 ± 67.7[a]	654.5 ± 103.19[b]
Naringenin (Nar)	nd	nd
Kaempferol (KP)	nd	nd
Apigenin (Api)	nd	nd

The same letter (s) on the top of the value of the same experiment did not differ significantly at the 5% level of significance

nd Not detected

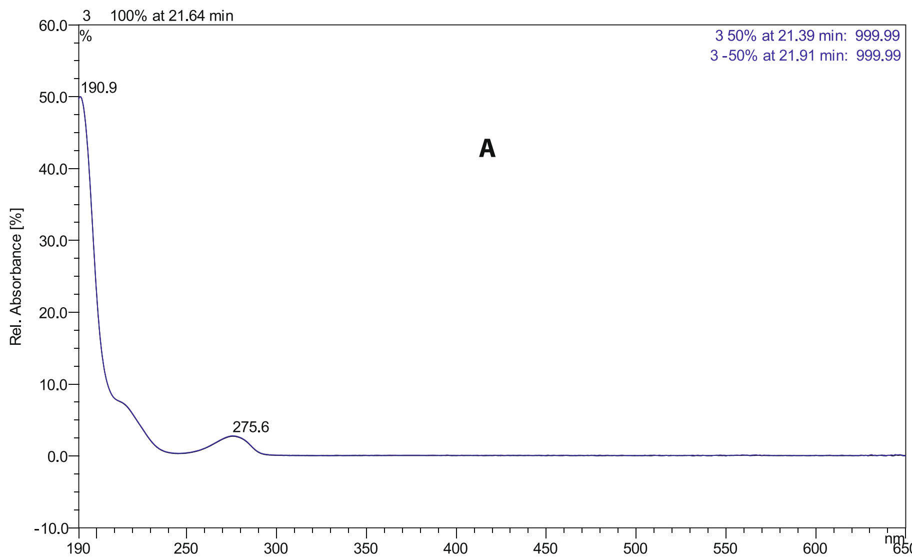
some leafy vegetable of Bangladesh like *A. gangeticus* (Sarker & Oba 2021) and *A. tricolor* (Sarker & Oba 2020f; Sarker and Oba 2018b).

In another study, Omale et al. found that the IC_{50} value of *I. aquatica* methanolic extracts was 672.37 μg/mL, which showed good agreement with this study (James et al., 2009). On the contrary, Dasgupta and De claimed that the IC_{50} value of hot water extract of *Ipomoea reptans* was 131 μg/mL (Dasgupta & De 2007).

IAE had a much higher flavonoid content than MCE, but MCE demonstrated significantly stronger bioactive characteristics in TTC, TPC, and TAA ($P < 0.001$). Additionally, MCE considerably outperformed IAE in terms of free radical scavenging activity ($P < 0.001$). At a 95% level of confidence, there was a significant association between total phenolic content and antioxidant ability ($r = 0.925$). The content of bioactive phyto-constituent depends on the extraction solvent and procedure (Khledkhoudja et al., 2014; Aryal et al. 2019).

Peak purity is a crucial characteristic used in HPLC analysis using PDA detectors to assess a chromatographic peak's quality and purity. The peak purity was measured using Dionix Chromeleon software (Version 6.80 RS 10) in this study and the peak purity match (PPM), serves as the acceptance criteria. The acceptance limit of PPM was > 98% which is indicative of a pure and homogeneous sample peak. For an example, the UV spectrum comparison (standard and sample) of chlorogenic acid was shown in Fig. 4. The photodiode array analysis and peak purity tests were used to show that each chromatographic peak for all standards was due to a single component (Papadoyannis & Gika 2004). Depending on the specific polyphenolic molecule being analysed, the acceptability criteria for peak purity in polyphenolic analysis utilising HPLC PDA detectors may change (Marchelak et al. 2020). The UV–vis measurements were gathered between 190 and 650 nm. Using Chromeleon software, the peak purity was determined based on how comparable the UV spectra of the peak were throughout the whole data collecting range. Advanced Chromeleon software tools enable instant online 3-D data inspection, and cutting-edge peak purity algorithms facilitate speedy peak purity assessment.

Kubola et al. also showed that *p*-coumaric acid, tannic acid, benzoic acid, gallic acid, caffeic acid, and catechin were identified in aqueous extracts of bitter gourd leaf and among them gallic acid (95.8 ± 0.31 μg/mL fraction) was the most predominant phenolic compound (Kubola & Siriamornpun, 2008). In another study conducted by Nagarani et al., it was observed that acidified aqueous methanolic extract of *M. charantia* leaves contained gallic acid (66.5 ± 0.45 mg/100 g), chlorogenic acid (2969 ± 1.80 mg/100 g), catechin (145.3 ± 4.2 mg/100 g) and quercetin (2601 ± 2.4 mg/100 g) (Nagarani et al. 2014). Svobodova et al. reported three phenolic acid derivatives (hydroxycinnamic acid derivatives) and eleven flavonoids (flavonol glycoside derivatives) in aqueous ethanolic extract (Svobodova et al. 2017). Several other works mentioned the presence of rutin, catechin, epicatechin, and epigallocatechin in *M. charantia* fresh leaves (Choi et al. 2012; Jia et al. 2017; Kenny et al. 2013; Kubola & Siriamornpun 2008). This difference in the content of bioactive phenolic compounds might be due to the origin and extraction procedures of the plant materials and analytical procedures. Shodehinde et al. also reported that some phenolic acids such as gallic acid, catechin, chlorogenic acid, caffeic acid, ellagic acid, epicatechin, and flavonoids such as quercetin, isoquercitrin, kaempferol, and rutin were present in the extract of *M. charantia* (Shodehinde et al. 2016). Hefny et al. confirmed the presence of nicotiflorin, ramnazin-3-O-rutinoside and dihydroxybenzoic acid pentoside by QTOF-MS and NMR in the extract of *I. aquatica* (Hefny et al. 2018).



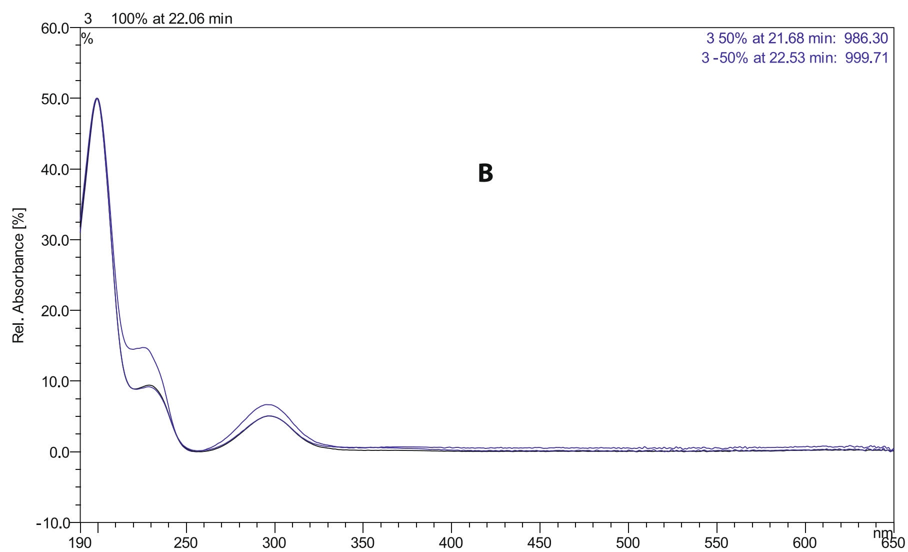


Fig. 4 UV spectrum comparison of compound analyzed by HPLC–DAD. As example- Chlorogenic acid: **A** standard and **B** sample

Bioactive phenolic compounds are the major contributors to the potent antioxidant capacity of the extracts. This investigation revealed the availability of polyphenolic compounds, which greatly support the traditional use of these leaves as folk medicine for different health ailments like hepatoprotective, antioxidant, anti-microbial, cardioprotective, anti-inflammatory, antipyretic, neuroprotective, anti-obesity, immunostimulating, and anti-analgesic activity. The molecular structure, availability, and functional properties of secondary metabolites (polyphenols) available in MCE and IAE were reviewed in Table 4. *M. charantia* and *I. aquatica* are two abundantly available green leafy vegetables that could be an important source of phenolic compounds for local residents to maintain good health as well as a source of raw materials for the development of new functional products.

The Pearson correlation analysis ($P<0.05$) uncovered some previously unknown connections between the parameters (Fig. 5). There was a significant negative connection between gallic acid and myricetin. Chlorogenic acid was found to have a strong positive association with vanillin, p-coumaric acid, trans-ferulic acid, rutin hydrate, rosmarinic acid, and myricetin. Whereas,

Table 4 Discussion on the functional properties of secondary metabolite available in MCE and IAE

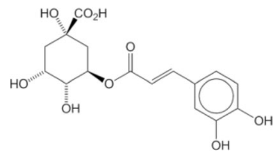
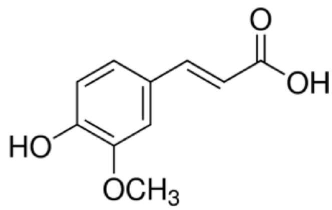
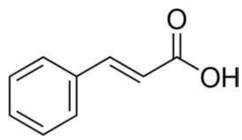
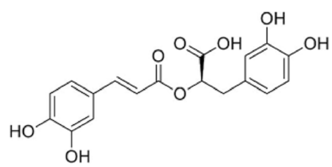
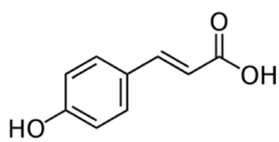
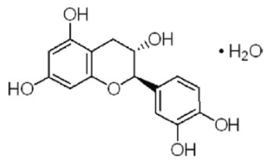
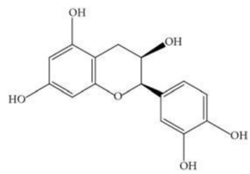
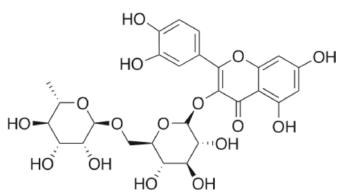
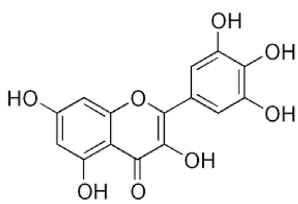
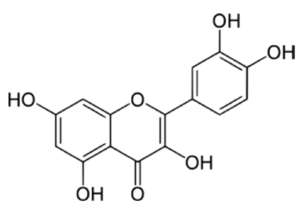
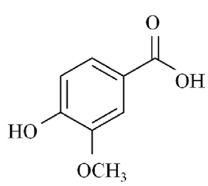
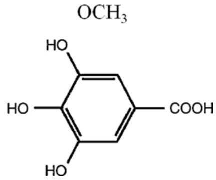
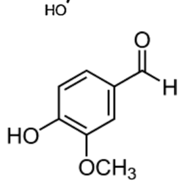
Group of Phenolic compounds	Phenolic compounds	Molecular Structure	Functional characteristic	Availability
Hydroxycinnamic acid	Chlorogenic Acid		hepatic steatosis, anti-carcinogenic anti-diabetic, anti-inflammatory, anti-obesity (Naveed et al. 2018; Tajik et al. 2017)	MCE < IAE
	trans-Ferulic acid		antithrombotic, vasodilatory effect, antioxidant, increase the viability of sperms, anticarcinogenic, antiallergic, anti-inflammatory, antimicrobial, hepatoprotective, antiviral (Kumar & Pruthi 2014)	MCE > IAE
	trans-Cinnamic acid		antioxidant, anti-inflammatory, anti-cancer activities, anti-diabetic (Adisakwattana 2017)	MCE < IAE
	Rosmarinic acid		anti-inflammatory, anti-diabetic,, anti-inflammatory,anti-allergic, renal and hepato protectant (Alagawany et al. 2017)	MCE
	p-Coumaric acid		antipyretic, anti-inflammatory, antiplatelet aggregation, anxiolytic, analgesic, anti-arthritis activities (Pei et al. 2016)	MCE > IAE

Table 4 (continued)

Group of Phenolic compounds	Phenolic compounds	Molecular Structure	Functional characteristic	Availability
Flavonoids	Catechin hydrate		potential chemo preventive agent, liver damage prevention, cholesterol lowering, anti-obesity, inhibiting the ovarian cancer (Lee et al. 2007; Kim & Heo 2022)	MCE < IAE
	(-) Epicatechin		prevention and treatment of Parkinson's disease, anti-diabetic, anti-carcinogenic (Abdulkhaleq et al. 2017)	MCE < IAE
	Rutin Hydrate		improve blood circulation,, antidepressant, neuroinflammation prevention, anticonvulsant (Ganeshpurkar & Saluja, 2017; Imani et al. 2021)	MCE > IAE
	Myricetin		antidiabetic, anticancer, anti-amyloidogenic, anti-inflammatory (Semwal et al. 2016)	MCE
	Quercetin		antiviral, anti-carcinogenic, reducing the risk of heart disease, anti-inflammatory, preventing neurological disorder (Li et al. 2016; Yang et al. 2020)	MCE < IAE
Hydroxybenzoic acid	Vanillic acid		Antioxidant, anti-inflammatory, anti-analgesic (Calixto-Camposet al. 2015; Sharma et al. 2020)	MCE > IAE
	Gallic acid		antihyperlipidemic, antioxidant, antineoplastic properties, cardioprotective, anti-inflammatory (Kahkeshani et al. 2019; Zanwar et al. 2014)	MCE < IAE
Phenolic aldehyde	Vanillin		anti-cancer, antioxidant, anti-inflammatory, antiviral, antibacterial, neuroprotective activity, antifungal, sickle cell anaemia recovery (Arya et al. 2021; Costantini et al. 2021)	MCE > IAE

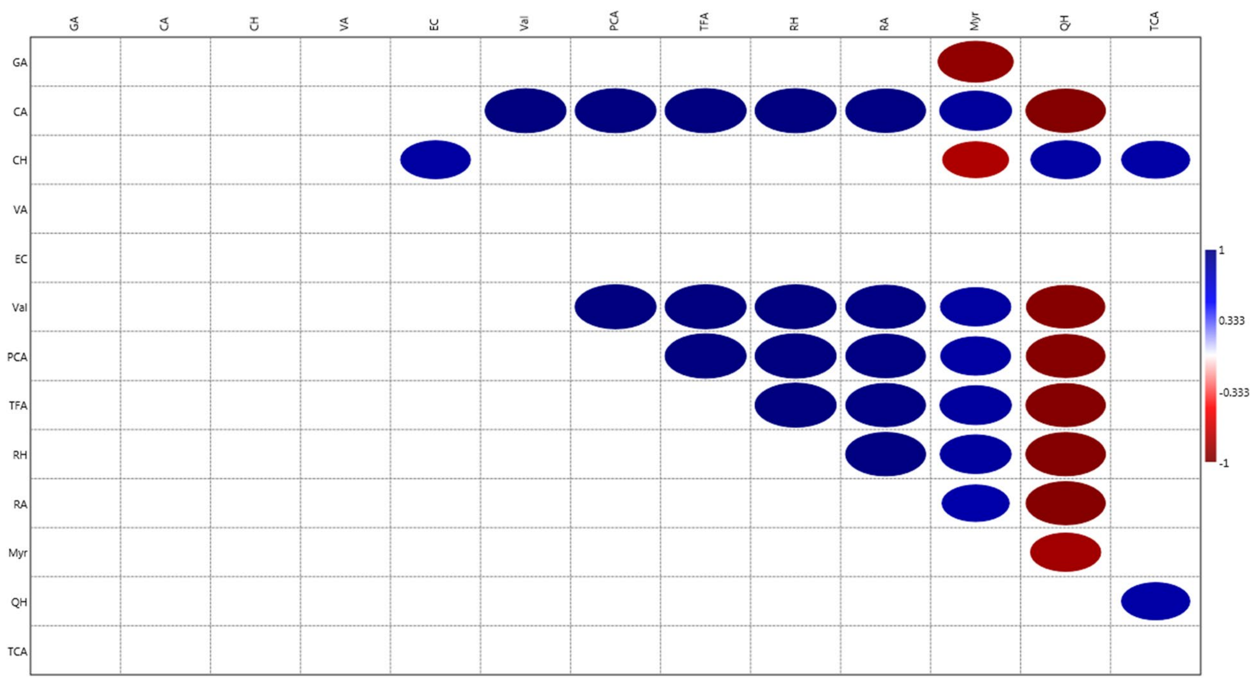


Fig. 5 Pearson's correlation analysis of polyphenolic compounds found in the extract of *M. charantia* and *I. aquatica*. Where, $p < 0.05$ and gallic acid = GA, chlorogenic acid = CA, catechin hydrate = CH, vanillic acid = VA, (-) epicatechin = EC, vanillin = Val, *p*-coumaric acid = PCA, *trans*-ferulic acid = TFA, rutin hydrate = RH, rosmarinic acid = RA, myricetin = Myr, quercetin = QH, *trans*-cinnamic acid = TCA

quercetin had a strong inverse connection with chlorogenic acid, vanillin, p-coumaric acid, trans-ferulic acid, rutin hydrate, rosmarinic acid, and myricetin, and a positive association with catechin hydrate and trans-cinnamic acid, respectively. At the same time, RH was found to have a strong positive association with vanillin, p-coumaric acid, and trans-ferulic acid. Catechin hydrate was positively correlated with epicatechin, quercetin, and trans-cinnamic acid. A strong correlation was also observed between p-coumaric acid and vanillin in the plant extract.

Conclusion

In the course of our research, we found that the ethanolic extracts of *M. charantia* and *I. aquatica* demonstrated significant antioxidant activity against free radicals. This study also showed that *M. charantia* and *I. aquatica* leaves contained a wide variety of bioactive polyphenolic compounds having documented medicinal and nutraceutical activity. The leaf extract of *I. aquatica* could be regarded a source of flavonols, such as quercetin, as well as flavan-3-ol, which includes catechins and epicatechin. The extract of the leaves of *M. charantia* is a potential source of flavonols: quercetin; flavan-3-ol: catechins and epicatechin; and hydroxycinnamic acid: chlorogenic acid and ferrulic acid. Further research is needed to investigate the various solvent systems present in these green crops to identify additional beneficial compounds. The ethanolic extract may decide to manufacture functional food by highlighting the major bioactive polyphenols in to promote the health benefits of such a source of green leafy vegetables.

Abbreviations

AAE	Ascorbic acid equivalent
DPPH	2,2-Diphenyl-1-picrylhydrazyl
GAE	Gallic acid equivalent
HPLC-DAD	High-Performance Liquid Chromatography with Diode-Array Detection
IAE	*Ipomoea aquatica* Leaf extract
IC	Inhibition Concentration
MCE	*Momordica charantia* Leaf extract
TAA	Total antioxidant activity
TAE	Tannic acid equivalent
TFC	Total flavonoid content
TPC	Total phenolic content
QE	Quercetin equivalent

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Authors' contributions

ATMA: Conceptualization, Methodology, Investigation, Writing—Original Draft, Visualization and Formal analysis MMR: Formal analysis and Writing—Review & Editing, MS: Formal analysis and Writing—Review & Editing, TAK: Formal analysis and Validation, SNI: Resources, Writing—Review & Editing and Project administration.

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Availability of data and materials
The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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