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Effect of extraction solvent on the recovery of bioactive compounds and antioxidant activity in Momordica Charantia: Towards sustainable processes

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Abstract: *Momordica charantia*, a climbing plant of the Cucurbitaceae family, has recognized potential in traditional medicine. Ethanolic and aqueous extracts from ripe fruits, seeds, roots, stems, and leaves were analyzed. Qualitative phytochemical analysis was performed following standard protocols, while quantitative phenol and flavonoid contents and antioxidant activity were assessed by colorimetric methods (ABTS and DPPH assays). Significant differences in compound concentration and composition were observed, depending on the plant part and solvent used. Secondary metabolites detected included saponins, catechins, flavonoids, cardiotonic glycosides, alkaloids, free amino acids, reducing sugars, and terpenoids. The ethanol extract of ripe fruit rind showed the highest phenol content (53.42 ± 3.62 mg GAE/g DW), while the ethanol extract of leaves had the highest flavonoid content (62.69 ± 1.87 mg QE/g DW). Ripe fruit ethanolic extracts also exhibited the strongest antioxidant activity, with DPPH and ABTS values of 868.41 ± 39.99 and 857.41 ± 12.60 µmol Trolox equivalents/g DW, respectively. These results confirm that ethanol is a highly effective solvent for extracting antioxidant compounds from M. charantia. In this way, the integration of sustainable cultivation practices and ecological extraction processes could contribute to the generation of nutraceutical and pharmaceutical products with lower environmental impact, promoting sustainable value chains.

Keywords: *Extraction solvents, Extracts, Maceration, Phytochemicals.*

1. Introduction

Momordica charantia, a Cucurbitaceae species known as achochilla or bitter melon, is a climbing plant with a history of medicinal use in traditional systems across Asia, Africa, and the Americas. Its bioactive compounds contribute to its therapeutic applications in managing chronic diseases such as diabetes, infections, cancer, and cardiovascular and gastrointestinal disorders [1, 2].

Secondary metabolites present in M. charantia including phenols, flavonoids, saponins, terpenoids, and alkaloids, are key contributors to its reported pharmacological activities, such as anti-inflammatory, antimicrobial, anticancer, and antioxidant effects [1, 3, 4]. However, the concentration and composition

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of the bioactive compounds may vary depending on the plant material and the extraction solvent used. Consequently, the choice of solvent is crucial in terms of process efficiency. Polar solvents such as water and ethanol are common for extracting a wide range of secondary metabolites [5, 6].

Phytochemical compounds can be identified and quantified by advanced analytical techniques such as high-performance liquid chromatography, gas chromatography, mass analysis, and nuclear magnetic resonance spectroscopy. However, UV-Visible spectroscopy remains a useful and widely applied tool for identifying and quantifying phenolic compounds and flavonoids [7-9].

The aim of this study was to characterize the phytochemical and functional extracts obtained from different parts of M. charantia (ripe fruit rind, seeds, roots, stems and leaves) using ethanol and water as extraction solvents. Bioactive compounds were identified using UV-Vis spectroscopy. Antioxidant capacity was measured and their respective total phenolic and flavonoid contents were determined. This data will contribute to a better appreciation of the therapeutic benefits of M. charantia and will provide crucial information to advance the extraction of phytopharmaceuticals and nutrapharmaceuticals from this plant, in line with sustainable approaches and circular economy principles.

2. Materials and Methods

2.1. Plant Material

Plant samples of M. charantia were collected at the Research and Technology Transfer Center "Los Pinos", located in Chone canton, Manabí province, Ecuador. The coordinates of the sampling points are presented in Figure 1.

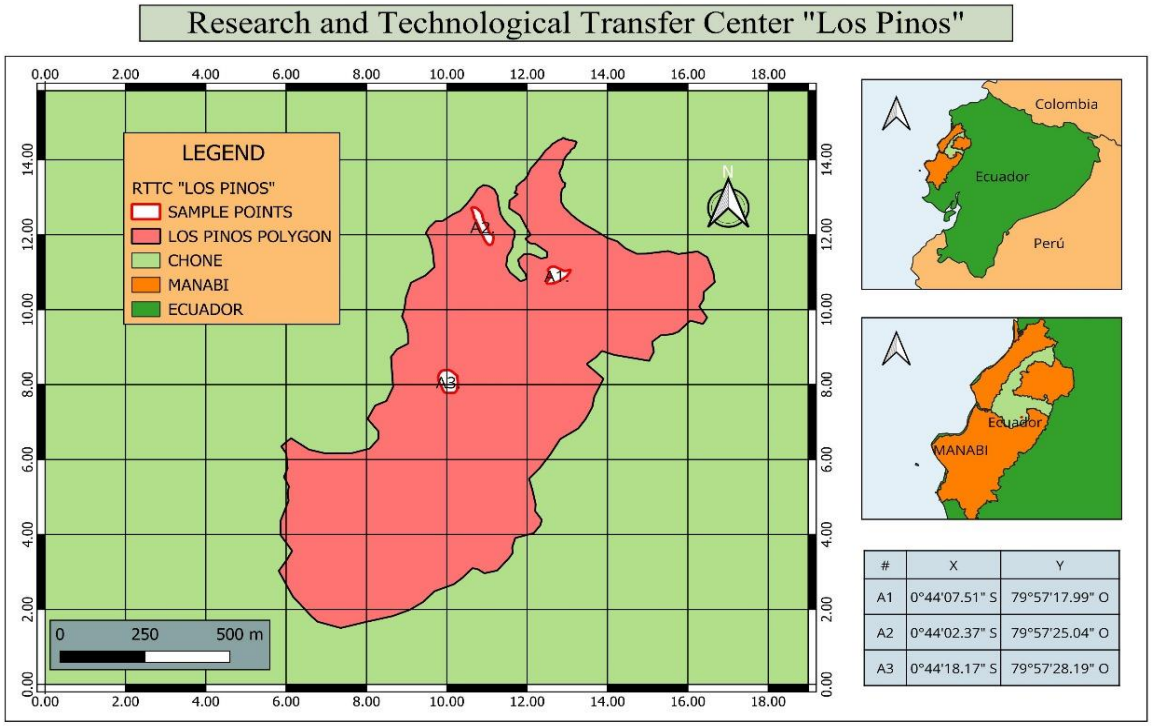

Figure 1.
Location of the Research and Technology Transfer Center 'Los Pinos' in Manabí, Ecuador. The coordinates A1, A2, and A3 were the sampling points. Image obtained with Q-GIS.

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2.2. Preparation of Plant Extracts

The collected plant material of M. charantia was classified into ripe fruit bark, seeds, roots, stems and leaves. The classified material was washed with running water and finally rinsed with distilled water, then dried at 35°C for 96 hours in a forced air drying oven until the plant material reached a humidity of 10 ± 2 %, and ground in a knife mill at 25,000 rpm.

Extracts from different plant structures (ripe fruit, bark, seeds, roots, stems and leaves) were obtained by ultrasound-assisted maceration. Sterile water and 96% ethanol were used as solvents for the extraction of secondary metabolites. Mixtures of plant material and solvent in a 1:10 ratio were prepared in amber flasks and placed in an ultrasound bath (ISOLAB, Model:621.05.006) for 1 hour at 35°C and then left to macerate for 72 hours at 35 ± 1°C and 60 rpm in a shaking incubator (STUARD, Model:SI500). After the maceration period, the samples were filtered with Whatman No. 1 filter paper and stored in amber containers at 4°C (Figure 2).

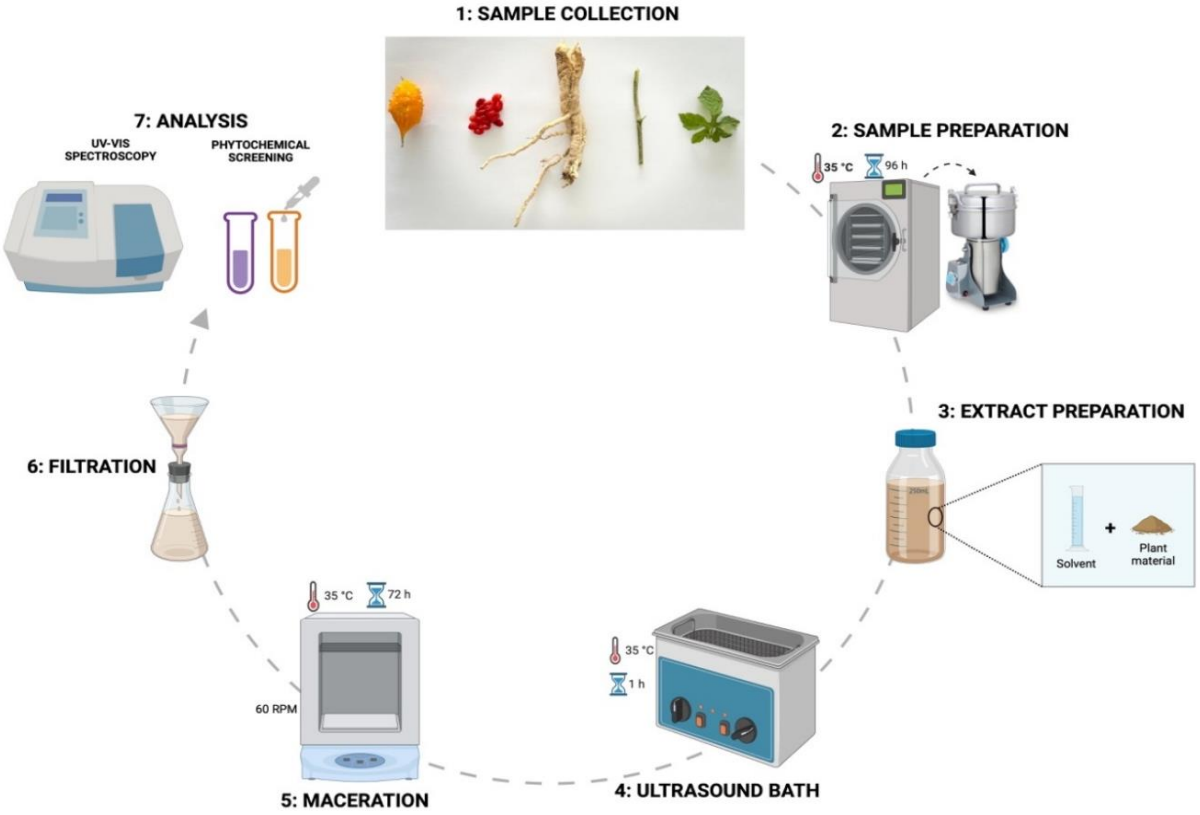

Figure 2.
Graphic representation of the process of obtaining ethanol and aqueous extracts of ripe fruits, seeds, roots, stems and leaves of M. charantia created in BioRender.

2.3. Chemicals and Reagents

Analytical grade reagents and chemicals used in this study were: Folin-Ciocalteu reagent, 1,1-diphenyl-2-picryl-hydrazyl (DPPH), (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) (ABTS), Trolox (6-hydroxy-2,5,7,8-tetramethyl-3,4-dihydro-2H-1-benzopyran-2-carboxylic acid), sodium carbonate, gallic acid, ferric chloride (Sigma-Aldrich), methanol, hydrochloric acid, acetic acid, and absolute ethanol (Supelco). Other chemicals were obtained from local suppliers.

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2.4. Phytochemical Screening of Extracts

A phytochemical analysis was conducted on the plant extracts to identify the secondary metabolites present in M. charantia The methodologies used are derived from those described by Alqethami and Aldhebiani [10] and Valarmathi, et al. [4] with some modifications, where the following were analyzed: alkaloids (Dragendorff test), tannins (Braymer test), flavonoids (Shinoda test), reducing sugars (Fehling test), triterpenes (test Liebermann-Burchard), catechins (UV development), saponins (foam test), free amino acids (test Ninhydrin) and cardiac glycosides (Kedde test). These tests employ colorimetric assays in which functional groups interact with specific chemical reagents, generating distinctive reactions [11].

2.5. Determination of Total Phenolic Content

Ethanol and aqueous extracts of bark, leaves, seeds and roots was determined using the Folin-Ciocalteu colorimetric method, according to the methodology described by Kupina, et al. [12] with some modifications. From the dilutions of the extracts, according to their characteristics, a volume of 200 µL per extract were taken, mixed with 1500 µL of distilled water and 100 µL of Folin-Ciocalteu reagents in a 2 mL microcentrifuge tube. Then, after allowing the reaction to proceed for five minutes under ambient conditions, 200 µL of a 20% (w/v) sodium carbonate solution was added. The samples were vortexed for 30 seconds and left to incubate in the dark for 30 min at room temperature. The absorbance at 760 nm was recorded using a spectrophotometer (Thermo Scientific GENESYS 180 UV-Vis). The values were obtained using a calibration curve, where gallic acid served as the standard at concentrations of 5, 10, 20, 40, 80, and 160 mg L^{-1}. The resulting equation was $y = 0.017x + 0.028$, with a coefficient of determination of 0.999. The values were expressed in milligrams of gallic acid equivalent (GAE) per gram of dry weight (mg GAE/g DW).

2.6. Determination of Flavonoid Content

Total flavonoid contents were determined using the aluminum chloride method using quercetin as standard, according to a method described by Matić, et al. [13] with some modifications. 200 µL of the previously prepared sample dilution using methanol as solvent was used, then 800 µL of 70% (v/v) methanol was added to the dilution, and then homogenized in a vortex for 10 seconds, then 1000 µL of a 2% aluminum chloride ($AlCl_3$) solution in 5% (v/v) acetic acid in 2% (v/v) methanol of aluminum chloride was added in a microcentrifuge tube (2 ml). After 15 minutes of incubation in the dark at room temperature, the absorbance of the sample was measured at 430 nm (Thermo UV-Vis Spectrophotometer Scientific GENESYS 180). The values were obtained using a calibration curve in which quercetin was used as a standard with concentrations of (5, 10, 20, 40, 80, 100) mg L-1 obtaining the equation: $y= 0.026x + 0.034$ with a coefficient of determination of 0.997. The total flavonoid content was expressed as milligrams of quercetin equivalents (QE) per gram of dry weight (mg QE/g DW).

2.7. Determination of Antioxidant Activity

To assess the antioxidant activity, the ABTS and DPPH methods were applied, which measure the suppression of the free radical DPPH• (2,2-diphenyl-1-picrylhydrazyl) and ABTS•+ (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)), following a protocol adapted from Cano and Arnao [14] with some modifications. For the ABTS assay, an aqueous solution containing 7 mM ABTS was mixed in equal proportions with a 2.45 mM potassium persulfate solution and left to react under light-protected conditions for 18 hours at ambient temperature to generate the radical. Similarly, the DPPH radical was prepared by dissolving DPPH in methanol to obtain a 0.1 mM solution, which was also incubated in the absence of light at ambient temperature for 18 hours. Volumes of 1 mL of the dilutions were then taken according to the intrinsic characteristics of each extract, then 1 mL of the working solution of each of the radicals was added and they were homogenized and then incubated for 1 hour at room temperature and darkness.

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The solutions were measured in a spectrophotometer (Thermo Scientific GENESYS 180 UV-Vis) at a wavelength of 734 nm for ABTS and 517 nm for DPPH, the values were obtained by calibration curves in which Trolox was used as a standard for ABTS with concentrations of (2.5, 5, 10, 15, 25) µM obtaining the equation: y= -0.012x + 0.375 with a coefficient of determination of 0.998. and for DPPH with concentrations of (1.25, 2.5, 5, 10, 15, 20, 25) µM obtaining the equation: y= -0.0069x + 0.408 with a coefficient of determination of 0.999. The results of the antioxidant activity were expressed in micromoles equivalent of Trolox corresponding to each gram of dry weight (µmol Trolox equivalents/g DW).

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2.8. Experimental Design

A 2x5 Completely Randomized Factorial Experiment (CRFE) was applied. The study factors were: the type of extract with 2 levels (alcoholic and aqueous) and the plant structures of M. charantia with 5 levels (ripe fruits, seeds, roots, stems and leaves), thus forming 10 treatment combinations, with three replicates per treatment, generating a total of 30 experimental units. The response variables were the phenolic content, flavonoid content and antioxidant activity in the extracts.

2.9. Statistical Analysis

The data are presented as mean ± standard deviation (SD) of three experiments (n = 3). A one-way analysis of variance (ANOVA) was performed to evaluate significant statistical differences in the mean values of total phenolic content, flavonoid content, and antioxidant activity in M. charantia extracts. Normality was assessed through the Shapiro-Wilk test, while variance homogeneity was assessed with Levene's test. Tukey's HSD test was applied for multiple comparisons at a 95% confidence level. Data analysis was conducted using SPSS software version 29.0.2.0.

3. Results and Discussion

3.1. Phytochemical Content

Table 1 shows the presence and absence of 8 groups of secondary metabolites in extracts from different parts of M. charantia (ripe fruit rind, seeds, roots, stems and leaves).

The results revealed a considerable variability in the presence of various phytochemical groups depending on the type of solvent used (ethanol or aqueous) as well as the plant material used. Thus, in all plant structures the ethanol solvent was able to capture triterpenes with a strong presence, which were absent in the aqueous extract, while cardiotonic glycosides, in lower presence, were only present in aqueous extracts of fruits, roots and stems. It is also possible to indicate that reducing sugars were present more in aqueous extracts than in alcoholic extracts.

Phytochemical groups were identified in the extracts from the peel of ripe fruits, six in the extracts with ethanol solvent and six in the extracts with aqueous solvent. A strong presence of alkaloids and free amino acids was observed in both aqueous and alcoholic extracts, while flavonoids and saponins stood out with a moderate presence in the aqueous extract.

Phytochemical groups were identified in the seed extracts: four in extracts with ethanol solvents and four in extracts with aqueous solvents. As in the fruit peel extract, alkaloids and free amino acids showed a strong presence in both types of extracts. In addition, saponins were especially abundant in extracts with aqueous solvents.

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Table 1.
Phytochemical analysis of extracts from ripe fruits, seeds, roots, stems and leaves of M. charantia.

Phytochemical Groups	Ripe Fruit		Seeds		Roots		Stems		Leaves	
	Ethanol	Water	Ethanol	Water	Ethanol	Water	Ethanol	Water	Ethanol	Water
Saponins	+	++	-	+++	++	++	++	++	+	+
Catechins	-	-	-	-	-	-	-	-	-	+
Flavonoids	+	++	-	-	-	+	+++	+++	+++	+++
Cardiotonics	-	+	-	-	-	+	-	+	-	-
Alkaloids	++	+++	+++	+++	+	++	+	+++	+++	+++
Free Amino Acids	+++	+++	+++	+++	+++	+++	+++	+++	-	+++
Sugar Reducers	+	+	+	+++	+	+++	+	++	-	-
Triterpenes	+++	-	+++	-	+++	-	+++	-	+++	-

Note: +++ (Strong presence); ++ (Moderate presence); + (Low presence); and − (Absence).

Regarding root extracts, six phytochemical groups were detected: five in extracts with ethanol solvents and six in extracts with aqueous solvents. In these extracts, alkaloids and free amino acids were particularly prominent in extracts treated with aqueous solvents, as were triterpenes in extracts treated with ethanol solvents. Saponins were moderately present in both types of extracts.

In the stem extracts, seven phytochemical groups were found, six in the extracts with ethanol solvents and six in the extracts with aqueous solvents. Flavonoids stood out with high concentration, especially in the extracts with aqueous solvents. Moderate to high levels of saponins, alkaloids and free amino acids were also observed. Triterpenes, as mentioned above, showed a high presence in the extracts obtained with ethanol.

Finally, in the leaf extracts, five phytochemical groups were identified, three in extracts with ethanol solvents and four in the extracts with aqueous solvents. Flavonoids were strongly present in both extracts, while alkaloids and free amino acids were found in high concentrations, especially in the extracts with aqueous solvents. In contrast, catechins were detected in low amounts in the extracts with aqueous solvents, highlighting the variability in the phytochemical profile of the leaves compared to other tissues.

The results show a variable distribution of phytochemical groups and their concentrations in the different plant structures of M. charantia. However, an apparently uniform distribution of saponins, alkaloids, free amino acids, and triterpenes was observed in all the parts of the plant analyzed. Likewise, flavonoids prevail in the aerial parts, such as fruits, stems and leaves. In particular, catechins are only present in aqueous leaf extracts in very low quantities. This variability is consistent with that reported in several studies and influences the functional and biological properties that the different extracts may display [15-17].

3.2. Total Phenols and Flavonoids Content

The results showed the presence of total phenolic compounds (Figure 3) and flavonoids (Figure 4) in the extracts of M. charantia obtained using ethanol and aqueous solvent. These demonstrated significant statistical differences in all treatments for both bioactive compounds.

In general, the extracts obtained with the ethanol solvent stand out for their content of total phenolic compounds and flavonoids. For these extracts, the highest values of total phenolic compounds were detected in the extracts from ripe fruit peels (53.42 mg GAE/g DW) and stems (47.92 mg GAE/g DW). Meanwhile, in the flavonoid content, the leaf extracts stood out (62.69 mg QE/g DW) followed by the stem extracts (11.31 mg QE/g DW), results in accordance with what was shown in the phytochemical analysis described in Table 1.

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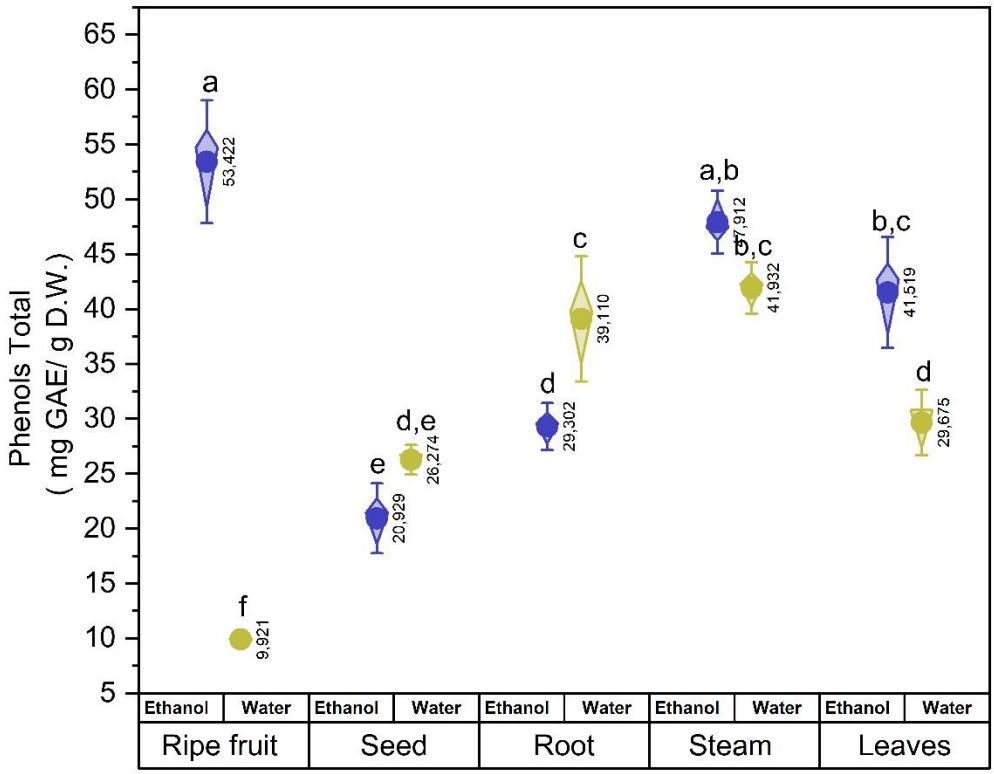

Figure 3.
Interaction of ethanol and aqueous solvents with M. charantia plant material on the mean phenols total (mg GAE/g DW). Error bars indicate standard deviation (± SD). Different lowercase letters indicate significant differences between treatments (Tukey's HSD, $p < 0.05$).

However, the content of total phenolic compounds in the aqueous extracts of seeds and roots was higher than that of the ethanol extract of both parts. Likewise, in the content of flavonoids, the aqueous extract of roots was the exception, since this extract obtained a higher value of flavonoids than the ethanol extract of roots. The latter is related to the phytochemical analysis carried out on the extracts, where the aqueous extract of roots registered the presence of flavonoids, while in the ethanol extract they were not detected qualitatively.

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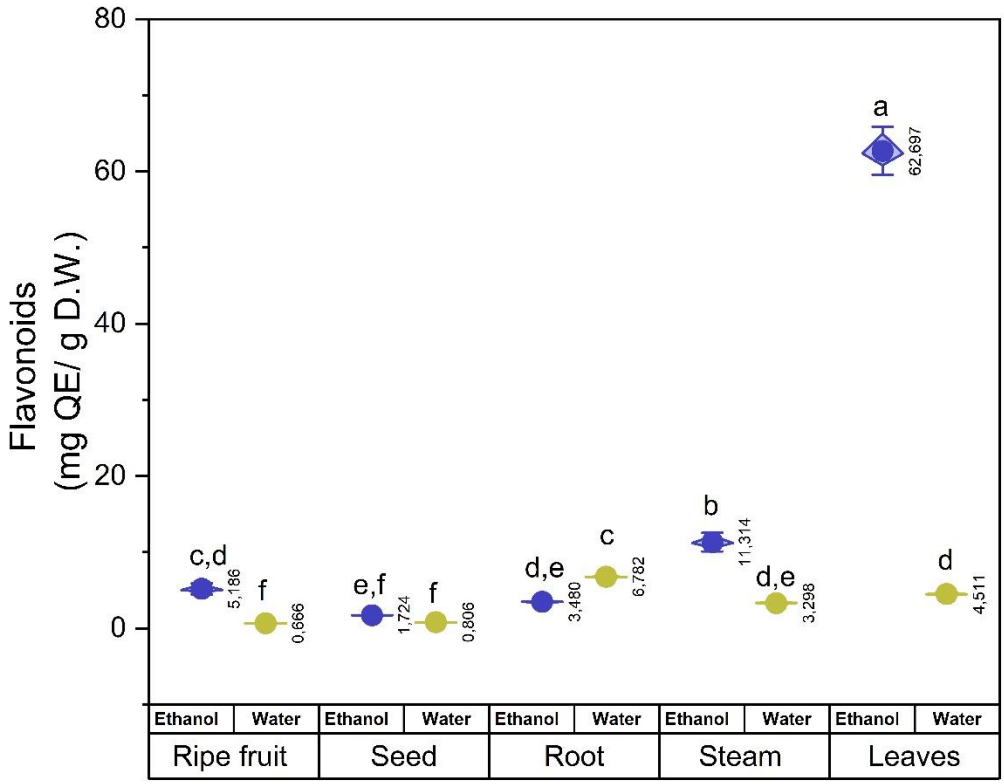

Figure 4.
Interaction of ethanol and aqueous solvents with M. charantia plant material on the mean flavonoids (mg QE/g DW). Error bars indicate standard deviation (± SD) Different lowercase letters indicate significant differences between treatments (Tukey's HSD, $p < 0.05$)

In relation to the presence of total phenols recorded in other cucurbit extracts, as in this study, the peel of the fruit is where the highest concentration of total phenols and flavonoids have been extracted, using ethanol as a solvent [18, 19].

The phenolic content found in *M. charantia* is higher than the total phenolic content of zucchini peels (*Cucurbita pepo;* 3.12 ± 0.06 mg GAE/g DW), cucumber peel (*Cucumis sativus;* 23.08 mg GAE/g), snake gourd leaves (*Trichosanthes cucumerina;* 32.2 ± 0.49 mg GAE/g DW), watermelon (*Citrullus lanatus;* 215 ± 1.24 mg GAE/100 g); melon seed (615 to 850 mg GAE/kg). However, it is lower than the total phenolic content of other cucurbits such as pumpkin peel extract (*Cucurbita maxima*; 55.91 ± 5.67 to 336.19 ± 0.89 mg GAE/g) [18-23].

The flavonoid content shown by the ethanol extracts of the leaves and stems is higher than the flavonoid content of extracts from cucurbits, snake gourd leaves (*Tricosanthes cucumerina;* 7.82 ± 0.67 mg QE/g DW), cucumber peel (*Cucumis sativus*; 14.02 mg QE/g) [19, 20].

These results highlight the importance of choosing a specific solvent to extract these types of components and the plant material from which they are extracted [18]. The variability of concentrations in the different parts of the plant is related to the physiological role of the different plant structures in response to the different environmental factors and their survival [17].

3.3. Total Antioxidant Capacity

The antioxidant capacity of M. charantia extracts by ABTS and DPPH methods are shown in Table 2. The extracts of the ripe fruits in ethanol showed higher values in both methods as a reflection of the

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high phenolic content recorded, reaching 857.41 µmol Trolox equivalents/g DW in the ABTS assay and 868.41 µmol Trolox equivalents/g DW in the DPPH assay. In contrast, the same plant material with aqueous solvent presented a significantly lower antioxidant activity, with values of 32.71 µmol Trolox equivalents/g DW and 11.61 µmol Trolox equivalents/g DW for the ABTS and DPPH assays, respectively.

The seed extracts in ethanol also demonstrated a high antioxidant capacity despite their lower total phenol content compared to the other plant structures. These extracts showed values of 703.78 µmol Trolox equivalents/g DW in the ABTS assay and 301.76 µmol Trolox equivalents/g DW in the DPPH assay. However, when using aqueous solvent, the antioxidant activity decreased significantly reaching values of 35.09 µmol Trolox equivalents/g DW in the ABTS assay and 113.41 µmol Trolox equivalents/g DW in the DPPH assay.

Table 2.
Antioxidant capacity by ABTS and DPPH methods of extracts of ripe fruits, seeds, roots, stems and leaves of *M. charintia*.

Plant material	Solvent	ABTS (µmol Trolox equivalents/g DW)	DPPH (µmol Trolox equivalents/g DW)
Ripe fruits	Ethanol	857,41 ±12,60 a	868,41 ± 39,99 a
	Water	32,71 ± 1,80 g	11,61 ± 0,80 g
Seeds	Ethanol	703,78 ± 18,88 b	301,76 ± 9,41 b
	Water	35,09 ± 3,28 fg	113,41 ± 1,30 e
Roots	Ethanol	57,30 ± 1,90 ef	50,30 ± 3,04 f
	Water	43,91 ± 1,56 fg	28,03 ± 1,23 fg
Stems	Ethanol	208,63 ± 7,06 d	151,62 ± 10,21 d
	Water	77,63 ± 2,16 e	46,88 ± 2,70 f
Leaves	Ethanol	497,33 ± 38,03 c	117,86 ± 12,15 e
	Water	207,14 ± 11,34 d	181,86 ± 31,45 c

Source: Mean ± standard deviation. Different lowercase letters indicate significant differences between treatments (Tukey's HSD, $p < 0.05$).

On the other hand, roots, stems and leaves showed a lower antioxidant capacity compared to mature fruits and seeds. The ethanol extracts of roots reached values of 57.30 µmol Trolox equivalents/g DW. in the ABTS assay and 50.30 µmol Trolox equivalents/g DW in the DPPH assay, while the aqueous extracts presented values of 43.91 µmol Trolox equivalents/g DW in the ABTS assay and 28.03 µmol Trolox equivalents/g DW in the DPPH assay.

Finally, leaf extracts in ethanol stood out with moderate activity, obtaining values of 497.33 µmol Trolox equivalents/g DW. in the ABTS assay and 117.86 µmol Trolox equivalents/g DW in DPPH assay. The aqueous leaf extracts also showed considerable antioxidant capacity of 207.14 µmol Trolox equivalent /g DW in ABTS assay and 181.86 µmol Trolox equivalents/g DW in DPPH assay.

Thus, the ethanol extracts of the aerial parts of M. charantia presented an antioxidant activity that even surpassed the activity recorded for substances recognized for this functionality, such as the extract of Jamaican flower (*Hibiscus sabdariffa*) of 143 µmol Trolox equivalents/g [24]. These results allow presenting the ethanol extracts of M. charantia as a natural source of antioxidants for therapeutic and nutraceutical purposes.

4. Conclusions

The present study confirms that the extraction solvent has a significant impact on the efficiency of obtaining bioactive compounds from M. charantia. Results demonstrate that ethanol facilitates the most efficient extraction of phenols and flavonoids, with the highest levels detected in ripe fruits and stems. In addition, the type of plant material used in the extraction significantly influences the content of bioactive compounds and antioxidant activity, which was manifested in greater proportion in ripe fruits and seeds.

The use of *M. charantia* in obtaining bioactive compounds represents a promising alternative for the

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valorization of natural resources with high added value. The selection of ethanol as extraction solvent not only optimizes the obtaining of bioactive compounds and their expression as antioxidant activity, but is also an ecological option, since it is a renewable, biodegradable and less toxic solvent compared to other organic solvents. In this way, the harmony between sustainable cultivation practices and ecological extraction processes could contribute to the generation of nutraceutical and pharmaceutical products with lower environmental impact, promoting sustainable value chains.

Transparency:

The authors confirm that the manuscript is an honest, accurate, and transparent account of the study; that no vital features of the study have been omitted; and that any discrepancies from the study as planned have been explained. This study followed all ethical practices during writing.

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