AppliedChem

MDPI

Article

Phenolic Composition and Antioxidant Activity of Three Underexplored Non-Conventional Food Plants (*Rumex acetosa* L., *Tropaeolum majus* L., and *Stachys byzantina* K. Koch): Toward Their Valorization as Dietary Sources of Bioactive Compounds

Marinez Marçal Martins [1], Leonardo Assis Campos Jaymes [1], Vinícius Oliveira de Faria [1], Dhionne Correia Gomes [1], Ana Luiza Santos Vieira [1], Ana Luiza Coeli Cruz Ramos [1], Maysa do Vale-Oliveira [2], Ailton Junior Antunes da Costa [1], Marinalva Woods Pedrosa [3], Renata Adriana Labanca [1] and Raquel Linhares Bello de Araújo [1,*]

[1] Department of Foods, Faculty of Pharmacy, Federal University of Minas Gerais—UFMG, Belo Horizonte 31270-901, MG, Brazil; marinezmarcal@yahoo.com.br (M.M.M.); leojaymes@ufmg.br (L.A.C.J.); vinicius.faria@outlook.com.br (V.O.d.F.); dhionne@gmail.com (D.C.G.); anavieiranutricionista@gmail.com (A.L.S.V.); analuizacoeli@gmail.com (A.L.C.C.R.); ailtonjracosta@gmail.com (A.J.A.d.C.); renatalabanca@ufmg.br (R.A.L.)

[2] Federal University of Espírito Santo—UFES, São Mateus Campus, São Mateus 29932-540, ES, Brazil; maysa.v.oliveira@ufes.br

[3] Minas Gerais Agricultural Research Corporation—EPAMIG, Centro-Oeste, Prudente de Morais 35701-970, MG, Brazil; marinalva@epamig.br

* Correspondence: raquellba.linhares@gmail.com; Tel.: +55-(31)-34096722

Abstract

Non-conventional food plants (NCFPs) are edible species that remain underutilized in the human diet despite being potential sources of phenolic compounds. This study characterized the phenolic composition and antioxidant activity of three botanically distinct NCFPs: sorrel (*Rumex acetosa* L.), nasturtium (*Tropaeolum majus* L.), and lamb's ear (*Stachys byzantina* K. Koch.). We evaluated how extraction conditions influence the recovery of these compounds. We compared ten extraction protocols that differed in solvent, acidification, and ultrasound assistance. Total phenolic content and antioxidant activity (ABTS, DPPH, FRAP) were determined spectrophotometrically, and UPLC-DAD quantified eleven individual phenolic compounds. Recovery depended on both the protocol and the plant matrix. The highest total phenolic contents were obtained with ultrasound-assisted acidified methanol extraction, reaching 3540.73, 3940.83, and 4497.19 mg GAE/100 g DW for *Rumex acetosa*, *Tropaeolum majus*, and *Stachys* byzantina, respectively; however, the integrated phenolic analysis revealed species-specific responses. Chlorogenic acid was the predominant compound across all species and protocols, indicating that these plants are relevant dietary sources of hydroxycinnamic acids. Principal component analysis explained 89.5%, 82.8%, and 75.3% of the total variance for the three species. These findings support valorizing these edible NCFPs as functional food sources and highlight the need for matrix-specific analytical characterization.

Keywords: phenolic compounds; antioxidant activity; non-conventional food plants

Check for updates

Academic Editor: José P. Coelho

Received: 7 August 2026
Revised: 25 September 2026
Accepted: 29 September 2026
Published: 5 October 2026

Copyright: © 2026 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license.

1. Introduction

Phenolic compounds are secondary metabolites widely distributed throughout the plant kingdom; they are responsible for functions such as imparting color and flavor, as well

as promoting plant resistance to environmental and biotic stress [1–3]. Consuming these compounds is important for human health [4] because of their antimutagenic, antidiabetic, and anti-inflammatory properties [5,6].

From a chemical standpoint, phenolic compounds are characterized by the presence of one or more hydroxyl groups attached to aromatic rings [7–9]. Depending on the number of carbon atoms and their structural characteristics, these compounds can be classified into different groups, such as phenolic acids, flavonoids, and condensed tannins [8]. Structural variations among phenolics can influence important physicochemical properties, including polarity, degree of conjugation, solubility in different solvents, and interaction with the sample matrix, consequently affecting extraction and separation processes [10,11]. Furthermore, phenolic compounds may vary in stability among plant species because of differing susceptibilities to oxidation and degradation, making extraction and preservation challenging [12].

Solvent extraction is one of the most widely used methods for obtaining phenolic compounds due to its simplicity, efficiency, and broad applicability. However, its performance depends on several parameters that require preliminary optimization to ensure efficient recovery from plant matrices [13,14]. Key factors in selecting the extraction method include the class of analytes of interest and the polarity of the compounds and the extraction solution [13]. In addition, operational conditions such as agitation, extraction time, solute-to-solvent ratio, temperature, mass transfer efficiency, and particle size can also significantly affect the yield and composition of the extracts obtained [15–18]. Thus, different combinations of these parameters may enhance the extraction of various phenolic compounds [19].

After extraction, it is necessary to identify and quantify the phenolic compounds in the extracts. In this context, liquid chromatography stands out as a main analytical technique, especially for analyzing substances in complex matrices [20]. To reduce analysis time and improve chromatographic separation efficiency, several studies have used ultra-high-performance liquid chromatography (UPLC) to determine phenolic compounds [21,22]. Due to the presence of multiple conjugated double bonds in their chemical structures, phenolic compounds exhibit characteristic absorption in the ultraviolet–visible (UV-vis) region. In this context, the diode array detector (DAD) is widely used in chromatographic analyses of different classes of phenolic compounds because it allows simultaneous acquisition of complete absorption spectra in the UV-vis range, generally between 200 and 800 nm [23].

Applying these analytical techniques, combined with appropriate extraction methods, is essential for accurately determining phytochemicals in complex plant matrices and improving understanding of phenolic profiles across plant species, including Non-Conventional Food Plants (NCFPs). NCFPs refer to plant species that have one or more edible parts but, for various cultural, social, or economic reasons, remain underutilized in the human diet [24]. These species have antioxidant potential associated with phenolic compounds [25]. However, knowledge of these compound profiles remains limited [26], and few studies have demonstrated the potential of these species, necessitating further research to reveal their functional properties [27].

Thus, considering the relevance of investigating different extraction methodologies and the need to expand knowledge regarding the profile of phenolic compounds present in NCFPs, the present study aimed to critically evaluate and compare the extraction efficiency of phenolic compounds using 10 different extraction methods, followed by the identification and quantification of the compounds via ultra-performance liquid chromatography (UPLC).

For this study, three NCFP species were selected as plant matrices: *Rumex acetosa* L., commonly known as sorrel; *Tropaeolum majus* L., known as nasturtium; and *Stachys byzantina* K.Koch, commonly known as lamb's ear. We selected these species based on complementary

criteria. First, EPAMIG (Minas Gerais Agricultural Research Corporation) cultivates and characterizes these three species, ensuring standardized plant material with documented agronomic origin, a critical factor for analytical reproducibility in phytochemical studies. Second, a preliminary literature review confirmed that the phenolic compounds for which certified reference standards were available in our laboratory, including chlorogenic acid, rutin, quercetin, and catechin, among others, are reported as relevant constituents in these three species, enabling rigorous quantification by UPLC. Third, the species belong to distinct botanical families (*Polygonaceae*, *Tropaeolaceae*, and *Lamiaceae*), allowing a comparative phytochemical perspective across taxonomically diverse NCFPs with potential relevance for applications in human nutrition and food science.

2. Materials and Methods

2.1. Reagents and Materials

Reference standards of caffeic acid, chlorogenic acid, ferulic acid, p-coumaric acid, apigenin, kaempferol, catechin, epicatechin, myricetin, quercetin, and rutin were purchased from Sigma-Aldrich® (St. Louis, MO, USA). Acetonitrile, methanol, and 98–100% formic acid (chromatographic grade for UPLC analysis) were purchased from Dinâmica (São Paulo, Brazil), Êxodo Científica (São Paulo, Brazil), and Merck (Darmstadt, Germany), respectively. Acetone (AR grade), Ethanol, Iron(III) Chloride Hexahydrate, and iron (III) Chloride (ICO) (AR grade) were purchased from Êxodo Científica. Glacial acetic acid (AR grade), hydrochloric acid, and cyclohexane were obtained from Synth (São Paulo, Brazil), while hydrochloric acid and gallic acid monohydrate (AR grade) were obtained from Neon (São Paulo, Brazil). Anhydrous sodium acetate (AR grade), ethyl alcohol, methanol (AR grade), ferrous sulfate heptahydrate (AR grade), and granular activated carbon were obtained from CRQ Química (São Paulo, Brazil), Sciavicco (Minas Gerais, Brazil), Vertec (Rio de Janeiro, Brazil), Química Moderna (São Paulo, Brazil), and Brascarbo (Paraná, Brazil), respectively. Ultrapure water was produced using a Milli-Q water purification system (Direct-Q® 3 UV Water Purification System, Millipore, ZRQSVP300) (Molsheim, França).

Samples were collected in August and September 2023 at the Bank of Non-Conventional Vegetables at EPAMIG's Santa Rita Experimental Farm in Prudente Morais, Minas Gerais, Brazil. The research involving genetic material and/or associated traditional knowledge was registered in the National System for the Management of Genetic Heritage and Associated Traditional Knowledge (SisGen), under registration number AC4BDD3, in accordance with current Brazilian legislation.

2.2. Sample Preparation

The leaves of sorrel (*Rumex acetosa* L.), nasturtium (*Tropaeolum majus* L.), and lamb's ear (*Stachys byzantina* K. Koch.) were dried in a forced-air oven at 35 °C ± 5 °C for 48 h and ground in a blender (ARNO, model L.S.) (São Paulo, Brazil) until a fine, homogeneous powder was obtained (standardized using a 16-mesh sieve). The Stachys byzantina leaves were only ground, as the characteristics of the plant matrix did not allow for the production of a fine, homogeneous powder.

2.3. Extraction of Extracts

Extractions were performed in triplicate in a light-protected environment, following the experimental conditions described in Table 1, which presents the 10 adapted extraction methods used [28–37]. We stored the extracts at −25 °C until analysis.

Table 1. Experimental conditions for the extraction of total phenolic compounds in non-conventional food plants: *Rumex acetosa* L., *Tropaeolum majus* L., and *Stachys byzantina* K.Koch.

Method	Solvent System	Sample: Solvent Ratio	Ultrasound * (min)	Orbital Incubation	Centrifugation	Extraction Cycles	Final Volume (mL)	Ref.
1	Methanol: water (50:50 *v*/*v*); acetone: water (70:30 *v*/*v*)	1:20	30	-	15 min; 2810× *g*	4	50	[28]
2	Ethanol: water (80:20 *v*/*v*)	1:20	10	-	5 min; 1970× *g*	2	10	[29]
3	Methanol: water (85:15 *v*/*v*)	1:75	-	120 rpm; 42 °C; 10 min	10 min; 2000× *g*	2	25	[30]
4	Methanol: water (50:50 *v*/*v*) at pH 2 (acidified with acetic acid) and acetone: water (70:30 *v*/*v*)	1:40	-	120 rpm; 25 °C; 60 min	10 min; 2500× *g*	2 [1]	50	[31]
5	Methanol: water: hydrochloric acid (80:19:1 *v*/*v*/*v*)	1:100	30	-	15 min; 2810× *g*	2	25	[32]
6	Acetonitrile: water acidified with 2% acetic acid (50:50, *v*/*v*)	1:100	5	120 rpm; 25 °C; 15 min	10 min; 1970× *g*	1	10	[33]
7	Water:acetone:ethanol (33.4:33.3:33.3, *v*/*v*/*v*)	1:20	-	120 rpm; 45 °C; 45 min	10 min; 2810× *g*	1	25	[34]
8	Methanol: water (80:20, *v*/*v*)	1:100	-	-	10 min; 2810× *g*	5	50	[35]
9	Acetone: water (80:20, *v*/*v*)	1:100	-	-	15 min; 2810× *g*	1	10	[36]
10	Methanol: water (50:50 *v*/*v*), acetone: water (70:30 *v*/*v*)	1:20	-	-	15 min; 2810× *g*	2 [1]	10	[37]

* Frequency 40 kHz; temp. 35 °C; [1] one extraction cycle was performed for each solvent system; - not used.

2.4. Purification of the Extract for Analysis of Total Phenolic Compounds

The extracts used to determine total phenolic content (TPC) by spectrophotometry were purified with activated charcoal, following the method proposed by Peres et al. [38], with modifications. This purification step reduced pigments and other reducing compounds in the matrix, particularly chlorophyll, which can interfere with the Folin–Ciocalteu reaction and overestimate spectrophotometrically determined total phenolic content. However, because activated charcoal may also adsorb phenolic compounds, potential losses of phenolic analytes during this step cannot be excluded.

For this purification, different aliquots of crude extract ranging from 2 to 5 mL were used, depending on the requirements of each extract, maintaining a plant mass: activated carbon ratio of 1:1. In a Falcon tube, granular activated carbon was weighed, raw extract was added, and the mixture was homogenized for 10 s in a tube shaker and placed in an orbital incubator at 130 rpm at room temperature for 20 min. The mixture was then filtered through quantitative filter paper (blue band; Quanty, 8 µm) (Paraná, Brazil) into a 5 mL volumetric flask, and the final volume was adjusted to the mark with deionized water.

2.5. Determination of Total Phenolic Content and Antioxidant Activity

We determined the phenolic content of the purified extracts using the Folin–Ciocalteu spectrophotometric method [37,39]. The results were expressed as gallic acid equivalents (mg GAE/100 g DW). The antioxidant activity of the crude extracts was determined using three methods: ABTS*+ free radical scavenging; reaction with 2,2′-diphenyl-1-

picrylhydrazine (DPPH); and ferric reducing antioxidant power (FRAP) [37]. We evaluated antioxidant activity using the ABTS, DPPH, and FRAP assays. The ABTS results were expressed as µmol Trolox equivalents/g DW, while the DPPH results were expressed as (EC50 g DW sample/g). We expressed FRAP results as µmol $FeSO_4$ equivalents/g DW, based on a calibration curve prepared with a standard ferrous sulfate solution. We performed the analyses in triplicate under low-light conditions.

2.6. Qualitative Analysis by Ultra-Performance Liquid Chromatography (UPLC)

We determined the phenolic compound profile in triplicate using the chromatographic method described by Felix Ávila et al. [40], with modifications. The analysis was performed by ultra-performance liquid chromatography coupled with diode array detection (UPLC-DAD) on a Waters ACQUITY UPLC system (Waters Corporation, Milford, MA, USA) consisting of a quaternary pump, online degasser, autosampler, column temperature controller (35 °C), and a diode array detector configured for 271 and 320 nm. Separation was performed on an Acquity BEH C18 column (1.7 µm × 2.1 × 100 mm). To obtain a more accurate and efficient assessment of phenolic compound composition, this study employed two distinct separation methods. The first method identified catechin and chlorogenic acid, and the chromatographic conditions used were: injection volume of 1 µL, flow rate of 0.3 mL/minute, and mobile phase consisting of water/formic acid (99.75:0.25, *v*/*v*) as eluent A and acetonitrile/formic acid (99.75:0.25, *v*/*v*) as eluent B. The mobile phase gradient conditions were as follows: Method A (0–3.5 min, 100% A, 0% B; 3.5–4.0 min, 100–92% A, 0–8% B; 4.0–10.0 min, 92% A, 8% B). The second method identified nine phenolic compounds: caffeic acid, ferulic acid, p-coumaric acid, apigenin, kaempferol, epicatechin, myricetin, quercetin, and rutin. We used the same eluents, flow rate, and injection volume as in the first method. However, the mobile phase gradient conditions were as follows: Method B (0.0–10.0 min, 90% A, 10% B; 10.0–10.5 min, 82% A, 18% B; 10.5–16.0 min, 82% A, 18% B; 16.0–16.5 min, 85% A, 15% B; 16.5–25.0 min, 75% A, 25% B).

We filtered the samples using a PVDF syringe filter membrane (25 mm diameter, 0.22 µm pore size) (Guangzhou Jet Bio-Filtration Co., Ltd., Guangzhou, China). To quantify the fractionated phenolic compounds, we used the peak areas of each compound and established calibration curves for the injected standards (see Figures S1 and S2—Supplementary Material). We identified compounds by comparing retention times and UV-VIS spectra with reference standards. Quantification used calibration curves in the range of 1 to 7 µg/mL for all compounds, except myricetin and kaempferol, with ranges of 1 to 6 µg/mL and 1 to 4 µg/mL, respectively. We processed the data using Empower® 3.0 software and expressed the results in mg/100 g dry weight.

We established the sensitivity of the analytical methods by determining the limits of detection (LOD) and quantification (LOQ), in accordance with RDC No. 166/2017 [41] and ICH Q2(R2) [41,42]. (see Tables S1 and S2 in the Supplementary Material). We estimated the LODs statistically, and their values were visually distinguishable from the equipment's baseline noise. Although the LOQs could have been calculated using parameters from the calibration curve, in this study, the value of the lowest point on the curve was conservatively adopted as the lower limit of quantification, fulfilling the requirement for experimental validation [41], ensuring that the reported LOQ exhibits validated precision and accuracy, demonstrating analytical sensitivity superior to that required by the study's working range.

2.7. Statistical Analysis

For each extraction method, we performed three independent extractions, and we carried out each analytical determination in triplicate. The results are expressed as mean ± standard deviation. We subjected the data to one-way analysis of variance

(ANOVA), followed by Tukey's post hoc test, with statistical significance set at $p < 0.05$, to compare the means [43]. We also performed a multivariate analysis to summarize data patterns using principal component analysis (PCA). We used R version 4.4.1 and RStudio 2025.05.0 for the statistical analyses [44–47]. Non-detected (ND) values represented concentrations below the limit of detection (LOD) of the analytical method and were reported as ND in the tables. We excluded these values from the statistical analyses.

3. Results and Discussion

3.1. Total Phenolic Content (TPC)

The results obtained from the TPC analyses, presented in Table 2, were analyzed and compared with one another, allowing identification of the experimental conditions most favorable for the extraction of phenolic compounds, as they yielded the highest TPC values, expressed in gallic acid equivalents (mg GAE per 100 g of sample, dry weight).

Table 2. Total phenolic content of non-conventional food plants: *Rumex acetosa* L., *Tropaeolum majus* L., and *Stachys byzantina* K.Koch.

Extraction Method	*Rumex acetosa*	*Tropaeolum majus*	*Stachys byzantina*
	Total Phenolic Content (mg GAE/100 g DW)		
1	1546.69 ± 187.99 ^{b}	2015.85 ± 16.80 ^{b}	1927.83 ± 119.34 ^{c}
2	860.04 ± 140.84 ^{cd}	2028.14 ± 129.24 ^{c}	1139.76 ± 270.16 ^{d}
3	1011.35 ± 44.40 ^{bcd}	1394.48 ± 67.90 ^{c}	1862.23 ± 95.18 ^{c}
4	1388.41 ± 15.70 ^{bc}	1862.07 ± 102.94 ^{b}	1627.44 ± 87.04 ^{cd}
5	3540.73 ± 437.09 ^{a}	3940.83 ± 121.02 ^{a}	4497.19 ± 238.54 ^{a}
6	942.65 ± 122.39 ^{bcd}	1121.08 ± 69.28 ^{c}	1670.90 ± 153.35 ^{c}
7	3135.94 ± 866.65 ^{a}	3586.55 ± 423.27 ^{a}	3246.65 ± 279.57 ^{b}
8	638.46 ± 136.05 ^{d}	1123.60 ± 26.46 ^{c}	3481.29 ± 378.78 ^{b}
9	787.97 ± 96.21 ^{bcb}	1025.44 ± 37.99 ^{c}	1060.24 ± 163.36 ^{d}
10	1138.61 ± 27.70 ^{bcd}	2120.50 ± 215.24 ^{b}	1894.85 ± 60.99 ^{c}

NOTE: Extraction conditions for Methods 1–10 are described in Table 1. We analyzed purified extracts with activated charcoal. Mean values ± standard deviation ($n = 3$). Values expressed in the same column followed by different letters indicate statistical differences ($p \leq 0.05$ according to Tukey's test). Results are expressed on a dry-weight basis. GAE = gallic acid equivalent.

The experimental conditions of Method No. 5 yielded the highest numerical TPC values for sorrel (3540.73 mg GAE/100 g), nasturtium (3940.83 mg GAE/100 g DW), and lamb's ear (4497.19 mg GAE/100 g DW). Method No. 7 yielded similar results for sorrel (3135.94 mg GAE/100 g DW) and nasturtium (3586.55 mg GAE/100 g DW), with no significant differences compared with Method No. 5 for these species.

Feduraev et al. reported a total phenolic content of 2300 mg GAE/100 g DW in *Rumex acetosa* leaves extracted with 70% ethanol under reflux at 60 °C for 1 h [48]. Ceccanti et al. valuated four *R. acetosa* cultivars using a methanol–water mixture (80:20, *v*/*v*) and ultrasound-assisted extraction for 30 min at 4 °C, reporting values below 1000 mg GAE/100 g DW [49]. Likewise, Cornara et al. obtained 644.71 mg GAE/100 g DW after extracting *R. acetosa* with 70% ethanol through three successive ultrasound-assisted extraction cycles. In the present study, Methods No. 5 and 7 yielded the highest total phenolic contents for *R. acetosa*, exceeding the values reported in these previous studies [50]. Although direct comparisons should be interpreted cautiously because of differences in plant material and extraction

procedures, these findings indicate that Methods 5 and 7 were particularly effective for recovering phenolic compounds from *R. acetosa*.

Sytar et al. reported a total phenolic content of 1864 ± 69.9 mg GAE/100 g DW in *Stachys byzantina* leaves, using 70% methanol and two successive extraction steps at 70 °C for 10 min. In the present study, Method No. 5 yielded 4497.19 mg GAE/100 g DW, which was substantially higher than the value reported by Sytar et al. [51]. This difference may be attributed to the distinct extraction conditions, particularly the use of acidified 80% methanol combined with acetone and ultrasound-assisted extraction in Method 5, which may have enhanced phenolic recovery from *S. byzantina*. These findings further support the effectiveness of Method No. 5 for recovering phenolic compounds from this species.

For *Tropaeolum majus*, direct comparisons with previous studies remain challenging, as relatively few investigations have reported results under directly comparable conditions. Differences in plant material, plant parts, extraction procedures, and units of expression hinder meaningful quantitative comparisons across studies. Therefore, the available literature was primarily used to support the role of extraction conditions in phenolic compound recovery rather than to enable direct numerical comparisons.

The superior performance of the methanol-based extraction methods may be attributed, at least in part, to the physicochemical properties of methanol, including its high polarity and ability to act as both a hydrogen-bond donor and acceptor [52]. Its shorter carbon chain than ethanol and acetone may also contribute to its greater affinity for highly polar phenolic compounds, favoring their solubilization and recovery [53].

Another relevant factor in Method No. 5 was the addition of 1% hydrochloric acid. Acidifying the extraction medium can enhance phenolic recovery by altering the chemical environment and analyte solubility, improving extraction efficiency [54–56].

For Method No. 7, its effectiveness may be linked to a mixed solvent system of water, acetone, and ethanol. Because solvent polarity and the chemical structure of phenolic compounds influence their solubility, different solvent systems can affect the recovery of these compounds [12].

The extracts subjected to total phenolic compound analysis were purified with activated charcoal to minimize interference from chlorophyll and other reducing compounds. However, we cannot rule out adsorption and loss of phenolic compounds. Because no recovery experiment was conducted, we could not quantify the magnitude of these potential losses.

3.2. Antioxidant Activity

The antioxidant activity assays (ABTS, DPPH, and FRAP) in Table 3 were evaluated to determine which extraction conditions produced extracts with the highest antioxidant activity.

Unlike the findings from the TPC analysis, the experimental conditions of Method No. 8 allowed for the extraction of a higher content of compounds with antioxidant activity, according to the ABTS method, for the extracts of sorrel (330.55 μM Trolox/g DW), nasturtium (519.62 μM Trolox/g DW), and lamb's ear (393.33 μM Trolox/g DW). This result may reflect more effective extraction of other antioxidant compounds under the experimental conditions used in Method No. 8. Because the ABTS assay reflects the overall radical-scavenging capacity of the extracts and is not specific to phenolic compounds, these antioxidant constituents may also contribute to the observed antioxidant response.

As for the DPPH method, the best results for the antioxidant activity of the extracts were obtained under the experimental conditions applied in method No. 1 for sorrel (172.21 EC50 g DW sample/g DPPH). For nasturtium, the best results were obtained using Method No. 1 (196.84 EC50 g DW sample/g DPPH) and Method No. 8 (220.88 EC50 g DW sample/g DPPH), while for the lamb's ear, the conditions applied by Method No. 3

(175.25 EC50 g DW sample/g DPPH) and Method No. 8 (156.02 EC50 g DW sample/g DPPH) stood out.

Table 3. Antioxidant activity of non-conventional food plants: *Rumex acetosa* L., *Tropaeolum majus* L., and *Stachys byzantina* K.Koch.

Extraction Method	*Rumex acetosa*	*Tropaeolum majus*	*Stachys byzantina*
	ABTS (µmol Trolox equivalents/g DW)		
1	217.04 ± 22.08 ^{c}	243.32 ± 19.55 ^{cd}	198.42 ± 15.23 ^{cd}
2	103.98 ± 4.49 ^{e}	93.65 ± 3.28 ^{f}	106.03 ± 6.19 ^{e}
3	144.27 ± 11.16 ^{d}	177.40 ± 6.27 ^{e}	194.99 ± 0.84 ^{cd}
4	263.54 ± 8.96 ^{b}	318.46 ± 13.23 ^{b}	230.14 ± 9.35 ^{bc}
5	125.84 ± 4.06 ^{de}	114.51 ± 14.75 ^{f}	180.79 ± 21.98 ^{d}
6	130.56 ± 5.24 ^{de}	195.83 ± 5.55 ^{de}	210.84 ± 13.26 ^{cd}
7	153.12 ± 9.23 ^{d}	234.26 ± 10.48 ^{cd}	91.34 ± 7.44 ^{e}
8	330.55 ± 26.17 ^{a}	519.62 ± 40.95 ^{a}	393.33 ± 24.81 ^{a}
9	241.66 ± 3.70 ^{bc}	273.25 ± 16.78 ^{bc}	215.34 ± 9.03 ^{cd}
10	257.60 ± 10.85 ^{b}	308.55 ± 23.75 ^{b}	267.33 ± 11.65 ^{b}
	DPPH (EC50 g DW sample/g)		
1	172.21 ± 10.65 ^{g}	196.84 ± 8.88 ^{h}	244.68 ± 5.76 ^{e}
2	536.15 ± 14.03 ^{c}	455.89 ± 13.44 ^{c}	313.71 ± 9.00 ^{cd}
3	385.08 ± 12.23 ^{de}	342.79 ± 11.15 ^{e}	175.25 ± 4.48 ^{f}
4	295.65 ± 14.46 ^{ef}	273.55 ± 7.60 ^{f}	284.38 ± 5.71 ^{cde}
5	1187.56 ± 88.58 ^{a}	1009.43 ± 30.70 ^{a}	873.69 ± 61.74 ^{a}
6	472.82 ± 19.51 ^{cd}	283.10 ± 6.90 ^{f}	309.47 ± 18.59 ^{cde}
7	712.95 ± 17.52 ^{b}	588.76 ± 16.59 ^{b}	480.05 ± 13.83 ^{b}
8	292.41 ± 12.45 ^{f}	220.88 ± 7.02 ^{gh}	156.02 ± 6.86 ^{f}
9	455.51 ± 2.48 ^{cd}	386.35 ± 19.82 ^{d}	334.73 ± 6.58 ^{c}
10	341.77 ± 14.48 ^{ef}	243.28 ± 1.23 ^{fg}	256.23 ± 21.11 ^{de}
	FRAP (µmol $FeSO_4$ equivalents/g DW)		
1	403.97 ± 7.03 ^{a}	401.75 ± 12.30 ^{a}	353.50 ± 5.70 ^{b}
2	159.95 ± 2.63 ^{h}	155.52 ± 4.97 ^{e}	199.93 ± 10.82 ^{g}
3	261.45 ± 5.03 ^{b}	283.79 ± 19.12 ^{d}	404.12 ± 14.42 ^{a}
4	258.33 ± 5.95 ^{b}	373.59 ± 10.47 ^{ab}	278.92 ± 8.75 ^{cd}
5	247.14 ± 5.57 ^{bc}	343.54 ± 12.31 ^{bc}	359.51 ± 24.12 ^{de}
6	216.84 ± 3.91 ^{ef}	259.34 ± 0.81 ^{d}	344.09 ± 5.23 ^{b}
7	197.23 ± 4.01 ^{g}	254.94 ± 4.51 ^{d}	248.35 ± 13.45 ^{ef}
8	229.77 ± 11.01 ^{de}	337.04 ± 18.57 ^{c}	366.16 ± 8.79 ^{b}
9	200.57 ± 5.57 ^{fg}	220.80 ± 8.54 ^{e}	225.95 ± 10.37 ^{fg}
10	234.26 ± 3.27 ^{cd}	393.94 ± 9.50 ^{a}	298.85 ± 5.49 ^{c}

NOTE: Extraction conditions for Methods 1–10 are described in Table 1. Analysis was performed on crude extracts without activated charcoal purification. Mean values ± standard deviation (n = 3). Values expressed in the same column followed by different letters indicate statistical differences ($p \leq 0.05$ according to Tukey's test). Results are expressed on a dry-weight basis.

The FRAP method yielded the highest results under the analytical conditions of method No. 1 for sorrel (403.97 μM ferrous sulfate/g) and nasturtium (401.75 μM ferrous sulfate/g). In comparison, method No. 10 also yielded higher antioxidant activity for nasturtium (393.94 μM ferrous sulfate/g). In contrast, the extraction conditions applied in method No. 3 were more favorable for obtaining extracts with higher antioxidant activity, according to the FRAP method, for lamb's ear (404.12 μM ferrous sulfate/g).

Mollica et al., when analyzing *Alstonia boonei* extracts, found that methanol can extract higher levels of phenolic compounds and yield greater antioxidant activity than water and ethyl acetate [57]. Similarly, Herrera-Pool et al. found the best antioxidant activity using 50% and 80% methanol and 80% acetone when analyzing chili pepper leaves [58]. These findings corroborate those of the present study, since the best results for total phenolic content and antioxidant activity were obtained primarily from extracts whose experimental conditions used aqueous solutions of 50%, 80%, and 85% as the extraction solvent, demonstrating that these solutions are more efficient in extraction compared to solutions using other solvents. The superior extraction efficiency of methanol can be explained by its shorter carbon chain compared to other solvents [59]. Furthermore, methanol is a polar solvent that can form hydrogen bonds with the hydroxyl groups in phenolic compounds, facilitating their extraction.

The differences observed among the antioxidant activity assays may reflect their distinct chemical principles. The ABTS and DPPH assays are mixed-mode methods that involve both hydrogen atom transfer and electron transfer mechanisms, whereas the FRAP assay is based on electron transfer [60,61]. Responses from these assays may also depend on the chemical properties of the antioxidants and on reaction conditions, such as pH, solvent, and reaction kinetics. Therefore, differences in antioxidant responses obtained using the ABTS, DPPH, and FRAP assays may arise because compounds in the extracts can exhibit different reactivities under the specific conditions of each assay [62].

3.3. Profile of Phenolic Compounds Obtained by Different Extraction Methods

Of the 11 phenolic compounds studied, only kaempferol was not identified in any of the extracts from the three NCFPs. Some chromatograms of the extracts from sorrel, nasturtium, and lamb's ear obtained by Methods A and B are presented in the Supplementary Material—Figure S3.

The results of the UPLC determination of individual phenolic compounds present in the different extracts of the three NCFPs are presented in Table 4.

The most efficient experimental conditions for the extraction of phenolic acids and flavonoids used an aqueous methanol solution at different concentrations as the solvent, since this solvent acts as both a hydrogen donor and acceptor in the molecule, promoting the recovery of phenolic compounds compared to less polar solvents such as ethanol and acetone [52]. Similarly, the experimental conditions of method No. 6, in which the extraction solvent was a solution of acetonitrile in acidified water, also stood out in the recovery of phenolic compounds, which can be explained by the fact that the polarity of the extractant solvents and the solubility of the particles in the solvent influence the extraction of phenolic compounds in different matrices [63].

Table 4. Profile of phenolic compounds of non-conventional food plants—sorrel, nasturtium and lamb's ear—by Ultra-High-Performance Liquid Chromatography (UPLC).

Extraction Method	*Rumex acetosa*	*Tropaeolum majus*	*Stachys byzantina*
	Chlorogenic Acid (mg/100 g)		
1	113.96 ± 3.20 ^{a}	909.17 ± 64.07 ^{a}	523.75 ± 26.60 ^{a}
2	71.76 ± 4.62 ^{a}	708.12 ± 30.94 ^{c}	326.94 ± 6.42 ^{b}
3	99.56 ± 1.86 ^{a}	818.02 ± 29.88 ^{ab}	514.37 ± 5.53 ^{a}
4	105.31 ± 4.65 ^{a}	834.13 ± 45.08 ^{ab}	548.14 ± 29.07 ^{a}
5	90.70 ± 4.48 ^{a}	315.27 ± 29.68 ^{d}	222.22 ± 12.74 ^{c}
6	80.24 ± 7.57 ^{a}	872.39 ± 25.95 ^{a}	503.95 ± 49.95 ^{a}
7	72.56 ± 0.65 ^{a}	707.58 ± 57.36 ^{c}	351.00 ± 11.53 ^{b}
8	100.47 ± 2.95 ^{a}	823.17 ± 17.01 ^{ab}	542.67 ± 17.56 ^{a}
9	77.42 ± 5.19 ^{a}	751.17 ± 17.04 ^{bc}	376.32 ± 8.45 ^{b}
10	104.25 ± 1.38 ^{a}	819.32 ± 25.00 ^{ab}	495.65 ± 21.09 ^{a}
	Caffeic acid (mg/100 g)		
1	2.67 ± 0.09 ^{b}	0.47 ± 0.24 ^{ab}	1.87 ± 0.21 ^{cd}
2	1.88 ± 0.01 ^{bc}	ND	1.49 ± 0.03 ^{e}
3	2.52 ± 0.13 ^{b}	0.48 ± 0.22 ^{ab}	2.08 ± 0.07 ^{bc}
4	2.16 ± 0.13 ^{bc}	0.44 ± 0.03 ^{b}	1.75 ± 0.13 ^{cde}
5	9.18 ± 0.97 ^{a}	0.88 ± 0.26 ^{a}	2.62 ± 0.15 ^{a}
6	2.01 ± 0.02 ^{bc}	ND	1.96 ± 0.03 ^{bcd}
7	1.74 ± 0.04 ^{bc}	0.50 ± 0.05 ^{ab}	1.11 ± 0.04 ^{f}
8	2.37 ± 0.14 ^{b}	0.87 ± 0.08 ^{a}	2.29 ± 0.11 ^{ab}
9	1.37 ± 0.10 ^{c}	0.76 ± 0.10 ^{ab}	1.72 ± 0.25 ^{cde}
10	2.51 ± 0.07 ^{b}	0.64 ± 0.02 ^{ab}	1.59 ± 0.07 ^{de}
	P-coumaric acid (mg/100 g)		
1	14.67 ± 0.20 ^{bc}	1.38 ± 0.13 ^{a}	3.10 ± 0.16 ^{a}
2	9.87 ± 1.03 ^{e}	1.00 ± 0.05 ^{b}	1.59 ± 0.08 ^{de}
3	12.82 ± 0.56 ^{cd}	1.30 ± 0.05 ^{a}	3.26 ± 0.09 ^{a}
4	13.13 ± 0.27 ^{c}	1.36 ± 0.16 ^{a}	2.42 ± 0.16 ^{bc}
5	88.61 ± 1.69 ^{a}	ND	2.00 ± 0.15 ^{cd}
6	15.69 ± 1.27 ^{b}	1.29 ± 0.04 ^{a}	3.11 ± 0.28 ^{a}
7	10.52 ± 0.82 ^{de}	1.05 ± 0.06 ^{b}	2.07 ± 0.04 ^{cd}
8	12.82 ± 0.60 ^{cd}	ND	2.11 ± 0.38 ^{cd}
9	10.13 ± 0.73 ^{e}	0.40 ± 0.03 ^{c}	1.16 ± 0.06 ^{e}
10	13.57 ± 0.53 ^{bc}	1.28 ± 0.02 ^{a}	2.76 ± 0.13 ^{ab}

Table 4. *Cont.*

Extraction Method	*Rumex acetosa*	*Tropaeolum majus*	*Stachys byzantina*
	Ferulic acid (mg/100 g)		
1	52.29 ± 1.67 ^{a}	ND	0.76 ± 0.15 ^{c}
2	45.01 ± 1.15 ^{bc}	ND	ND
3	52.03 ± 1.84 ^{a}	ND	1.26 ± 0.12 ^{c}
4	48.53 ± 2.28 ^{ab}	ND	1.17 ± 0.22 ^{c}
5	47.15 ± 2.53 ^{ab}	ND	ND
6	34.,10 ± 3.37 ^{e}	ND	5.72 ± 0.54 ^{a}
7	37.95 ± 0.82 ^{de}	ND	1.23 ± 0.06 ^{c}
8	51.75 ± 1.21 ^{a}	ND	ND
9	41.29 ± 2.78 ^{cd}	ND	ND
10	48.78 ± 0.36 ^{ab}	ND	2.65 ± 0.22 ^{b}
	Catechin (mg/100 g)		
1	8.37 ± 0.22 ^{bc}	20.88 ± 2.55 ^{a}	ND
2	5.86 ± 0.40 ^{d}	13.27 ± 0.30 ^{b}	ND
3	8.86 ± 0.29 ^{bc}	12.71 ± 0.47 ^{b}	ND
4	7.10 ± 0.66 ^{cd}	15.21 ± 2.05 ^{b}	ND
5	29.46 ± 0.67 ^{a}	12.53 ± 0.55 ^{b}	ND
6	7.35 ± 0.41 ^{cd}	15.47 ± 1.29 ^{b}	ND
7	5.71 ± 0.42 ^{d}	15.91 ± 1.23 ^{b}	ND
8	10.02 ± 1.62 ^{b}	15.19 ± 3.09 ^{b}	ND
9	6.18 ± 0.62 ^{d}	16.00 ± 1.34 ^{b}	ND
10	8.65 ± 0.31 ^{bc}	15.23 ± 0.39 ^{b}	ND
	Epicatechin (mg/100 g)		
1	ND	ND	ND
2	ND	ND	ND
3	ND	ND	ND
4	ND	ND	ND
5	14.23 ± 2.90	ND	ND
6	ND	ND	ND
7	ND	ND	ND
8	ND	ND	ND
9	ND	ND	ND
10	ND	ND	ND

Table 4. *Cont.*

Extraction Method	*Rumex acetosa*	*Tropaeolum majus*	*Stachys byzantina*
		Rutin (mg/100 g)	
1	172.82 ± 4.43 ^{abc}	2.86 ± 0.19 ^{a}	ND
2	152.47 ± 2.74 ^{bcd}	1.74 ± 0.04 ^{d}	ND
3	178.20 ± 6.85 ^{ab}	2.10 ± 0.09 ^{cd}	ND
4	155.70 ± 6.85 ^{bc}	2.79 ± 0.08 ^{ab}	ND
5	169.02 ± 11.41 ^{abc}	0.98 ± 0.25 ^{e}	ND
6	185.64 ± 15.14 ^{ab}	2.43 ± 0.37 ^{abc}	ND
7	132.40 ± 2.33 ^{e}	2.02 ± 0.15 ^{dc}	ND
8	190.40 ± 5.36 ^{a}	2.33 ± 0.23 ^{abc}	ND
9	142.66 ± 8.04 ^{de}	2.28 ± 0.12 ^{bc}	ND
10	167.63 ± 1.85 ^{bc}	2.37 ± 0.05 ^{abc}	ND
		Myricetin (mg/100 g)	
1	4.81 ± 0.31 ^{ab}	1.29 ± 0.10 ^{abc}	6.10 ± 0.26 ^{ab}
2	4.08 ± 0.26 ^{bc}	1.13 ± 0.15 ^{bc}	1.77 ± 0.12 ^{e}
3	4.56 ± 0.50 ^{ab}	1.29 ± 0.12 ^{abc}	3.72 ± 0.46 ^{cd}
4	5.01 ± 0.22 ^{a}	1.14 ± 0.12 ^{abc}	5.25 ± 0.55 ^{b}
5	2.21 ± 0.12 ^{e}	0.98 ± 0.29 ^{c}	6.51 ± 0.38 ^{a}
6	4.20 ± 0.22 ^{bc}	1.25 ± 0.17 ^{abc}	4.34 ± 0.19 ^{c}
7	3.19 ± 0.27 ^{d}	1.13 ± 0.08 ^{bc}	3.25 ± 0.08 ^{d}
8	4.76 ± 0.25 ^{ab}	1.52 ± 0.00 ^{ab}	3.85 ± 0.31 ^{cd}
9	3.48 ± 0.27 ^{cd}	1.55 ± 0.12 ^{a}	1.98 ± 0.04 ^{e}
10	4.18 ± 0.15 ^{bc}	1.23 ± 0.11 ^{abc}	3.15 ± 0.11 ^{d}
		Quercetin (mg/100 g)	
1	ND	1.11 ± 0.16 ^{bc}	ND
2	0.53 ± 0.03 ^{b}	1.05 ± 0.17 ^{bc}	ND
3	ND	1.32 ± 0.08 ^{b}	ND
4	ND	1.18 ± 0.12 ^{b}	ND
5	4.50 ± 0.28 ^{a}	13.26 ± 1.15 ^{a}	ND
6	ND	1.14 ± 0.37 ^{bc}	ND
7	ND	1.06 ± 0.04 ^{bc}	ND
8	ND	ND	ND
9	ND	0.84 ± 0.10 ^{bc}	ND
10	ND	0.88 ± 0.02 ^{bc}	ND

Table 4. *Cont.*

Extraction Method	*Rumex acetosa*	*Tropaeolum majus*	*Stachys byzantina*
		Apigenin (mg/100 g)	
1	ND	ND	51.21 ± 1.03 ^{a}
2	ND	ND	7.01 ± 0.81 ^{e}
3	ND	ND	18.76 ± 1.03 ^{de}
4	ND	ND	34.19 ± 7.28 ^{bc}
5	ND	ND	41.18 ± 3.86 ^{ab}
6	ND	ND	33.29 ± 3.53 ^{bc}
7	ND	ND	26.09 ± 0.79 ^{cd}
8	ND	ND	18.11 ± 10.43 ^{de}
9	ND	ND	9.96 ± 0.14 ^{e}
10	ND	ND	24.72 ± 2.10 ^{cd}

NOTE: Extraction conditions for Methods 1–10 are described in Table 1. Analysis was performed on crude extracts without activated charcoal purification. Mean values ± standard deviation (n = 3). Values expressed in the same column followed by different letters indicate statistical differences ($p \leq 0.05$ according to Tukey's test). Results are expressed on a dry-weight basis. ND—not detected.

A study by Herrera-Pool et al. found significant differences in the recovery of specific phenolic compounds in chromatographic analyses: extraction with 50% methanol in an ultrasonic bath provided better recovery of phenolic acids, and the use of 80% acetone, with an ultrasonic bath and water bath, proved more favorable for the recovery of flavonoids [58]. These results differ from those presented in the present study, since although Method No. 9, whose extraction solution is 80% acetone, extracted flavonoids such as catechin, rutin, myricetin, and apigenin, its extracts did not exhibit the highest levels, demonstrating that a method will not always be capable of extracting a greater number of phenolic compounds at their highest concentrations. This study observed that extractions with solutions containing methanol, methanol and acetone, and acidified methanol promoted the highest recovery of catechin, rutin, myricetin, and apigenin, because the hydroxyl groups in the solutions formed bonds with the hydroxyl groups of the flavonoids, favoring better recovery.

In addition to polar solvents, the ultrasonic bath, as used in the study by Herrera-Pool et al. and under the extraction conditions of methods No. 1, 2, 5, and 6 in the present study, may have contributed to superior extraction of phenolic compounds, particularly flavonoids. Sonication increases plant-tissue disintegration and solvent penetration through rapid bubble collapse and cavitation [64]. During ultrasonic bath use, repeated compressions and rarefactions in the extraction solvent induce bubble formation due to temperature and pressure changes, which can help release phenolic compounds from plant matrices and improve extraction efficiency [65]. In addition, collisions between particles and ultrasonic waves can reduce particle size, promoting mass transfer and allowing the solvent to access plant cellular materials more quickly [66].

Vieira et al. compared different chayote leaf extracts prepared by maceration, microwaving, and ultrasonic extraction and found that the latter was more effective at extracting TPC [67]. Another study examined the morphological structure of kiwi leaf surfaces after ultrasonic extraction and observed cell disruption, which led to more efficient extraction than maceration and microwave treatment [68]. In the present study, methods No. 1 and 5, with a sonication time of 30 min, showed better flavonoid extraction efficiency for the nasturtium and lamb's ear samples.

For solvent selection, polar mixtures can also be used to recover phenolic compounds. Solvents such as water, acetone, methanol, ethanol, and acetonitrile, alone or in mixtures, can be used to obtain phenolics in high yield. Still, the choice may influence the extract's final composition and antioxidant activity [69–72]. Similarly, the presence of different hydroxyl groups in the structures of phenolic compounds, as observed in the structure of chlorogenic acid, as well as conjugation with sugars—present, for example, in the structure of rutin—acids, or alkyl groups, considerably influences the polarity of these compounds and, consequently, the composition and antioxidant activity of the extracts [73].

Solvents such as methanol have significantly lower polarity compared to water, which favors the solubility and diffusion of phenolic compounds by reducing the solvent's dielectric constant. Polar solvents exhibit greater efficiency in the solvation process due to interactions (hydrogen bonds) between the polar sites of the compounds and the solvent [53].

Among the extraction methods applied in this study, three utilized an acidified extraction solution. In methods 4 and 6, acetic acid was added, and the extraction solutions had pH values of 2.00 $\pm$ 0.01 and 2.82 $\pm$ 0.01, respectively; in method No. 5, hydrochloric acid was added, and the extraction solution had a pH of 1.01 $\pm$ 0.01. These methods yielded good extraction results for phenolic acids (methods No. 4 and 6) and for flavonoids (method No. 5). Acids such as hydrochloric, acetic, and formic acids are frequently used to improve extraction efficiency [54,55]. pH is a critical factor influencing extraction efficiency because it affects analyte solubility and the charge density of extracts and solvents [55]. Furthermore, aqueous solvents acidified with hydrochloric acid and formic acid can rupture plant vacuoles and release phenolic compounds [74,75]. Xia et al., when extracting phenolic acids from the plant *Prunella vulgaris*, observed that at pH 1, the extraction yield of rosmarinic acid increased, demonstrating that pH can significantly affect the extraction rate of phenolic acids [76].

Another factor that can optimize extraction is combining extraction techniques when a single technique is insufficient, providing access to additional phenolic compounds [77,78]. The experimental conditions in method No. 6 produced this synergistic effect by combining ultrasonication with agitation, which contributed to the extraction process.

Regarding temperature, increasing this parameter correlates with improved phenolic-compound extraction yields because it induces breaks in matrix bonds [79]; increases compound solubility, solvent diffusion rate, and mass transfer; and reduces solvent viscosity and surface tension [80]. Therefore, the 35 °C temperature achieved in methods No. 1 and 6 and the 42 °C temperature in method 3 positively influenced the extraction process.

Thus, the combination of the aforementioned parameters—specifically, the use of 80% methanol acidified with hydrochloric acid and the 30 min ultrasonic bath step—was key to making method No. 5 the most efficient for recovering phenolic acids from sorrel.

Among the compounds analyzed, chlorogenic acid was the predominant phenolic acid quantified in all extracts obtained from the three plant matrices studied. The extraction conditions of methods No. 1, 3, 4, 6, 8, and 10 yielded the best chlorogenic acid extractions, with no significant difference among them ($p > 0.05$) for nasturtium and lamb's ear. There was no significant difference ($p > 0.05$) in chlorogenic acid content among the sorrel extracts obtained by the ten methods used.

The predominance of chlorogenic acid in all extraction methods suggests that these species may represent relevant dietary sources of antioxidant phenolic compounds. Chlorogenic acid is one of the most abundant hydroxycinnamic acids in the human diet and has been widely associated with antioxidant, anti-inflammatory, and cardioprotective effects. Its antioxidant activity is related both to its direct ability to scavenge free radicals and to its capacity to modulate endogenous antioxidant defense pathways [81,82]. Therefore, the consistent detection of chlorogenic acid, regardless of extraction conditions, indicates that

the plant matrices analyzed may contribute to dietary antioxidant intake and hold potential for the development of functional foods and nutraceutical products.

Previous studies have highlighted the potential of chlorogenic acid for food applications, particularly in seafood, meat, and bakery products. Its antioxidant properties may contribute to controlling oxidative processes, including lipid oxidation and protein degradation, which are important factors affecting the stability, nutritional value, and sensory quality of foods. These characteristics support the investigation of chlorogenic acid as a naturally occurring phenolic compound with potential technological relevance for food preservation [83].

The highest levels of caffeic acid were found when the extraction conditions of method No. 5 were applied to sorrel, while the conditions applied in methods No. 5 and 8 allowed for the recovery of higher levels in lamb's ear, with no significant difference ($p < 0.05$) between them. In contrast, methods No. 1, 3, 7, 9, 8, and 10 yielded higher caffeic acid levels in the nasturtium extracts, with no significant differences among them ($p < 0.05$).

There was a higher recovery of p-coumaric acid in the sorrel extract when extraction method No. 5 was applied. For the nasturtium and lamb's ear extracts, the highest p-coumaric acid recoveries occurred with extraction methods No. 10, 3, 6, and 1, with no significant differences among them ($p < 0.05$).

Extraction methods No. 1, 3, 4, 5, 8, and 10 yielded higher ferulic acid levels in sorrel, with no significant differences among them ($p < 0.05$). Extraction method No. 6 allowed for the recovery of higher levels of this acid in the lamb's-ear sample. Meanwhile, extraction methods No. 2, 5, 8, and 9 did not extract ferulic acid in sufficient quantities for quantification.

A study by Benedec et al. used a 70% ethanol extract of *Stachys byzantina* prepared in an ultrasonic bath and reported phenolic compound concentrations that, although lower than the highest values in the present study, were comparable to those observed under some extraction conditions [84]. In particular, Method No. 5 resulted in an extract containing 222.25 mg/100 g of chlorogenic acid; Method No. 9 enabled the quantification of 1.16 mg/100 g of p-coumaric acid; Method No. 1 yielded 0.76 mg/100 g of ferulic acid; and Method No. 2 resulted in 7.01 mg/100 g of apigenin.

Kucekova et al. investigated the content of p-coumaric acid, ferulic acid, caffeic acid, rutin, and catechin in sorrel flowers using 90% methanol (2 mL/g) for extraction for 30 min at 4 °C, with three extraction cycles. In that study, only catechin was quantified (7.54 mg/100 g, dry basis), whereas this study identified all compounds in sorrel leaves [85]. Feduraev et al., when investigating the levels of chlorogenic, caffeic, p-coumaric, and catechin acids, found chlorogenic acid and p-coumaric acid at 121 mg/100 g and 15 mg/100 g on a dry basis, respectively [48]. These values are very close to those found in this study.

The highest catechin concentrations were obtained under the experimental conditions used in method No. 5 for sorrel and in method No. 1 for nasturtium. In contrast, it was not possible to quantify catechin in any of the lamb's ear extracts.

Epicatechin was identified and quantified only in the extract obtained by method No. 5 from sorrel. Apigenin was identified only in the lamb's ear extracts, particularly under the extraction conditions used in methods No. 1 and 5, which showed no significant difference ($p < 0.05$). Bahadori et al. investigated the levels of phenolic compounds in the leaves, stems, and flowers of lamb's ear through decoction with boiling water and quantified catechin, epicatechin, rutin, and kaempferol, compounds that were not found in the present study, suggesting that the extraction conditions of the ten methods were not efficient for extracting these compounds [86]. This highlights the importance of optimizing extraction conditions to obtain a more complete profile of the phenolic compounds present in plants.

High levels of rutin were quantified in the extracts obtained by extraction methods No. 1, 3, 5, 6, and 8 for sorrel, among which there was no significant difference ($p < 0.05$). For nasturtium, the highest rutin levels were quantified in the extracts obtained using extraction methods No. 1, 4, 6, 8, and 10, with no significant difference ($p < 0.05$) among them.

High levels of myricetin were quantified in the sorrel extracts obtained under the extraction conditions of methods No. 3, 8, 1, and 4, with no significant difference among them ($p < 0.05$). Meanwhile, methods No. 4, 10, 6, 1, 3, 8, and 9 allowed recovery of high levels of these flavonoids in the nasturtium extracts. In contrast, lamb's ear extracts obtained using Methods No. 1 and 5 showed the highest myricetin quantification.

High levels of quercetin were extracted when the experimental conditions of Method No. 5 were applied to sorrel and nasturtium. However, none of the extraction methods used were effective in extracting quercetin from the lamb's ear samples.

Overall, all experimental conditions of the 10 extraction methods reproduced in this study were capable of extracting phenolic compounds from NCFPs, with some being more efficient than others. The analysis showed that all extracts contained phenolic acids and flavonoids, with the highest concentrations in the methanol and acetonitrile extracts. In contrast, the ethanol and acetone extracts had lower concentrations of phenolic acids and flavonoids.

In practical terms, the choice of the most appropriate extraction method should be guided by the extract's intended use. For routine analyses of food composition, methanol extraction may be preferable because of its high extraction efficiency and greater recovery of phenolic compounds compared with other solvent systems. However, in nutraceutical applications, in addition to extraction yield, solvent toxicity and safety for human consumption must be considered. In this regard, ethanol and hydroethanolic mixtures stand out as more suitable alternatives, as they combine efficient extraction of bioactive compounds with the requirement for food-grade solvents, making them particularly suitable for obtaining extracts intended for the development of functional foods and nutraceutical products [87,88].

The present study showed that extraction performance depends on the species and should be selected according to the intended application, whether for routine compositional analysis or for producing extracts rich in phenolic compounds.

3.4. Principal Component Analysis

Principal component analysis (PCA) was applied to provide an integrated assessment of the extraction protocols and their influence on the recovery of phenolic compounds from the three plants evaluated. The graphs for *Rumex acetosa*, *Tropaeolum majus*, and *Stachys byzantina* are presented in Figures 1–3, respectively.

For *Rumex acetosa*, the first two principal components explained 89.5% of the total variance (PC1 = 61.0% and PC2 = 28.5%), indicating that a two-dimensional space effectively summarized the variability between extraction treatments. The positive association between caffeic acid, p-coumaric acid, catechin, epicatechin, and quercetin characterized method No. 5, suggesting that the extraction conditions employed in this protocol favored the recovery of these compounds. In contrast, myricetin was mainly associated with methods No. 1, 3, 4, and 8, while chlorogenic acid, ferulic acid, and rutin clustered together and showed a stronger association with methods No. 1, 3, 4, 8, and 10. Methods No. 2, 6, 7, and 9 were further removed from the phenolic variables, indicating lower extraction efficiency of the compounds evaluated.

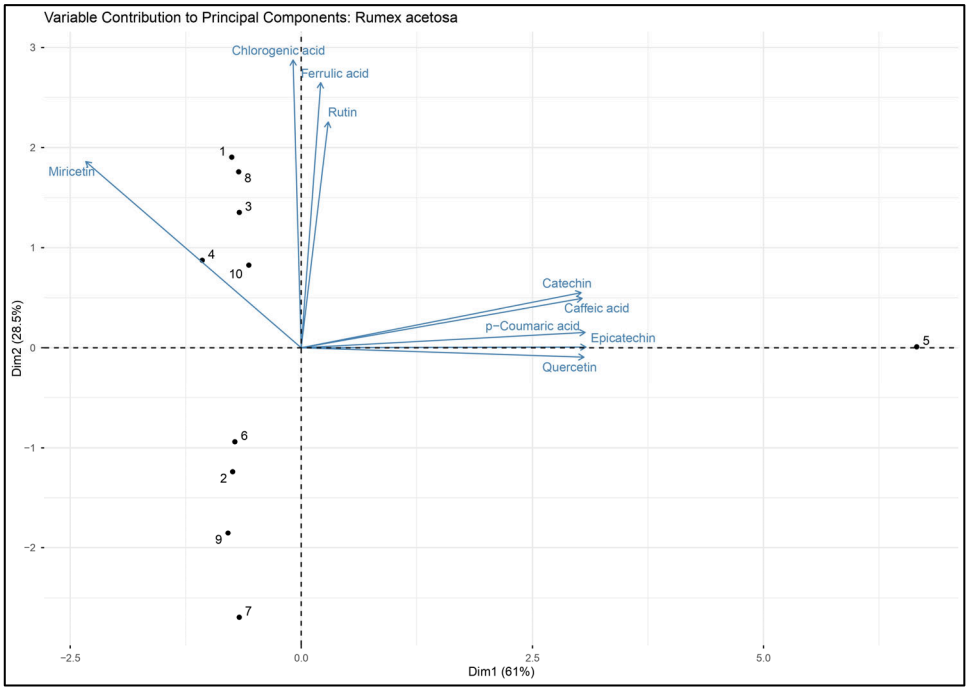


Figure 1. PCA of phenolic acids and flavonoids in extracts of *Rumex acetosa*.

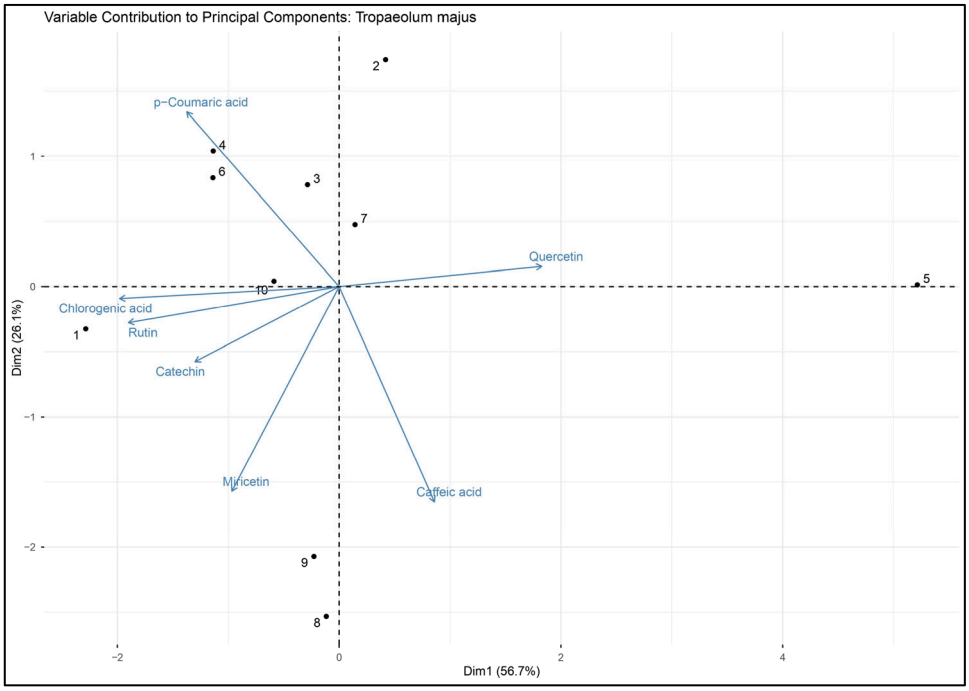


Figure 2. PCA of phenolic acids and flavonoids in extracts of *Tropaeolum majus*.

https://doi.org/10.3390/appliedchem6040074

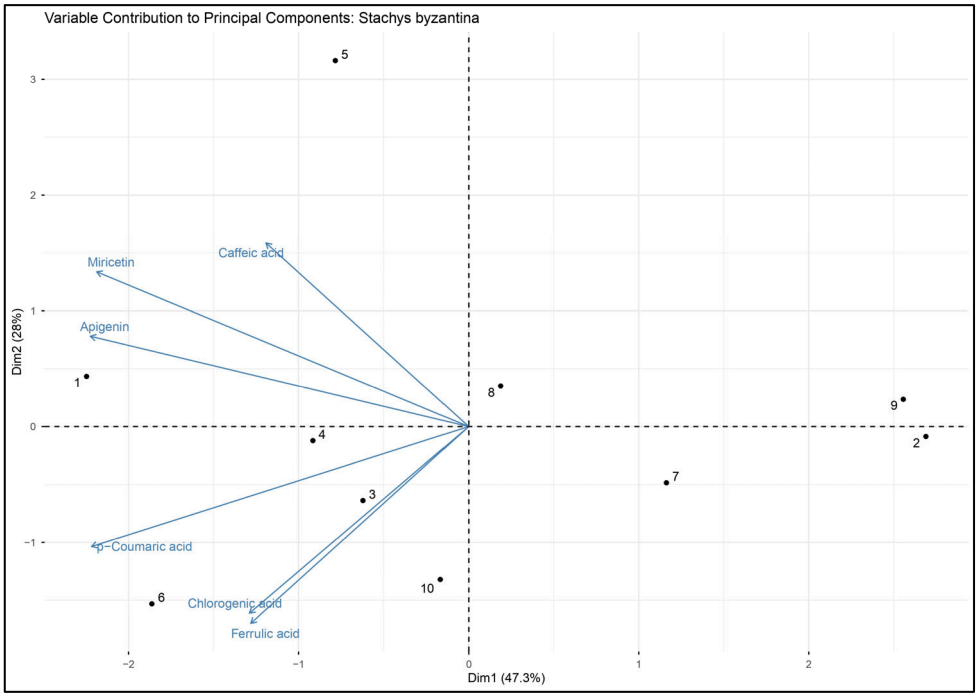


Figure 3. PCA of phenolic acids and flavonoids in extracts of *Stachys byzantina*.

For *Tropaeolum majus*, PC1 and PC2 explained 56.7% and 26.1% of the total variance, respectively, accounting for 82.8% of the cumulative variance. Similar to *Rumex acetosa*, the high explanatory power of the first two components indicates a well-defined multivariate structure and a relatively consistent response of phenolic compounds to extraction conditions. Chlorogenic acid, p-coumaric acid, rutin, catechin, and myricetin showed a positive association with methods No. 1, 3, 4, 6, 8, 9, and 10. In contrast, caffeic acid and quercetin showed a stronger association with method No. 5. These results reinforce the influence of extraction conditions on the selective recovery of phenolic compounds and highlight the existence of specific extraction patterns for each compound in the same botanical matrix.

In *Stachys byzantina*, the first two principal components explained 75.3% of the total variance (PC1 = 47.3% and PC2 = 28.0%), a smaller proportion than that observed in *Rumex acetosa* and *Tropaeolum majus*. Although this percentage still provides a satisfactory representation of the dataset, it suggests that a larger fraction of the variability was distributed among the subsequent principal components. Unlike the other species, in which up to nine phenolic compounds were quantified, only six were detected in quantifiable amounts in *Starchys byzantina* extracts. However, these compounds responded differently to the extraction treatments, resulting in a more dispersed multivariate distribution. The positive association between caffeic acid, myricetin, and apigenin characterized methods No. 1, 5, and 8, while chlorogenic acid, p-coumaric acid, and ferulic acid were associated with methods No. 1, 3, 4, 6, and 10. Furthermore, the lower explanatory power of principal components 1 and 2 may reflect a less uniform extraction behavior among the quantified compounds, potentially influenced by specific matrix characteristics, such as the densely pubescent leaf surface of *Stachys byzantina*, which could affect solvent penetration and mass transfer during extraction.

A notable finding across all three species was the recurring association of chlorogenic acid with the most efficient extraction protocols. Because chlorogenic acid was consistently among the predominant phenolic compounds recovered, regardless of the extraction strategy, this result suggests that these unconventional food plants may be relevant dietary sources of hydroxycinnamic acids.

Overall, Principal Component Analysis (PCA) showed that extraction performance depended strongly on interactions among the solvent system, extraction conditions, and plant matrix. While *Rumex acetosa* and *Tropaeolum majus* exhibited more compact and predictable multivariate structures, *Stachys byzantina* showed a more dispersed variability distribution among extraction treatments. These results reinforce the importance of optimizing species-specific extraction protocols and indicate that no single extraction strategy is equally effective across all plant matrices when the goal is to maximize phenolic compound recovery.

4. Conclusions

Extraction conditions significantly influenced the total phenolic content, antioxidant activity, and phenolic profile of the evaluated unconventional food plants, showing that no single extraction protocol was universally optimal for all species. This study showed that extraction performance depends on the species and extraction conditions and should therefore be selected based on the intended application, whether for routine compositional analysis or for producing extracts rich in phenolic compounds. These results reinforce the influence of extraction conditions on the selective recovery of phenolic compounds and highlight specific extraction patterns for each compound within the same botanical matrix.

In general, methanol-based solvent systems, characterized by their high polarity and small molecular size, promoted greater recovery of phenolic compounds. Furthermore, solvent acidification and ultrasound-assisted extraction improved extraction efficiency, highlighting the importance of both solvent composition and extraction technique in maximizing phenolic recovery.

Each species showed distinct responses across the evaluated matrices. For *Rumex acetosa*, extraction method 5, which used 80% methanol acidified with 1% hydrochloric acid and ultrasound, provided the highest phenolic recovery. In contrast, method No. 1 was most effective for *Tropaeolum majus* and *Stachys byzantina*. These results reinforce the need to optimize extraction protocols for each matrix and indicate that extraction conditions should consider both the botanical characteristics of the plant material and the intended analytical or industrial application.

The predominance of chlorogenic acid among the quantified phenolic compounds highlights the potential of these species as food sources of bioactive molecules with recognized antioxidant properties. In addition, the results contribute to the characterization of little-explored unconventional food plants and corroborate their potential application in functional foods and nutraceutical products. Future studies should expand the characterization of phenolic compounds and optimize extraction strategies for different plant matrices, strengthening the scientific basis for the nutritional valorization and wider use of these species.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/appliedchem6040074/s1, Figure S1: Chromatogram (A)—Standard calibration curve (Method A); Figure S2: Chromatogram (B)—Standard calibration curve (Method B); Figure S3: Chromatogram of the phenolic compound profile for Method A (271 nm) and Method B (277 nm); Table S1: Calibration curve of standards (Method A) of phenolic compounds used in the quantification of compounds present in *Rumex acetosa* L., Tropaeolum majus L., and *Stachys byzantina* K.Koch title; Table S2: Calibration curve of standards (Method B) of phenolic compounds used in the quantification of compounds present in *Rumex acetosa* L., *Tropaeolum majus* L., and *Stachys byzantina* K.Koch.

Author Contributions: Conceptualization: R.L.B.d.A., R.A.L., M.W.P. and M.M.M.; methodology: M.d.V.-O., A.L.C.C.R., A.L.S.V. and M.M.M.; validation: R.A.L., R.L.B.d.A., A.L.S.V. and M.M.M.; formal analysis: A.J.A.d.C. and M.M.M.; investigation: L.A.C.J., V.O.d.F., D.C.G. and M.M.M.; resources: R.L.B.d.A. and M.W.P.; data curation: M.M.M., A.J.A.d.C.; writing—original draft preparation: R.L.B.d.A., R.A.L., A.L.S.V. and M.M.M.; writing—review and editing: R.L.B.d.A., R.A.L. and M.M.M.; visualization: M.M.M.; supervision: R.L.B.d.A. and R.A.L.; project administration: R.L.B.d.A.; funding acquisition: R.L.B.d.A. All authors have read and agreed to the published version of the manuscript.

Funding: This research was supported by the Minas Gerais Research Foundation (FAPEMIG—APQ-01614-23) and the National Council for Scientific and Technological Development (CNPq; Grant No. 449213/2025-0).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The original contributions presented in this study are included in the article/Supplementary Materials. Further inquiries can be directed to the corresponding author(s).

Acknowledgments: The authors thank the Minas Gerais Agricultural Research Company for the institutional support and infrastructure provided for the realization of this work. They also thank the Minas Gerais State Research Support Foundation and the National Council for Scientific and Technological Development for the financial support and encouragement of scientific research.

Conflicts of Interest: Author Marinalva Woods Pedrosa was employed by the company Minas Gerais Agricultural Research Corporation—EPAMIG Centro-Oeste. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Abbreviation

NCFPs Non-conventional food plants

References

1. Rana, A.; Samtiya, M.; Dhewa, T.; Mishra, V.; Aluko, R.E. Health Benefits of Polyphenols: A Concise Review. *J. Food Biochem.* **2022**, *46*, e14264. [CrossRef]
2. Šamec, D.; Karalija, E.; Šola, I.; Vujčić Bok, V.; Salopek-Sondi, B. The role of polyphenols in abiotic stress response: The influence of molecular structure. *Plants* **2021**, *10*, 118. [CrossRef]
3. Zhou, M.; Fakayode, O.A.; Li, H. Green extraction of polyphenols via deep eutectic solvents and assisted technologies from agri-food by-products. *Molecules* **2023**, *28*, 6852. [CrossRef]
4. Domínguez-Avila, J.A.; Villa-Rodriguez, J.A.; Montiel-Herrera, M.; Pacheco-Ordaz, R.; Roopchand, D.E.; Venema, K.; González-Aguilar, G.A. Phenolic Compounds Promote Diversity of Gut Microbiota and Maintain Colonic Health. *Dig. Dis. Sci.* **2021**, *66*, 3270–3289. [CrossRef]
5. Biglari, F.; AlKarkhi, A.F.M.; Easa, A.M. Antioxidant Activity and Phenolic Content of Various Date Palm (*Phoenix dactylifera*) Fruits from Iran. *Food Chem.* **2008**, *107*, 1636–1641. [CrossRef]
6. Shi, L.; Zhao, W.; Yang, Z.; Subbiah, V.; Suleria, H.A.R. Extraction and Characterization of Phenolic Compounds and Their Potential Antioxidant Activities. *Environ. Sci. Pollut. Res.* **2022**, *29*, 81112–81129. [CrossRef]
7. Hättenschwiler, S.; Vitousek, P.M. The Role of Polyphenols in Terrestrial Ecosystem Nutrient Cycling. *Trends Ecol. Evol.* **2000**, *15*, 238–243. [CrossRef]
8. Vermerris, W.; Nicholson, R. Phenolic compounds and their effects on human health. In *Phenolic Compound Biochemistry*; Springer: Dordrecht, The Netherlands, 2006; pp. 235–255. [CrossRef]
9. Zainal-Abidin, M.H.; Hayyan, M.; Hayyan, A.; Jayakumar, N.S. New Horizons in the Extraction of Bioactive Compounds Using Deep Eutectic Solvents: A Review. *Anal. Chim. Acta* **2017**, *979*, 1–23. [CrossRef]
10. Bohlin, L. Natural Products Isolation. *Drug Discov. Today* **1998**, *3*, 536–537. [CrossRef]
11. Robards, K. Strategies for the Determination of Bioactive Phenols in Plants, Fruit and Vegetables. *J. Chromatogr. A* **2003**, *1000*, 657–691. [CrossRef]

12. Alara, O.R.; Abdurahman, N.H.; Ukaegbu, C.I. Extraction of Phenolic Compounds: A Review. *Curr. Res. Food Sci.* **2021**, *4*, 200–214. [CrossRef]
13. Dai, J.; Mumper, R.J. Plant Phenolics: Extraction, Analysis and Their Antioxidant and Anticancer Properties. *Molecules* **2010**, *15*, 7313–7352. [CrossRef]
14. Haminiuk, C.W.I.; Maciel, G.M.; Plata-Oviedo, M.S.V.; Peralta, R.M. Phenolic Compounds in Fruits—An Overview. *Int. J. Food Sci. Technol.* **2012**, *47*, 2023–2044. [CrossRef]
15. Haminiuk, C.W.I.; Plata-Oviedo, M.S.V.; Guedes, A.R.; Stafussa, A.P.; Bona, E.; Carpes, S.T. Chemical, Antioxidant and Antibacterial Study of Brazilian Fruits. *Int. J. Food Sci. Technol.* **2011**, *46*, 1529–1537. [CrossRef]
16. Hurtado-Fernández, E.; Gómez-Romero, M.; Carrasco-Pancorbo, A.; Fernández-Gutiérrez, A. Application and Potential of Capillary Electroseparation Methods to Determine Antioxidant Phenolic Compounds from Plant Food Material. *J. Pharm. Biomed. Anal.* **2010**, *53*, 1130–1160. [CrossRef]
17. Luthria, D.L. Influence of Experimental Conditions on the Extraction of Phenolic Compounds from Parsley (*Petroselinum crispum*) Flakes Using a Pressurized Liquid Extractor. *Food Chem.* **2008**, *107*, 745–752. [CrossRef]
18. Yang, B.; Jiang, Y.; Shi, J.; Chen, F.; Ashraf, M. Extraction and Pharmacological Properties of Bioactive Compounds from Longan (*Dimocarpus longan* Lour.) Fruit—A Review. *Food Res. Int.* **2011**, *44*, 1837–1842. [CrossRef]
19. Velázquez-Martínez, V.; Valles-Rosales, D.; Rodríguez-Uribe, L.; Laguna-Camacho, J.R.; López-Calderón, H.D.; Delgado, E. Effect of Different Extraction Methods and Geographical Origins on the Total Phenolic Yield, Composition, and Antimicrobial Activity of Sugarcane Bagasse Extracts. *Front. Nutr.* **2022**, *9*, 834557. [CrossRef]
20. López-Fernández, O.; Domínguez, R.; Pateiro, M.; Munekata, P.E.S.; Rocchetti, G.; Lorenzo, J.M. Determination of Polyphenols Using Liquid Chromatography–Tandem Mass Spectrometry Technique (LC–MS/MS): A Review. *Antioxidants* **2020**, *9*, 479. [CrossRef]
21. Liu, Z.; Wu, H.-L.; Xie, L.-X.; Hu, Y.; Fang, H.; Sun, X.-D.; Wang, T.; Xiao, R.; Yu, R.-Q. Direct and Interference-Free Determination of Thirteen Phenolic Compounds in Red Wines Using a Chemometrics-Assisted HPLC-DAD Strategy for Authentication of Vintage Year. *Anal. Methods* **2017**, *9*, 3361–3374. [CrossRef]
22. Pereira, V.; Câmara, J.S.; Cacho, J.; Marques, J.C. HPLC-DAD Methodology for the Quantification of Organic Acids, Furans and Polyphenols by Direct Injection of Wine Samples. *J. Sep. Sci.* **2010**, *33*, 1204–1215. [CrossRef]
23. Motilva, M.-J.; Serra, A.; Macià, A. Analysis of Food Polyphenols by Ultra High-Performance Liquid Chromatography Coupled to Mass Spectrometry: An Overview. *J. Chromatogr. A* **2013**, *1292*, 66–82. [CrossRef]
24. Kinupp, V.F. Plantas Alimentícias Não-Convencionais da Região Metropolitana de Porto Alegre, RS. Ph.D. Thesis, Universidade Federal do Rio Grande do Sul, Faculdade de Agronomia, Porto Alegre, Brazil, 2007.
25. Manach, C.; Scalbert, A.; Morand, C.; Rémésy, C.; Jiménez, L. Polyphenols: Food Sources and Bioavailability. *Am. J. Clin. Nutr.* **2004**, *79*, 727–747. [CrossRef]
26. Iqbal, Y.; Ponnampalam, E.N.; Cottrell, J.J.; Suleria, H.A.R.; Dunshea, F.R. Extraction and Characterization of Polyphenols from Non-Conventional Edible Plants and Their Antioxidant Activities. *Food Res. Int.* **2022**, *157*, 111205. [CrossRef]
27. Clemente-Villalba, J.; Burló, F.; Hernández, F.; Carbonell-Barrachina, Á.A. Valorization of Wild Edible Plants as Food Ingredients and Their Economic Value. *Foods* **2023**, *12*, 1012. [CrossRef]
28. Azevedo, T.D. Propriedades Nutricionais, Antioxidantes, Antimicrobianas e Toxicidade Preliminar do Peixinho da Horta (*Stachys byzantina* K. Koch). Master's Thesis, Universidade Federal do Paraná, Curitiba, Brazil, 2018. Available online: https://acervodigital.ufpr.br/xmlui/bitstream/handle/1884/58899/R%20-%20D%20-%20THAISE%20DUDA%20DE%20AZEVEDO.pdf?sequence=1&isAllowed=y (accessed on 1 July 2023).
29. Nascimento, L.E.S.; Arriola, N.D.A.; da Silva, L.A.L.; Faqueti, L.G.; Sandjo, L.P.; de Araújo, C.E.S.; Biavatti, M.W.; Barcelos-Oliveira, J.L.; Dias de Mello Castanho Amboni, R. Phytochemical Profile of Different Anatomical Parts of Jambu (*Acmella oleracea* (L.) R.K. Jansen): A Comparison between Hydroponic and Conventional Cultivation Using PCA and Cluster Analysis. *Food Chem.* **2020**, *332*, 127393. [CrossRef]
30. de Souza, T.C.L.; da Silveira, T.F.F.; Rodrigues, M.I.; Ruiz, A.L.T.G.; Neves, D.A.; Duarte, M.C.T.; Cunha-Santos, E.C.E.; Kuhnle, G.; Ribeiro, A.B.; Godoy, H.T. A Study of the Bioactive Potential of Seven Neglected and Underutilized Leaves Consumed in Brazil. *Food Chem.* **2021**, *364*, 130350. [CrossRef]
31. Pérez-Jiménez, J.; Arranz, S.; Tabernero, M.; Díaz-Rubio, M.E.; Serrano, J.; Goñi, I.; Saura-Calixto, F. Updated Methodology to Determine Antioxidant Capacity in Plant Foods, Oils and Beverages: Extraction, Measurement and Expression of Results. *Food Res. Int.* **2008**, *41*, 274–285. [CrossRef]
32. de Morais, J.S.; Sant'Ana, A.S.; Dantas, A.M.; Silva, B.S.; Lima, M.S.; Borges, G.C.; Magnani, M. Antioxidant Activity and Bioaccessibility of Phenolic Compounds in White, Red, Blue, Purple, Yellow and Orange Edible Flowers through a Simulated Intestinal Barrier. *Food Res. Int.* **2020**, *131*, 109046. [CrossRef]

33. Grutzmann Arcari, S.; Arena, K.; Kolling, J.; Rocha, P.; Dugo, P.; Mondello, L.; Cacciola, F. Polyphenolic Compounds with Biological Activity in Guabiroba Fruits (*Campomanesia xanthocarpa* Berg.) by Comprehensive Two-Dimensional Liquid Chromatography. *Electrophoresis* **2020**, *41*, 1784–1792. [CrossRef]
34. Fidelis, M.; Vieira do Carmo, M.A.; Azevedo, L.; Cruz, T.M.; Marques, M.B.; Myoda, T.; Sant'Ana, A.S.; Furtado, M.M.; Wen, M.; Zhang, L.; et al. Response Surface Optimization of Phenolic Compounds from Jabuticaba (*Myrciaria cauliflora* [Mart.] O.Berg) Seeds: Antioxidant, Antimicrobial, Antihyperglycemic, Antihypertensive and Cytotoxic Assessments. *Food Chem. Toxicol.* **2020**, *142*, 111439. [CrossRef]
35. Rodrigues, E.; Mariutti, L.R.B.; Mercadante, A.Z. Carotenoids and Phenolic Compounds from *Solanum sessiliflorum*, an Unexploited Amazonian Fruit, and Their Scavenging Capacities against Reactive Oxygen and Nitrogen Species. *J. Agric. Food Chem.* **2013**, *61*, 3022–3029. [CrossRef]
36. Ajila, C.M.; Bhat, S.G.; Prasada Rao, U.J.S. Valuable Components of Raw and Ripe Peels from Two Indian Mango Varieties. *Food Chem.* **2007**, *102*, 1006–1011. [CrossRef]
37. Rufino, M.d.S.M.; Alves, R.E.; de Brito, E.S.; Pérez-Jiménez, J.; Saura-Calixto, F.; Mancini-Filho, J. Bioactive Compounds and Antioxidant Capacities of 18 Non-Traditional Tropical Fruits from Brazil. *Food Chem.* **2010**, *121*, 996–1002. [CrossRef]
38. Peres, R.L.; Moraes, S.C.S.; de Carvalho, C.A.; do Nascimento, P.C.; de Carvalho, L.M.; da Silva, M.B.; Rampelotto, P.H.; da Rosa, M.B. Achillea millefolium–Asteraceae: Phytochemical, Spectrophotometric, and Antifungal Activity Study (*Colletotrichum musae*). *Rev. Eletrônica Farmácia* **2009**, *6*, 81–93. [CrossRef]
39. Singleton, V.L.; Orthofer, R.; Lamuela-Raventós, R.M. Analysis of Total Phenols and Other Oxidation Substrates and Antioxidants by Means of Folin-Ciocalteu Reagent. In *Methods in Enzymology*; Academic Press: New York, NY, USA, 1999; Volume 299, pp. 152–178. [CrossRef]
40. Felix Ávila, P.; Pereira Todescato, A.; de Melo Carolo dos Santos, M.; Fernando Ramos, L.; Caroline Menon, I.; Oliveira Carvalho, M.; do Vale-Oliveira, M.; Beatriz Custódio, F.; Beatriz Abreu Gloria, M.; Martins Dala-Paula, B.; et al. *Annona crassiflora* Suppresses Colonic Carcinogenesis through Its Antioxidant Effects, Bioactive Amines, and Phenol Content in Rats. *Food Res. Int.* **2024**, *175*, 113666. [CrossRef]
41. Agência Nacional de Vigilância Sanitária (ANVISA). Collegiate Board Resolution—RDC No. 166, of July 24, Provides for the Validation of Analytical Methods and Other Provisions. 2017. Diário Oficial da União. Available online: https://www.gov.br/anvisa/pt-br (accessed on 10 April 2024).
42. International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH). *Validation of Analytical Procedures Q2(R2)*; International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use: Geneva, Switzerland, 2023; Available online: https://www.ich.org/page/quality-guidelines (accessed on 12 April 2024).
43. The Jamovi Project. jamovi (Version 2.5) [Computer Software]. 2024. Available online: https://www.jamovi.org (accessed on 20 May 2024).
44. Kassambara, A. factoextra: Extract and Visualize the Results of Multivariate Data Analyses (Version 2.0.0). CRAN, 2026. Available online: https://CRAN.R-project.org/package=factoextra (accessed on 20 May 2024).
45. R Core Team. *R: A Language and Environment for Statistical Computing (Version 4.3)*; R Foundation for Statistical Computing: Vienna, Austria, 2024; Available online: https://www.R-project.org/ (accessed on 22 May 2024).
46. Wickham, H. *ggplot2: Elegant Graphics for Data Analysis*; Springer International Publishing: Cham, Switzerland, 2016. [CrossRef]
47. Posit Software, PBC. *RStudio: Integrated Development Environment for R, Version 2025.05.0*; Statistical Computing Software; Posit Software, PBC: Boston, MA, USA, 2025.
48. Feduraev, P.; Skrypnik, L.; Nebreeva, S.; Dzhobadze, G.; Vatagina, A.; Kalinina, E.; Pungin, A.; Maslennikov, P.; Riabova, A.; Krol, O.; et al. Variability of Phenolic Compound Accumulation and Antioxidant Activity in Wild Plants of Some Rumex Species (*Polygonaceae*). *Antioxidants* **2022**, *11*, 311. [CrossRef]
49. Ceccanti, C.; Guidi, L.; Tardelli, F.; Venturi, F.; Giusti, G.; Ricci, L.; Zinnai, A. Comparison of Three Domestications and Wild-Harvested Plants for Nutraceutical Properties and Sensory Profiles in Five Wild Edible Herbs: Is Domestication Possible? *Foods* **2020**, *9*, 1065. [CrossRef]
50. Cornara, L.; Ambu, G.; Alberto, A.; Trombetta, D.; Smeriglio, A. Characterization of Ingredients Incorporated in the Traditional Mixed-Salad of the Capuchin Monks. *Plants* **2022**, *11*, 301. [CrossRef]
51. Sytar, O.; Hemmerich, I.; Zivcak, M.; Rauh, C.; Brestic, M. Comparative Analysis of Bioactive Phenolic Compounds Composition from 26 Medicinal Plants. *Saudi J. Biol. Sci.* **2018**, *25*, 631–641. [CrossRef]
52. Lee, J.-E.; Jayakody, J.; Kim, J.-I.; Jeong, J.-W.; Choi, K.-M.; Kim, T.-S.; Seo, C.; Azimi, I.; Hyun, J.; Ryu, B. The Influence of Solvent Choice on the Extraction of Bioactive Compounds from *Asteraceae*: A Comparative Review. *Foods* **2024**, *13*, 3151. [CrossRef]
53. Thouri, A.; Chahdoura, H.; El Arem, A.; Omri Hichri, A.; Ben Hassin, R.; Achour, L. Effect of Solvents Extraction on Phytochemical Components and Biological Activities of Tunisian Date Seeds (Var. Korkobbi and Arechti). *BMC Complement. Altern. Med.* **2017**, *17*, 248. [CrossRef]

54. Tabart, J.; Kevers, C.; Evers, D.; Dommes, J. Ascorbic Acid, Phenolic Acid, Flavonoid, and Carotenoid Profiles of Selected Extracts from *Ribes nigrum*. *J. Agric. Food Chem.* **2011**, *59*, 4763–4770. [CrossRef]
55. Vagiri, M.; Ekholm, A.; Andersson, S.C.; Johansson, E.; Rumpunen, K. An Optimized Method for Analysis of Phenolic Compounds in Buds, Leaves, and Fruits of Black Currant (*Ribes nigrum* L.). *J. Agric. Food Chem.* **2012**, *60*, 10501–10510. [CrossRef]
56. Gao, W.; Sun, X.; Chen, T.; Lin, Y.; Chen, Y.; Lu, F.; Chen, Z. Preparation of Cyano-Functionalized Multiwalled Carbon Nanotubes as Solid-Phase Extraction Sorbent for Preconcentration of Phenolic Compounds in Environmental Water. *J. Sep. Sci.* **2012**, *35*, 1967–1976. [CrossRef]
57. Mollica, A.; Zengin, G.; Sinan, K.I.; Marletta, M.; Pieretti, S.; Stefanucci, A.; Etienne, O.K.; Jekő, J.; Cziáky, Z.; Bahadori, M.B.; et al. A Study on Chemical Characterization and Biological Abilities of *Alstonia boonei* Extracts Obtained by Different Techniques. *Antioxidants* **2022**, *11*, 2171. [CrossRef]
58. Herrera-Pool, E.; Ramos-Díaz, A.L.; Lizardi-Jiménez, M.A.; Pech-Cohuo, S.; Ayora-Talavera, T.; Cuevas-Bernardino, J.C.; García-Cruz, U.; Pacheco, N. Effect of Solvent Polarity on the Ultrasound-Assisted Extraction and Antioxidant Activity of Phenolic Compounds from Habanero Pepper Leaves (*Capsicum chinense*) and Its Identification by UPLC-PDA-ESI-MS/MS. *Ultrason. Sonochem.* **2021**, *76*, 105658. [CrossRef]
59. Freire, J.M.; Abreu, C.M.P.; Rocha, D.A.; Corrêa, A.D.; Marques, N.R. Quantification of Phenolic Compounds and Ascorbic Acid in Fruits and Frozen Pulps of Acerola, Cashew, Guava, and Strawberry. *Ciênc. Rural* **2013**, *43*, 2291–2295. [CrossRef]
60. Apak, R.; Özyürek, M.; Güçlü, K.; Çapanoğlu, E. Antioxidant Activity/Capacity Measurement. 2. Hydrogen Atom Transfer (HAT)-Based, Mixed-Mode (Electron Transfer (ET)/HAT), and Lipid Peroxidation Assays. *J. Agric. Food Chem.* **2016**, *64*, 1028–1045. [CrossRef]
61. Prior, R.L.; Wu, X.; Schaich, K. Standardized Methods for the Determination of Antioxidant Capacity and Phenolics in Foods and Dietary Supplements. *J. Agric. Food Chem.* **2005**, *53*, 4290–4302. [CrossRef]
62. Munteanu, I.G.; Apetrei, C. Analytical Methods Used in Determining Antioxidant Activity: A Review. *Int. J. Mol. Sci.* **2021**, *22*, 3380. [CrossRef]
63. Abozed, S.S.; El-Kalyoubi, M.; Abdelrashid, A.; Salama, M.F. Total Phenolic Contents and Antioxidant Activities of Various Solvent Extracts from Whole Wheat and Bran. *Ann. Agric. Sci.* **2014**, *59*, 63–67. [CrossRef]
64. Kumar, K.; Srivastav, S.; Sharanagat, V.S. Ultrasound Assisted Extraction (UAE) of Bioactive Compounds from Fruit and Vegetable Processing By-Products: A Review. *Ultrason. Sonochem.* **2021**, *70*, 105325. [CrossRef]
65. Shirsath, S.R.; Sonawane, S.H.; Gogate, P.R. Intensification of Extraction of Natural Products Using Ultrasonic Irradiations—A Review of Current Status. *Chem. Eng. Process. Process Intensif.* **2012**, *53*, 10–23. [CrossRef]
66. Ferraz, L.P.; Silva, E.K. Pulsed Electric Fields and Ultrasound for Enhanced Mass Transfer: A Review of Extraction and Drying in Food Processing. *ACS Food Sci. Technol.* **2025**, *5*, 3229–3253. [CrossRef]
67. Vieira, E.F.; Souza, S.; Moreira, M.M.; Cruz, R.; da Silva, A.B.; Casal, S.; Delerue-Matos, C. Valorization of Phenolic and Carotenoid Compounds of Sechium edule (Jacq. Swartz) Leaves: Comparison between Conventional, Ultrasound- and Microwave-Assisted Extraction Approaches. *Molecules* **2022**, *27*, 7193. [CrossRef]
68. Chen, Y.-H.; Yang, C.-Y. Ultrasound-Assisted Extraction of Bioactive Compounds and Antioxidant Capacity for the Valorization of *Elaeocarpus serratus* L. Leaves. *Processes* **2020**, *8*, 1218. [CrossRef]
69. Heleno, S.A.; Martins, A.; Queiroz, M.J.R.P.; Ferreira, I.C.F.R. Bioactivity of Phenolic Acids: Metabolites versus Parent Compounds: A Review. *Food Chem.* **2015**, *173*, 501–513. [CrossRef]
70. Khoddami, A.; Wilkes, M.; Roberts, T. Techniques for Analysis of Plant Phenolic Compounds. *Molecules* **2013**, *18*, 2328–2375. [CrossRef]
71. Martins, N.; Barros, L.; Henriques, M.; Silva, S.; Ferreira, I.C.F.R. Activity of Phenolic Compounds from Plant Origin against Candida Species. *Ind. Crops Prod.* **2015**, *74*, 648–670. [CrossRef]
72. Meneses, N.G.T.; Martins, S.; Teixeira, J.A.; Mussatto, S.I. Influence of Extraction Solvents on the Recovery of Antioxidant Phenolic Compounds from Brewer's Spent Grains. *Sep. Purif. Technol.* **2013**, *108*, 152–158. [CrossRef]
73. Mokrani, A.; Madani, K. Effect of Solvent, Time and Temperature on the Extraction of Phenolic Compounds and Antioxidant Capacity of Peach (*Prunus persica* L.) Fruit. *Sep. Purif. Technol.* **2016**, *162*, 68–76. [CrossRef]
74. Azman, E.M.; Nor, N.D.M.; Charalampopoulos, D.; Chatzifragkou, A. Effect of Acidified Water on Phenolic Profile and Antioxidant Activity of Dried Blackcurrant (*Ribes nigrum* L.) Pomace Extracts. *LWT* **2022**, *154*, 112733. [CrossRef]
75. Rodriguez-Saona, L.E.; Wrolstad, R.E. Extraction, Isolation, and Purification of Anthocyanins. *Curr. Protoc. Food Anal. Chem.* **2001**, *00*, F1.1.1–F1.1.11. [CrossRef]
76. Xia, B.-H.; Yu, Z.-L.; Lu, Y.-A.; Liu, S.-J.; Li, Y.-M.; Xie, M.-X.; Lin, L.-M. Green and Efficient Extraction of Phenolic Components from Plants with Supramolecular Solvents: Experimental and Theoretical Studies. *Molecules* **2024**, *29*, 2067. [CrossRef]
77. Alara, O.R.; Abdurahman, N.H.; Ukaegbu, C.I. Soxhlet Extraction of Phenolic Compounds from *Vernonia cinerea* Leaves and Its Antioxidant Activity. *J. Appl. Res. Med. Aromat. Plants* **2018**, *11*, 12–17. [CrossRef]

78. Osorio-Tobón, J.F. Recent Advances and Comparisons of Conventional and Alternative Extraction Techniques of Phenolic Compounds. *J. Food Sci. Technol.* **2020**, *57*, 4299–4315. [CrossRef]
79. Celli, G.B.; Ghanem, A.; Brooks, M.S.-L. Optimization of Ultrasound-Assisted Extraction of Anthocyanins from Haskap Berries (*Lonicera caerulea* L.) Using Response Surface Methodology. *Ultrason. Sonochem.* **2015**, *27*, 449–455. [CrossRef]
80. Hossain, M.B.; Barry-Ryan, C.; Martin-Diana, A.B.; Brunton, N.P. Optimisation of Accelerated Solvent Extraction of Antioxidant Compounds from Rosemary (*Rosmarinus officinalis* L.), Marjoram (*Origanum majorana* L.) and Oregano (*Origanum vulgare* L.) Using Response Surface Methodology. *Food Chem.* **2011**, *126*, 339–346. [CrossRef]
81. Lu, H.; Tian, Z.; Cui, Y.; Liu, Z.; Ma, X. Chlorogenic Acid: A Comprehensive Review of the Dietary Sources, Processing Effects, Bioavailability, Beneficial Properties, Mechanisms of Action, and Future Directions. *Compr. Rev. Food Sci. Food Saf.* **2020**, *19*, 3130–3158. [CrossRef]
82. Nguyen, V.; Taine, E.G.; Meng, D.; Cui, T.; Tan, W. Chlorogenic Acid: A Systematic Review on the Biological Functions, Mechanistic Actions, and Therapeutic Potentials. *Nutrients* **2024**, *16*, 924. [CrossRef]
83. Zhang, D.; Yu, F. Recent Advances in Chlorogenic Acids for Food Preservation and Shelf-Life Extension. *Antioxidants* **2026**, *15*, 633. [CrossRef]
84. Benedec, D.; Oniga, I.; Hanganu, D.; Tiperciuc, B.; Nistor, A.; Vlase, A.-M.; Vlase, L.; Pușcaș, C.; Duma, M.; Login, C.C.; et al. Stachys Species: Comparative Evaluation of Phenolic Profile and Antimicrobial and Antioxidant Potential. *Antibiotics* **2023**, *12*, 1644. [CrossRef]
85. Kucekova, Z.; Mlcek, J.; Humpolicek, P.; Rop, O.; Valasek, P.; Saha, P. Phenolic Compounds from *Allium schoenoprasum*, *Tragopogon pratensis* and *Rumex acetosa* and Their Antiproliferative Effects. *Molecules* **2011**, *16*, 9207–9217. [CrossRef]
86. Bahadori, M.B.; Zengin, G.; Dinparast, L.; Eskandani, M. The Health Benefits of Three Hedgenettle Herbal Teas (*Stachys byzantina, Stachys inflata*, and *Stachys lavandulifolia*)—Profiling Phenolic and Antioxidant Activities. *Eur. J. Integr. Med.* **2020**, *36*, 101134. [CrossRef]
87. Chemat, F.; Rombaut, N.; Sicaire, A.-G.; Meullemiestre, A.; Fabiano-Tixier, A.-S.; Abert-Vian, M. Ultrasound Assisted Extraction of Food and Natural Products. Mechanisms, Techniques, Combinations, Protocols and Applications. A Review. *Ultrason. Sonochem.* **2017**, *34*, 540–560. [CrossRef]
88. Lama-Muñoz, A.; Contreras, M.d.M. Extraction Systems and Analytical Techniques for Food Phenolic Compounds: A Review. *Foods* **2022**, *11*, 3671. [CrossRef]

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.