applied sciences

MDPI

Article

The Value of Using Green Extraction Techniques to Enhance Polyphenol Content and Antioxidant Activity in *Nasturtium officinale* Leaves

Eva Naoum, Aikaterini Xynopoulou, Konstantina Kotsou, Theodoros Chatzimitakos *, Vassilis Athanasiadis, Eleni Bozinou and Stavros I. Lalas

Department of Food Science and Nutrition, University of Thessaly, Terma N. Temponera Str., 43100 Karditsa, Greece; enaoum@uth.gr (E.N.); axynopoulou@uth.gr (A.X.); kkotsou@agr.uth.gr (K.K.); vaathanasiadis@uth.gr (V.A.); empozinou@uth.gr (E.B.); slalas@uth.gr (S.I.L.)

* Correspondence: tchatzimitakos@uth.gr

Abstract: Increasing research is being directed toward the production of value-added products using plant extracts that are super-fortified with antioxidants. In this study, the extraction parameters for bioactive compounds (such as polyphenols) from *Nasturtium officinale* leaves and their antioxidant properties were optimized using response surface methodology. The optimization procedure examined the effects of the extraction temperature, time, and solvent composition on conventional magnetic stirring (ST). In addition, the impacts of two green techniques—pulsed electric field (PEF) and ultrasound (US)—were evaluated individually and in combination to assess their potential to enhance the extraction of the compounds. According to our findings, under the proposed extraction conditions (a combination of PEF, US, and ST as a extraction technique, 50% ethanolic solvent, for 30 min at 80 °C). *N. officinale* leaf extract proved to be an excellent source of bioactive compounds, with extracts containing rosmarinic acid (3.42 mg/g dried weight (dw)), chlorogenic acid (3.13 mg/g dw), total polyphenol content (28.82 mg of gallic acid equivalents (GAE)/g dw), and strong antioxidant properties. The FRAP method measured 57.15 μmol ascorbic acid equivalents (AAE)/g dw, while the DPPH radical scavenging activity method measured 47.55 μmol AAE/g dw. This study was carried out to evaluate and improve the concentration of bioactive compounds in *N. officinale* leaf extract, resulting in a product with multiple applications across the food, cosmetic, and pharmaceutical industries.

Keywords: watercress; green extraction techniques; pulsed electric field; ultrasound; polyphenols; rosmarinic acid; antioxidants

Citation: Naoum, E.; Xynopoulou, A.; Kotsou, K.; Chatzimitakos, T.; Athanasiadis, V.; Bozinou, E.; Lalas, S.I. The Value of Using Green Extraction Techniques to Enhance Polyphenol Content and Antioxidant Activity in *Nasturtium officinale* Leaves. *Appl. Sci.* **2024**, *14*, 10739. https://doi.org/10.3390/app142210739

Academic Editor: Antony C. Calokerinos

Received: 7 September 2024
Revised: 30 October 2024
Accepted: 19 November 2024
Published: 20 November 2024

1. Introduction

Nasturtium officinale or watercress, a member of the Brassicaceae family [1], is native to western Asia, India, Europe, and Africa. In Europe, it is mainly found in Denmark, Belgium, the Czech Republic, Austria, Ireland, Sweden, Germany, and Hungary [2]. A plethora of phytochemicals and vitamins are contained in watercress. More specifically, it contains 2.4 g/80 g of crude protein, 0.8 g/80 g of fat, 1.2 g/80 g of fiber, and a total of 18 kcal [3]. In addition, it is a source of vitamins A (336 μg/80 g), B1 (0.13 mg/80 g), B6 (0.18 mg/80 g), C (50 mg/80 g), E (1.17 mg/80 g), and K (200 μg/ 80 g) [3]. It is also worth mentioning that watercress contains minerals such as calcium, iron, magnesium, phosphorus, potassium, and zinc in amounts of 136, 1.8, 12, 0.5, 42, 184, and 0.6 mg/80 g, respectively [4,5]. Finally, it contains sodium and copper in significant amounts [4,5].

Aside from their abundance of nutrients, watercress leaves are used in traditional medicine on account of their antioxidant, anti-cancer, antibacterial, anti-inflammatory, and cardioprotective properties [6]. These properties are mainly attributed to the total polyphenol content of watercress [6]. Despite the importance of polyphenols in watercress,

a low amount has been documented; in the vegetative period, the amount ranges between 8.03 and 9.35 mg gallic acid equivalents (GAE)/g, and in the generative period, the quantity is lower (between 6.5 and 7.65 mg GAE/g) [2].

Polyphenols, found in abundance in plants, have become an emerging field of interest in nutrition in recent decades since a rising body of research shows that polyphenol consumption plays a vital role in human health [7]. The solid–liquid method is one of the classical and conventional extraction techniques for the recovery of polyphenols [8]. This extraction method is based on the performance of different solvents or hot water extraction [9]. However, it also has some limitations, such as low efficiency and long extraction time, high solvent cost, and degradation of volatile compounds. Therefore, it is not considered "green" and attractive, and for this reason, some of the conventional solid–liquid extraction processes are well suited to be supported by green methods, such as ultrasound, to disrupt the cell wall structure of plant raw materials [8].

Nowadays, studies are being carried out to isolate the maximum amount of polyphenols from plant products using mainly green extraction methods [10–12]. A green extraction method or technique is an extraction process that has low energy consumption, a short extraction duration, and/or uses new-generation, non-harmful solvents, and ensures a safe and high-quality final product [13–15]. Among the green extraction methods, pulsed electric field (PEF), ultrasound (US), supercritical fluid extraction (SFE), pressurized liquid extraction (PLE), and microwave-assisted extraction (MAE) are the most renowned [16]. The advanced technologies of MAE, US, and PEF provide the basis for a significantly reduced cost of production of enhanced extracts [10].

Although the PEF system is an advanced extraction method, it has limitations in the food industry, such as the occurrence of electrochemical reactions at the electrode–rotor interface. These reactions can lead to significant alterations, such as electrolysis of water and chemical changes in the food product [17]. In general, the ultrasonic bath is one of the most widely used instruments in food analysis laboratories throughout the world. It is considered a very advanced extraction method and, indeed, the most economical ultrasonic irradiation system. It allows for a uniform distribution of energy within the vessel [18]; however, the placement of the vessel containing the matrix and solvent within the bath must be performed very carefully, as the effect of the ultrasonic waves varies with the location [19,20]. In light of the considerable nutritional value associated with watercress, coupled with the low amount of polyphenols, the primary objective of this research was the optimization of the extraction process so as to maximize the extraction yield of polyphenolic compounds. Moreover, given the importance of green extraction methods and their positive impact on the isolation of polyphenols, a multifactorial system was employed so as to examine both conventional extraction and two key green extraction methods, PEF and US, as well as their combination, taking into account all the parameters affecting extraction (temperature, time, and solvent composition) [21–23]. In this way, the superiority of green extraction methods, or a combination of them, for obtaining more antioxidant compounds from watercress was studied and analyzed. This research promotes the enrichment of a polyphenol-poor extract using green extraction techniques and facilitates the use of watercress extract in a wider range of applications.

2. Materials and Methods

2.1. Flower Collection and Preparation

Plants were collected from the Peloponnese region (Greece) when they were in full maturity according to the ripening data of watercress plant from previous studies [1,24–26]. The leaves were carefully cut off and then gently washed. All leaves employed for the experiment were at the maximum height encountered, 12 cm [24,25]. Before using the leaves, they were placed between absorbent paper for 48 h to absorb moisture and to be protected from light. More absorbent paper was used, when necessary, until the leaves were dried.

2.2. Chemicals and Reagents

All solvents used were of at least HPLC grade and sourced from Carlo Erba (Val de Reuil, France). The chemical standards for the polyphenolic compounds, including chlorogenic acid, caffeic acid, syringic acid, *p*-coumaric acid, ferulic acid, rosmarinic acid, rutin, quercetin 3-β-*D*-glucoside, luteolin-7-glucoside, narirutin, kaempferol-3-glucoside, apigenin-7-*O*-glucoside, and myricetin, were obtained from Sigma-Aldrich (Steinheim, Germany). Hydrochloric acid, ascorbic acid, trichloroacetic acid, ferric (III) chloride, aluminum chloride, and sodium acetate were also purchased from Sigma-Aldrich (Steinheim, Germany). Additionally, gallic acid, anhydrous sodium carbonate, Folin–Ciocalteu reagent, 2,2-diphenyl-1-picrylhydrazyl (DPPH), and 2,4,6-tri-2-pyridyl-1,3,5-triazine (TPTZ) were sourced from Penta (Prague, Czech Republic). Deionized water was used in all experiments.

2.3. Extraction Procedure

For the conventional extraction process (ST), mixtures (comprising 1 g of plant material and 20 mL of solvent) were stirred at 500 rpm under varying temperature and time conditions. Prior to the ST extraction, some samples underwent additional pretreatment with green extraction methods (PEF or US, or both). For the PEF treatment, a pulse period of 1 ms (frequency: 1 kHz), a pulse duration of 10 µs, and an electric field strength of 1.0 kV/cm were employed. This setup involved a mode/arbitrary waveform generator (UPG100, ELV Elektronik AG, Leer, Germany), a digital oscilloscope (Rigol DS1052E, Beaverton, OR, USA), a high-voltage power generator (Leybold, LD Didactic GmbH, Huerth, Germany), and custom stainless-steel chambers (Val-Electronic, Athens, Greece). The US pretreatment was conducted using an Elmasonic P70H machine, 180 W (Elma Schmidbauer GmbH, Singen, Germany), at 30 °C and 37 kHz. The dried material was pre-soaked in the solvent for 10 min before any treatment. Following extraction, the mixture was centrifuged at 4500 rpm for 10 min, and the supernatant was collected for subsequent analyses.

2.4. Response Surface Methodology (RSM) Extraction Optimization

The response surface methodology (RSM) was employed to optimize the total polyphenol content (TPC) and antioxidant potential. Optimization was achieved by refining the extraction process, particularly through the adjustment of parameters, such as the solvent concentration (ethanol in water), denoted as *C* (% *v/v*), to investigate solvents of varying polarities; the extraction time, labeled as *t* (min), based on preliminary experiments; and the extraction temperature, indicated as *T* (°C), with a cap of 80 °C to maintain the integrity of the compounds extracted. A main effects screening design, featuring twenty design points, was utilized to optimize the process. The variables underwent testing at five distinct levels, as outlined in Table 1, which presents both coded and actual levels. Analysis of variance (ANOVA) and summary-of-fit tests were performed to evaluate the model's overall significance (R^2, *p*-value), as well as the significance of the model coefficients (equations). Additionally, a second-order polynomial model was employed to forecast the dependent variable using the analyzed independent factors:

$$Y_k = \beta_0 + \sum_{i=1}^{2} \beta_i X_i + \sum_{i=1}^{2} \beta_{ii} X_i^2 + \sum_{i=1}^{2} \sum_{j=i+1}^{3} \beta_{ij} X_i X_j \quad (1)$$

where the independent variables are denoted by X_i and X_j, and the predicted response variable is defined by Y_k. In the model, the intercept and regression coefficients β_0, β_i, β_{ii} and β_{ij}, respectively, represent the linear, quadratic, and interaction terms.

Table 1. The actual and coded levels of the independent variables used to optimize the process.

Independent Variables	Code Units	Coded Variable Levels				
		1	2	3	4	5
Technique	X_1	ST	PEF + ST	US + ST	PEF + US + ST	–
C (%, *v*/*v*)	X_2	0	25	50	75	100
t (min)	X_3	30	60	90	120	150
T (°C)	X_4	20	35	50	65	80

2.5. Analyses of the Extracts

2.5.1. Total Polyphenol Content (TPC)

The total polyphenol contents (TPC) of the watercress extracts were determined following the procedure described by Lakka et al. [27]. In brief, 100 µL of the extracts were mixed with an equal volume of the Folin–Ciocalteu reagent in an Eppendorf tube. Two minutes later, 800 µL of Na_2CO_3 solution (5% *w*/*v*) was added, and the solutions were heated at 40 °C for 20 min. Finally, using a Shimadzu spectrophotometer (UV-1700, Shimadzu Europa GmbH, Duisburg, Germany), the absorbance at 740 nm was measured. A calibration curve was also prepared, using gallic acid as the standard compound. The concentration of the total polyphenols (C_{TP}) was expressed as mg gallic acid equivalents (GAE) per L. The TPC was expressed as mg GAE per g of dry weight (dw), using Equation (2):

$$\text{TPC (mg GAE/g dw)} = \frac{C_{TP} \times V}{w} \quad (2)$$

where V is the volume of the extraction medium (in L), and w is the dry weight of the sample (in g).

2.5.2. Ferric-Reducing Antioxidant Power (FRAP)

The antioxidant activity was measured using the ferric-reducing antioxidant power (FRAP) assay, according to the protocol previously outlined by Paleologou et al. [28]. The amount of 50 µL ferric (III) chloride solution (4 mM in 0.05 M HCl) was well mixed with 50 µL of the diluted sample extract and then incubated in a water bath at 37 °C for 30 min. After that, 900 µL of TPTZ solution (1 mM in 0.05 M HCl) was added, and the absorbance at 620 nm was measured after exactly 5 min. The ferric-reducing antioxidant power (P_R) was determined as µmol ascorbic acid equivalents (AAE) per g of dw, using an ascorbic acid calibration curve (C_{AA}, 50–500 µmol/L in 0.05 M HCl) calculated using Equation (3):

$$P_R \text{ (µmol AAE/g dw)} = \frac{C_{AA} \times V}{w} \quad (3)$$

where V is the volume of the extraction medium (in L), and w is the dry weight of the sample (in g).

2.5.3. Radical Scavenging Activity (DPPH)

The DPPH radical scavenging activity was evaluated using a modified version of the protocol previously outlined by Paleologou et al. [28]. A volume of 25 µL of diluted sample extract was mixed with 975 µL of DPPH solution (100 µmol/L in methanol), and the absorbance at 515 nm was measured immediately after mixing ($A_{515(i)}$) and exactly 30 min later ($A_{515(f)}$). To calculate the percentage of inhibition, Equation (4) was employed:

$$\text{Inhibition (\%)} = \frac{A_{515(i)} - A_{515(f)}}{A_{515(i)}} \times 100 \quad (4)$$

An ascorbic acid calibration curve in Equation (5) was used to evaluate the antiradical activity (A_{AR}), which was expressed as μmol AAE per gram of dw:

$$A_{AR}\ (\mu\text{mol AAE/g dw}) = \frac{C_{AA} \times V}{w} \quad (5)$$

where V is the volume of the extraction medium (in L), and w is the dry weight of the sample (in g).

2.5.4. High-Performance Liquid Chromatography (HPLC) Coupled with Diode Array Detector (DAD)

High-performance liquid chromatography (HPLC) coupled with diode array detector (DAD) was used to quantify individual polyphenolic compounds, as detailed in our previous study [29]. In more detail, the analysis utilized a Shimadzu CBM-20A liquid chromatograph connected to a Shimadzu SPD-M20A diode array detector, both provided by Shimadzu Europa GmbH in Duisburg, Germany. The separation of compounds occurred on a Phenomenex Luna C18 (2) column from Phenomenex Inc. in Torrance, CA, USA, maintained at 40 °C (100 Å, 5 μm, 4.6 mm × 250 mm). The mobile phase comprised 0.5% aqueous formic acid (A) and a mixture of 0.5% formic acid in acetonitrile/water (6:4) (B). The gradient program employed was as follows: starting at 0% B and increasing to 40% B, then a transition to 50% B over 10 min, further increasing to 70% B in the subsequent 10 min, and then maintaining this level for 10 min. The flow rate of the mobile phase was set at 1 mL/min. Retention time and absorbance spectrum comparisons were made against those of pure chemical standards for compound identification. Quantification was accomplished using calibration curves ranging from 0 to 50 μg/mL.

2.6. Statistical Analysis

The experimental design, statistical analysis related to the response surface methodology, and distribution analysis were all conducted using JMP® Pro 16 (SAS, Cary, NC, USA) software. All extraction procedures described above were performed three times, and each extract was analyzed in triplicate. The results are expressed as the mean values of all measurements (3 × 3 = 9 total measurements for each extract), with the standard deviation calculated from these nine samples.

3. Results and Discussion

3.1. Extraction Optimization

The main parameters affecting the extraction process of ST and the use of green extraction methods (US and PEF) were assessed to determine the maximum TPC yield. In order to minimize the number of conducted experiments and obtain a better overview, an RSM approach was employed. Using the RSM approach, a multi-factor system was constructed, as shown in Table 2, resulting in 20 different design points. From the results, the combination of the most suitable extraction parameters were identified to ensure the preparation of an extract from dried watercress leaves with the highest possible concentration of bioactive compounds and antioxidant activity. The experimental design used approximately 1 g of dried watercress plant and 20 mL of solvent for each extraction. This proportion was deliberately chosen to ensure the efficient extraction of all components. Preliminary research indicated that using a smaller volume of solvent led to significant absorption by the plant, whereas volumes exceeding 20 mL did not result in increased extraction yields.

The results obtained from the analyses of the 20 extracts (presented in Table 2) indicate that different combinations have a major impact on the measured responses. In particular, design point 13 is considered the most appropriate for acquiring the highest amount of total polyphenols, while design points 11 and 2 were considered the most suitable for enhancing the antioxidant activity by the FRAP and DPPH methods, respectively. Among the most interesting results is that neither PEF, US, nor the use of 100% ethanol was found

to enhance the FRAP activity. In general, ethanol is considered to favor the efficiency of an extraction, especially for bioactive compounds characterized by medium polarity, such as polyphenols [30]. This was substantiated in the present study, since, for maximum isolation of the total polyphenols, the use of 50% ethanolic solvent was found to be the most appropriate. Nevertheless, in a previous study, it was proven that the use of water as the solvent was the best extraction medium concerning the antioxidant activity in all studied fruits [31]. This finding was corroborated in the current study, where water emerged as the most suitable solvent for enhancing the antioxidant capacity of watercress leaves, as measured by the FRAP method. Despite the advantages associated with green extraction techniques, it is important to note that extraction processes, such as US at elevated temperatures, may lead to the degradation of heat-sensitive compounds, resulting in the loss of valuable elements [32,33], which occurred in the case of the antioxidant capacity via the FRAP method.

Table 2. The findings from the experiment involving four independent variables and the responses of the dependent variable.

Design Point	Independent Variables				Responses					
					TPC (mg GAE/g dw)		FRAP (μmol AAE/g dw)		DPPH (μmol AAE/g dw)	
	X_1	X_2	X_3	X_4	Actual	Predicted	Actual	Predicted	Actual	Predicted
1	3	1	3	4	7.59 ± 0.40	8.60	57.24 ± 1.66	54.96	29.33 ± 1.55	28.42
2	3	2	1	3	14.43 ± 0.87	13.79	48.67 ± 0.97	48.34	40.29 ± 2.70	37.77
3	2	3	4	3	7.29 ± 0.17	8.24	66.97 ± 2.01	68.89	35.08 ± 2.56	36.82
4	2	4	5	4	7.02 ± 0.31	5.74	34.56 ± 2.42	37.38	29.84 ± 1.10	30.38
5	3	5	4	2	3.61 ± 0.17	3.41	14.60 ± 0.55	14.56	6.14 ± 0.46	8.89
6	4	1	4	5	19.93 ± 0.44	18.93	23.63 ± 0.50	25.09	25.16 ± 1.28	24.57
7	4	2	3	1	20.22 ± 0.67	20.34	27.26 ± 1.74	28.83	28.84 ± 1.38	29.35
8	1	3	3	2	16.99 ± 0.83	15.44	64.03 ± 1.28	61.73	36.47 ± 1.93	33.45
9	1	4	4	1	8.62 ± 0.18	9.55	28.36 ± 1.50	29.18	25.74 ± 0.62	25.88
10	1	5	1	4	10.33 ± 0.22	11.07	55.48 ± 2.66	55.21	10.61 ± 0.36	12.16
11	1	1	2	3	16.15 ± 0.63	14.82	72.10 ± 3.10	75.31	28.74 ± 1.26	28.79
12	1	2	5	5	15.29 ± 0.73	16.31	51.20 ± 1.59	49.31	37.95 ± 2.39	38.80
13	4	3	2	4	24.67 ± 1.09	25.72	55.37 ± 3.43	52.78	38.79 ± 2.44	42.55
14	3	4	2	5	18.96 ± 0.89	19.03	58.94 ± 2.71	61.36	35.64 ± 2.46	36.02
15	2	5	3	5	10.59 ± 0.42	9.76	34.17 ± 1.54	32.75	18.30 ± 0.93	15.54
16	2	1	1	1	6.16 ± 0.44	6.68	31.23 ± 2.28	30.49	17.03 ± 1.11	18.80
17	2	2	2	2	9.52 ± 0.70	10.75	61.52 ± 3.01	60.22	31.56 ± 2.08	31.57
18	3	3	5	1	7.12 ± 0.19	6.29	66.54 ± 3.33	65.50	37.33 ± 1.79	36.33
19	4	4	1	2	19.42 ± 0.82	18.49	26.36 ± 1.16	27.58	28.80 ± 1.01	26.93
20	4	5	5	3	12.05 ± 0.83	13.01	20.03 ± 0.54	18.79	14.30 ± 0.57	12.92

Table 3 displays the statistical parameters, second-order polynomial equations, and coefficients ($R^2 > 0.97$) for each model, indicating a strong fit for the developed models. The plots comparing the actual response to the predicted response for each parameter, along with the desirability functions, are shown in Figures S1–S3. Three-dimensional response surface plots for TPC are depicted in Figure S4, and the remaining responses (FRAP and DPPH) are in Figures S5 and S6. Figures S4–S6 feature six 3D surface plots, labeled A to F. Each plot illustrates the relationships among three variables, with the x and y axes representing different variables (X_1, X_2, X_3, and X_4), and the z-axis corresponding to the response. The plots use a color gradient from blue (indicating low values) to red (indicating high values) to denote the response values. Figure S4 demonstrates how the TPC changes with various combinations of X_1, X_2, X_3, and X_4, which is beneficial for comprehending the interactions among these variables and for optimizing conditions to attain specific TPC levels.

Table 3. Mathematical models utilizing RSM were applied to optimize the extraction process from the watercress plant.

Responses	Second-Order Polynomial Equations (Models)	R^2 Predicted	R^2 Predicted	*p*-Value	Eq.
TPC	$Y = 23.16 - 19.37X_1 + 11.34X_2 - 2.88X_3 - 1.29X_4 + 3.8{X_1}^2 - 2.28{X_2}^2 - 0.76{X_3}^2 + 1.08{X_4}^2 + 0.4X_1X_2 + 0.88X_1X_3 - 0.59X_1X_4 + 0.88X_2X_3 - 0.5X_2X_4 + 0.08X_3X_4$	0.9731	0.8978	0.0053	(6)
FRAP	$Y = 13.88 - 13.93X_1 - 5.92X_2 + 34.77X_3 + 24.33X_4 - 2.7{X_1}^2 - 2.05{X_2}^2 + 0.79{X_3}^2 - 4.69{X_4}^2 + 5X_1X_2 - 0.27X_1X_3 + 2.69X_1X_4 - 6.44X_2X_3 + 4.96X_2X_4 - 5.32X_3X_4$	0.9901	0.9623	0.0005	(7)
DPPH	$Y = -2.98 - 1.07X_1 + 15.07X_2 + 5.7X_3 + 6.68X_4 + 0.45{X_1}^2 - 3.89{X_2}^2 + 0.54{X_3}^2 - 0.46{X_4}^2 + 0.68X_1X_2 - 1.42X_1X_3 + 0.41X_1X_4 - 0.02X_2X_3 + 0.74X_2X_4 - 1.59X_3X_4$	0.9662	0.8717	0.0090	(8)

3.2. Impact of Extraction Parameters on Assays as Analyzed Through Pareto Plots

An illustration of the correlation (positive or negative) of each extraction factor, including the extraction technique, is shown in Figure 1 through the Pareto plot. Through this visualization, the impact of each factor on the maximum recovery of the respective bioactive compound is also perceived. In view of the data provided in Table 2, a different extraction factor was expected for each predetermination method. Indeed, looking closely at Figure 1, the extraction technique seems to play the most decisive role, showing a positive correlation. This fact is fully in line with the results of Table 2, since the maximum TPC isolation required the application of all three extraction methods, PEF + US + ST. On the other hand, for the maximum free radical binding recovery (DPPH method), the solvent composition seems to be the most influential factor, exhibiting even a negative correlation. This means that neither 100% water nor 100% ethanol are the most suitable solvents; on the contrary, an excellent balance is found at a certain concentration of ethanol, 25%.

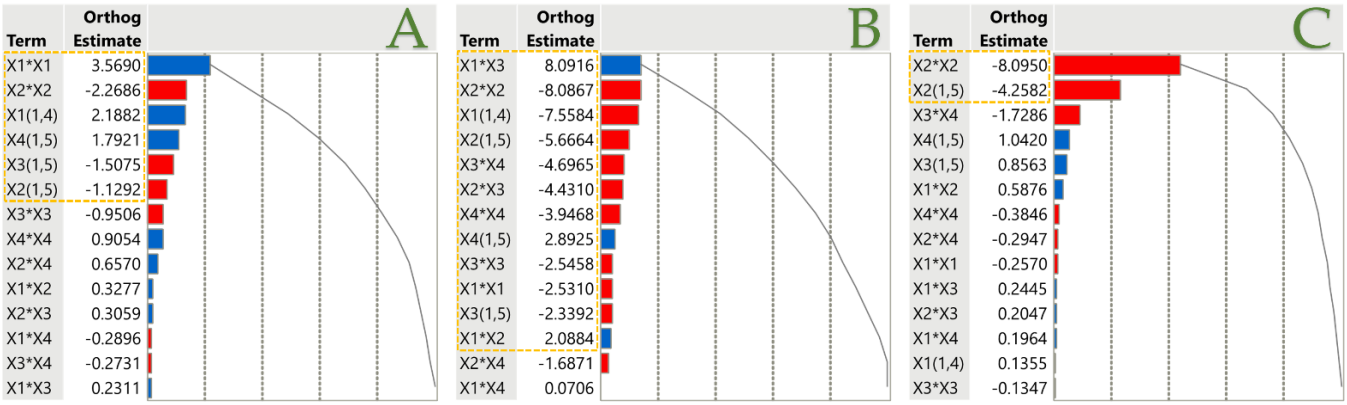

Figure 1. Pareto plots represent transformed estimates for TPC (**A**), FRAP (**B**), and DPPH (**C**) assays. A gold dashed rectangular reference line is included in the plot to denote the significance level ($p < 0.05$). Blue bars indicate positive values, while red bars represent negative values.

3.3. Optimal Extraction Conditions

3.3.1. Total Polyphenol Content of Watercress Extracts

As indicated in Tables 1 and 3, the experimentally applied optimal extraction parameters coincide with the predicted ones for maximum TPC recovery from watercress leaves. These parameters include extractions with a combination of green and conventional extraction techniques (PEF + US + ST), with 50% ethanolic solvent for 60 min at 65 °C. This temperature is reasonable, as TPC enhancement efficiencies start to decrease at temperatures above 80 °C due to their thermosensitive properties [34,35]. By following these

parameters, the TPC of watercress leaves can be enhanced from 3.61 to 24.67 mg GAE/g, i.e., 583.38%. The maximum TPC previously recorded in watercress leaves was 9.35 mg/g [2], i.e., 163.85% less than the maximum obtained in our case. In another study evaluating water and methanol separately as extraction solvents for watercress leaves, the results exhibited 2.31 mg GAE/g and 3.21 mg GAE/g, respectively [36]. Thus, the use of a combination of green extraction methods with conventional liquid–solid extraction proved to be the most suitable extraction method for obtaining the greatest amount of polyphenols from watercress. These results are fully consistent with previous studies on plant materials. For example, according to the research of Tan et al. [37], after optimizing the extraction parameters for litchi fruit peel, the total TPC of the sample extracted with the PEF-US combination was 2.30 times higher than that of the sample extracted with traditional extraction. Also, the effect of the PEF-US combination as an extraction technique on almond extract resulted in the highest concentration of total phenolics, total flavonoids, anthocyanins, and antioxidant activity (DPPH and FRAP) [38]. These outcomes demonstrate the importance of using green extraction techniques for unlocking the maximum bioactive value that a plant can yield and provide a highly beneficial extract with a plethora of applications. These techniques are "green" due to their lower energy requirements, reduced use of hazardous solvents, and greater yields with a minimal environmental footprint. Contrary to traditional extraction methods, which depend on high temperatures and chemical solvents, US and PEF employ physical mechanisms to improve extraction efficacy, rendering them more sustainable and eco-friendly.

3.3.2. Antioxidant Capacity of Watercress Extracts

Plant extracts have attracted the interest of industries and the scientific community worldwide owing to the functional benefits offered by their antioxidant activity [39]. A notable example is the cosmetics industry, where a plethora of cosmetics are preferred due to their natural antioxidant ingredients. Another great example is the food industry, where natural antioxidants [40], which are mainly derived from plant materials [39], are used as food ingredients [41]. This is the reason the present study focused on the antioxidant activity of watercress leaves. Regarding the results obtained by the FRAP method, by applying appropriate extraction procedures and parameters, the antioxidant activity increased from 14.60 to 72.10 μmol ascorbic acid equivalents (AAE)/g dw, i.e., a 393.84% increase, as shown in Table 2. These results are in perfect agreement with a previous study, where the antioxidant activity in watercress was found to be 74.54 ± 10.81 μmol AAE/g dw [42]. According to Tables 1 and 3, ST is the most suitable for enhancing the antioxidant activity of watercress leaf extracts. This finding may be attributed to the fact that the use of US and PEF results in extended processing time, increasing the risk of compound oxidation [43]. Moreover, this could also be the reason only the combination of US with ST was deemed appropriate for the maximum acquisition of antioxidant activity with DPPH, as shown in Tables 1 and 3. By following the conditions detailed in Table 2, an increase in the DPPH radical scavenging capacity of 556.19% can be achieved. At the same time, as shown in Table 4, applying US and ST as an extraction technique for 30 min at 65 °C with 50% ethanol solvent, a 43.98 ± 9.51 μmol AAE/g dw antioxidant capacity can be obtained, confirming, among others, the importance of an increased ethanol concentration to enhance the extraction efficiency [44]. Furthermore, it is of paramount importance that ethanol is not only an easily recoverable solvent but also deemed suitable for human consumption, making it a widely preferred choice in the food and pharmaceutical industries [45,46].

Table 4. Optimal extraction conditions and maximum predicted responses for the dependent variables.

Responses	Optimal Conditions				
	Maximum Predicted Response	**Technique (X_1)**	***C* (%, *v*/*v*) (X_2)**	***t* (min) (X_3)**	***T* (°C) (X_4)**
TPC (mg GAE/g dw)	25.72 ± 3.59	PEF + US + ST (4)	50 (3)	60 (2)	65 (4)
FRAP (µmol AAE/g dw)	79.82 ± 6.48	ST (1)	25 (2)	90 (3)	50 (3)
DPPH (µmol AAE/g dw)	43.98 ± 9.51	US + ST (3)	50 (3)	30 (1)	65 (4)

3.4. Principal Component Analysis (PCA) and Multivariate Correlation Analysis (MCA)

Principal component analysis (PCA) is a valuable statistical tool, as it enables the simplification of complex data sets. Three technical PCA iterations were performed to ensure the validity of the results. The three different tested variables, TPC, FRAP, and DPPH, in watercress leaf extract displayed a positive correlation, as shown in Figure 2. The variable X_4, temperature, also exhibits a positive correlation. These data are perfectly reasonable since, as described in Table 4, higher temperatures (up to 65 °C) seem to benefit the enhancement of the TPC, FRAP, and DPPH variables. In addition, the extraction technique was found to play the greatest role among the different extraction parameters. As found in Tables 1 and 3, different techniques and combinations of techniques appeared to strongly favor extraction for the amplification of each of the TPC, FRAP, and DPPH variables. This is the reason that, in Table 5, the different variables present low correlations with each other (with a peak correlation value of 1).

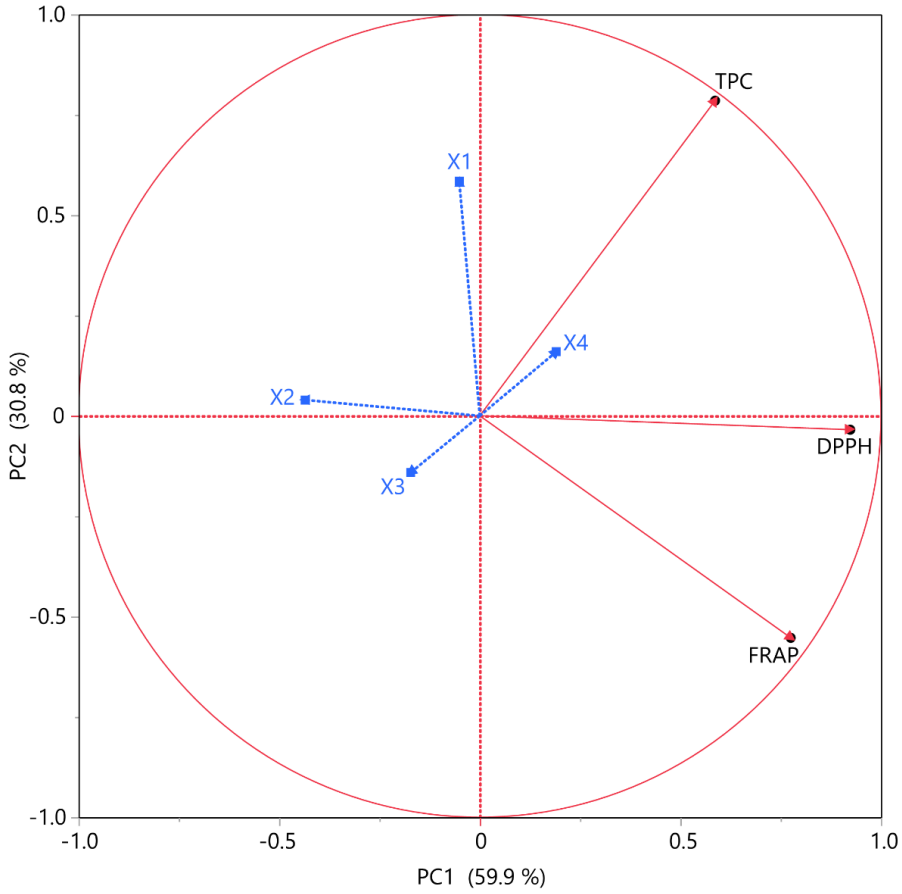

Figure 2. Principal component analysis (PCA) was applied to the measured variables, with each *X* variable represented in blue.

Table 5. Multivariate correlation analysis among the measured variables.

Responses	TPC	FRAP	DPPH
TPC	-	0.0810	0.4385
FRAP		-	0.6179
DPPH			-

3.5. Partial Least Squares (PLS) Analysis

A partial least squares (PLS) model was applied in order to determine and propose an optimal extraction mode for the maximum recovery of the total polyphenols and antioxidant activity, as illustrated in the correlation loading plot depicted in Figure 3A and the variable importance plot (VIP) option graphs, where values for each predictor variable are shown in Figure 3B. Considering the concentration of ethanol as a solvent and the extraction technique as the main extraction factors (Figure 3B), the optimum enhanced values were obtained by applying all extraction techniques, (PEF, US, and ST), with 50% ethanolic solvent for the shortest extraction time, 30 min, at the maximum temperature of 80 °C, as also exhibited in Table 6.

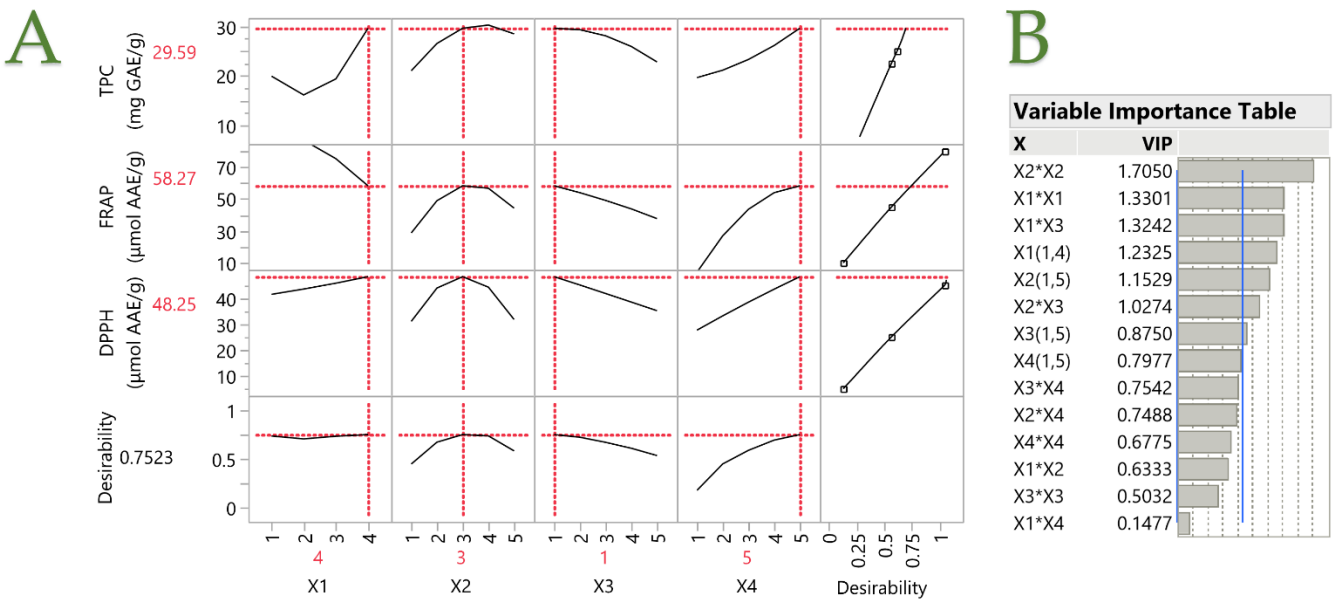

Figure 3. Plot (**A**) illustrates the desirability function with extrapolation control and the partial least squares (PLS) prediction profiler for optimizing watercress plant. The variable importance plot (VIP) option in Plot (**B**) displays the VIP values for each predictor variable. The blue dashed line at the 0.8 mark on the VIT indicates the significance level for each variable.

Table 6. The maximum desirability under the optimal extraction conditions (X_1: 4, X_2: 3, X_3: 1, and X_4: 5) for all variables using the partial least squares (PLS) prediction profiler.

Variables	PLS Model Values	Experimental Values
TPC (mg GAE/g dw)	29.59	28.82 ± 1.59
FRAP (µmol AAE/g dw)	58.27	57.15 ± 3.31
DPPH (µmol AAE/g dw)	48.25	47.55 ± 2.90

The high determination coefficient (R^2) of 0.9659 and the strong correlation coefficient of 0.9828 show that the experimental data and the predictions from the PLS model are in high agreement. Additionally, a *p*-value of less than 0.0001 indicates that there was no statistically significant difference in the variations between the experimental and PLS model (predicted) values.

Adopting the optimal extraction conditions, individual polyphenolic compounds contained in the optimum watercress leaf extracts were analyzed by HPLC-DAD. In accordance with previous research, some of the polyphenolic compounds contained in watercress are chlorogenic acid, caffeic acid, rutin, ferulic acid, apigenin, *p*-coumaric acid, quercetin, and kaempferol [36,47]. Substantial quantities of these compounds were also detected in the present investigation, as shown in Table 7. Figure 4 graphically illustrates an exemplary chromatogram. Despite the challenges in separation, the quantification of polyphenols in the chromatogram was reliably determined by the peak area and resolution. The methodology employed was robust, ensuring accurate analysis of the polyphenolic content, even in cases of suboptimal chromatographic separation. The most abundant polyphenolic compounds present in the optimized extract of watercress were rosmarinic acid and chlorogenic acid, with rutin and apigenin following. Rosmarinic acid is primarily detected in plants of the Lamiaceae family; however, in the present study, it was revealed that it may also be found in plants of the Brassicaceae family [48]. Rosmarinic acid is well known for its anticancer and antioxidant properties, as it is utilized to mitigate the risk of various cancers by preventing cellular damage caused by free radicals [49]. Although the extracts of watercress leaves revealed high antioxidant activity through the DPPH method, no strong correlation between the variables TPC and DPPH was detected. Chlorogenic acid is a natural polyphenolic acid compound displaying remarkable properties according to experiments carried out in mice. In particular, there is evidence that it potentiates osteogenic differentiation of human dental pulp stem cells and promotes the proliferation of intestinal stem cells and epithelial regeneration [50]. At the same time, rutin has been shown to have excellent medicinal properties, since it is indicated for the treatment of various heart diseases [51]. Lastly, apigenin also exhibits remarkable therapeutic properties. Depending on the dosage, apigenin offers muscle relaxation and sedation [52]. Additionally, it could represent a new tool for delaying the onset of Alzheimer's disease or slowing its progression [53]. Taking everything into account, a beneficial extract can be produced from the watercress leaves, with a multitude of health-promoting properties.

Table 7. The polyphenolic compounds under the optimal extraction conditions (X_1: 4, X_2: 3, X_3: 1, and X_4: 5). The percentages (%) of total identified polyphenols in watercress plant are also displayed.

A/A	Polyphenolic Compound	Optimal Extract (mg/g dw)	Quantity (%)
1.	Chlorogenic acid	3.13 ± 0.23	17.1
2.	Caffeic acid	0.17 ± 0.01	1.0
3.	Syringic acid	0.16 ± 0.01	0.9
4.	*p*-Coumaric acid	0.55 ± 0.04	3.0
5.	Ferulic acid	0.19 ± 0.01	1.0
6.	Rutin	2.54 ± 0.18	13.9
7.	Quercetin 3-β-*D*-glucoside	0.95 ± 0.03	5.2
8.	Luteolin-7-glucoside	1.37 ± 0.10	7.5
9.	Narirutin	0.96 ± 0.05	5.3
10.	Kaempferol-3-glucoside	1.72 ± 0.04	9.4
11.	Apigenin-7-*O*-glucoside	2.55 ± 0.13	13.9
12.	Myricetin	0.59 ± 0.04	3.2
13.	Rosmarinic acid	3.42 ± 0.09	18.7
	Total identified	18.3 ± 0.97	

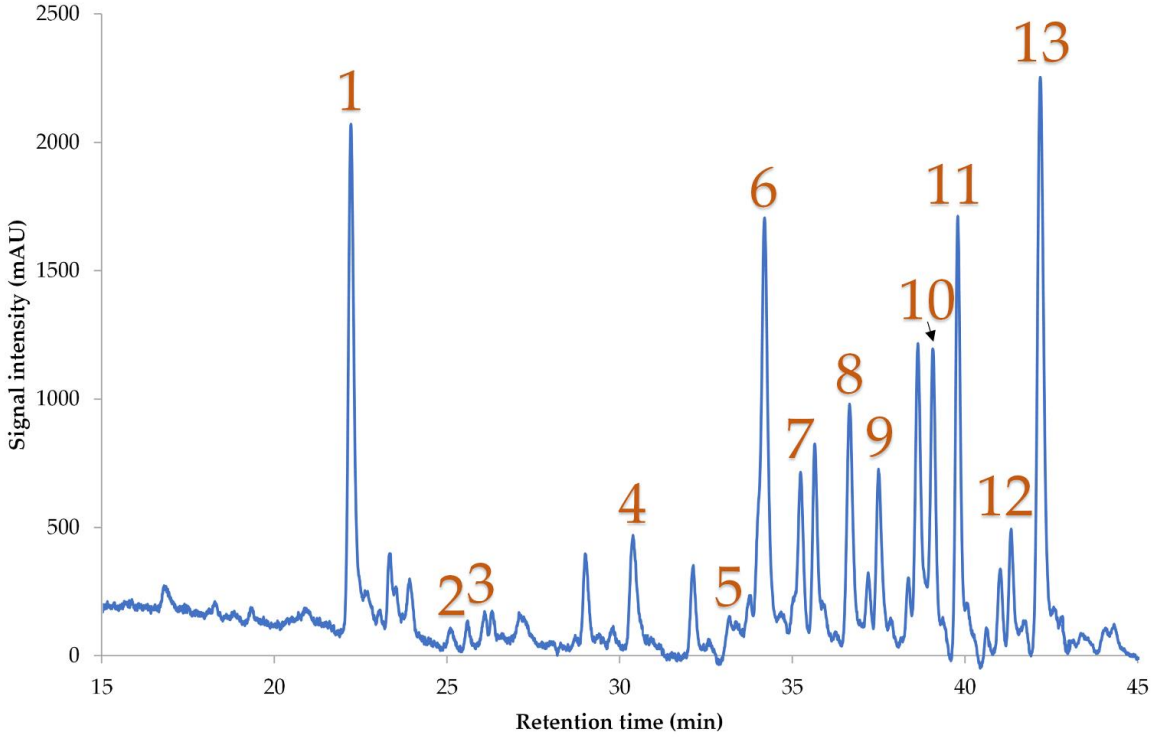

Figure 4. Exemplary HPLC chromatogram at 320 nm of optimal extract of watercress demonstrating identified polyphenolic compounds. (1) Chlorogenic acid; (2) caffeic acid; (3) syringic acid; (4) *p*-coumaric acid; (5) ferulic acid; (6) rutin; (7) quercetin 3-β-*D*-glucoside; (8) luteolin-7-glucoside; (9) narirutin; (10) kaempferol-3-glucoside; (11) apigenin-7-*O*-glucoside; (12) myricetin; (13) rosmarinic acid.

4. Conclusions

In conclusion, this study highlights the effectiveness and value of optimizing the extraction parameters for plant materials with limited bioactive compounds, such as watercress leaves. Utilizing RSM and PLS, this research comprehensively assessed the impact of the extraction temperature, time, solvent composition, and different extraction techniques (PEF, US, and ST). Green extraction methods, when used with an ethanolic solvent, continue to prove their vital importance in improving extraction efficiency. For instance, the TPC of watercress leaves can be increased by 583.38%. Moreover, the combination of PEF, US, and ST techniques, with 50% ethanolic solvent at 80 °C for 30 min, significantly improved the extraction process, yielding great results for TPC (29.59 mg GAE/g dw), FRAP (58.27 µmol AAE/g dw), and DPPH (48.25 µmol AAE/g dw). Under these optimized conditions, the extracts also exhibited a rich profile of bioactive compounds, including significant polyphenolic compounds with remarkable medicinal properties, and demonstrated substantial antioxidant activity. This confirms the potential of the extracts as a valuable source of antioxidants. This research emphasizes the efficacy of using combined green extraction methods to enhance the polyphenol content extracted from watercress leaves, providing promising avenues for the development of high-value-added products with enhanced bioactivity, given that the watercress plant already provides a wealth of other essential nutrients.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/app142210739/s1, Figures S1–S3 comprise the plots for TPC, FRAP, and DPPH that illustrate the comparison between the actual response and the predicted response for each parameter under examination, accompanied by the desirability functions. Figures S4–S6 present the 3D response plots for the responses (TPC, FRAP and DPPH).

Author Contributions: Conceptualization, T.C. and S.I.L.; methodology, V.A. and T.C.; software, V.A.; validation, T.C., K.K. and V.A.; formal analysis, T.C., E.B. and V.A.; investigation, E.N., A.X. and K.K.; resources, S.I.L.; data curation, V.A.; writing—original draft preparation, K.K. and T.C.; writing—review and editing, E.N., A.X., K.K., T.C., V.A., E.B. and S.I.L.; visualization, V.A.; supervision, T.C. and S.I.L.; project administration, S.I.L.; funding acquisition, S.I.L. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

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 Material; further inquiries can be directed to the corresponding author.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Chaudhary, S.; Hisham, H.; Mohamed, D. A Review On Phytochemical And Pharmacological Potential Of Watercress Plant. *Asian J. Pharm. Clin. Res.* **2018**, *11*, 102. [CrossRef]
2. Al-Snafi, A.E. A Review on *Nasturtium officinale*: A Potential Medicinal Plant. *IOSR J. Pharm.* **2020**, *10*, 33–43.
3. Pandey, Y.; Bhatt, S.; Debbarma, N. Watercress (*Nasturtium officinale*): A Potential Source of Nutraceuticals. *Int. J. Curr. Microbiol. Appl. Sci.* **2018**, *7*, 2685–2691. [CrossRef]
4. Robertson, C.E. McCance and Widdowson's The Composition of Foods—Sixth Summary Edition. *Nutr. Bull.* **2003**, *28*, 81–83. [CrossRef]
5. Pradhan, S.; Manivannan, S.; Tamang, J.P. Proximate, Mineral Composition and Antioxidant Properties of Some Wild Leafy Vegetables. *J. Sci. Ind. Res.* **2015**, *74*, 155–159.
6. Klimek-Szczykutowicz, M.; Szopa, A.; Ekiert, H. Chemical Composition, Traditional and Professional Use in Medicine, Application in Environmental Protection, Position in Food and Cosmetics Industries, and Biotechnological Studies of *Nasturtium officinale* (Watercress)—A Review. *Fitoterapia* **2018**, *129*, 283–292. [CrossRef]
7. Cory, H.; Passarelli, S.; Szeto, J.; Tamez, M.; Mattei, J. The Role of Polyphenols in Human Health and Food Systems: A Mini-Review. *Front. Nutr.* **2018**, *5*, 87. [CrossRef]
8. Sirohi, R.; Tarafdar, A.; Singh, S.; Negi, T.; Gaur, V.K.; Gnansounou, E.; Bharathiraja, B. Green Processing and Biotechnological Potential of Grape Pomace: Current Trends and Opportunities for Sustainable Biorefinery. *Bioresour. Technol.* **2020**, *314*, 123771. [CrossRef]
9. Maran, J.P.; Nivetha, C.V.; Priya, B.; Al-Dhabi, N.A.; Ponmurugan, K.; Manoj, J.J.B. Modeling of Polysaccharide Extraction from *Gossypium arboreum* L. Seed Using Central Composite Rotatable Design. *Int. J. Biol. Macromol.* **2016**, *86*, 857–864. [CrossRef]
10. Panja, P. Green Extraction Methods of Food Polyphenols from Vegetable Materials. *Curr. Opin. Food Sci.* **2018**, *23*, 173–182. [CrossRef]
11. Gil-Martín, E.; Forbes-Hernández, T.; Romero, A.; Cianciosi, D.; Giampieri, F.; Battino, M. Influence of the Extraction Method on the Recovery of Bioactive Phenolic Compounds from Food Industry By-Products. *Food Chem.* **2022**, *378*, 131918. [CrossRef] [PubMed]
12. Ameer, K.; Shahbaz, H.M.; Kwon, J.-H. Green Extraction Methods for Polyphenols from Plant Matrices and Their Byproducts: A Review. *Compr. Rev. Food Sci. Food Saf.* **2017**, *16*, 295–315. [CrossRef] [PubMed]
13. Chemat, F.; Vian, M.A.; Cravotto, G. Green Extraction of Natural Products: Concept and Principles. *Int. J. Mol. Sci.* **2012**, *13*, 8615–8627. [CrossRef] [PubMed]
14. Martins, R.; Barbosa, A.; Advinha, B.; Sales, H.; Pontes, R.; Nunes, J. Green Extraction Techniques of Bioactive Compounds: A State-of-the-Art Review. *Processes* **2023**, *11*, 2255. [CrossRef]
15. Raj, B.; John, S.; Chandrakala, V.; Kumari, G.H. Green Extraction Techniques for Phytoconstituents from Natural Products. In *Medicinal Plants*; Kumar, S., Ed.; IntechOpen: Rijeka, Croatia, 2022.
16. Picot-Allain, C.; Mahomoodally, M.F.; Ak, G.; Zengin, G. Conventional versus Green Extraction Techniques—A Comparative Perspective. *Curr. Opin. Food Sci.* **2021**, *40*, 144–156. [CrossRef]
17. Pataro, G.; Ferrari, G. Limitations of Pulsed Electric Field Utilization in Food Industry. In *Pulsed Electric Fields to Obtain Healthier and Sustainable Food for Tomorrow*; Barba, F.J., Parniakov, O., Francisco, B., Oleksii, P., Artur, W., Eds.; Elsevier: Amsterdam, The Netherlands, 2020; pp. 283–310. ISBN 978-0-12-816402-0.
18. Carreira-Casais, A.; Otero, P.; Garcia-Perez, P.; Garcia-Oliveira, P.; Pereira, A.G.; Carpena, M.; Soria-Lopez, A.; Simal-Gandara, J.; Prieto, M.A. Benefits and Drawbacks of Ultrasound-Assisted Extraction for the Recovery of Bioactive Compounds from Marine Algae. *Int. J. Environ. Res. Public Health* **2021**, *18*, 9153. [CrossRef]
19. Nascentes, C.C.; Korn, M.; Arruda, M.A. A Fast Ultrasound-Assisted Extraction of Ca, Mg, Mn and Zn from Vegetables. *Microchem. J.* **2001**, *69*, 37–43. [CrossRef]

20. Nascentes, C.C.; Korn, M.; Sousa, C.S.; Arruda, M.A.Z. Use of Ultrasonic Baths for Analytical Applications: A New Approach for Optimisation Conditions. *J. Braz. Chem. Soc.* **2001**, *12*, 57–63. [CrossRef]
21. Che Sulaiman, I.S.; Basri, M.; Fard Masoumi, H.R.; Chee, W.J.; Ashari, S.E.; Ismail, M. Effects of Temperature, Time, and Solvent Ratio on the Extraction of Phenolic Compounds and the Anti-Radical Activity of Clinacanthus Nutans Lindau Leaves by Response Surface Methodology. *Chem. Cent. J.* **2017**, *11*, 54. [CrossRef]
22. Venson, R.; Korb, A.S.; Cooper, G. A Review of the Application of Hollow-Fiber Liquid-Phase Microextraction in Bioanalytical Methods—A Systematic Approach with Focus on Forensic Toxicology. *J. Chromatogr. B Anal. Technol. Biomed. Life Sci.* **2019**, *1108*, 32–53. [CrossRef]
23. Drăghici-Popa, A.-M.; Boscornea, A.C.; Brezoiu, A.-M.; Tomas, Ș.T.; Pârvulescu, O.C.; Stan, R. Effects of Extraction Process Factors on the Composition and Antioxidant Activity of Blackthorn (*Prunus spinosa* L.) Fruit Extracts. *Antioxidants* **2023**, *12*, 1897. [CrossRef] [PubMed]
24. Franzke, A.; Lysak, M.A.; Al-Shehbaz, I.A.; Koch, M.A.; Mummenhoff, K. Cabbage Family Affairs: The Evolutionary History of Brassicaceae. *Trends Plant Sci.* **2011**, *16*, 108–116. [CrossRef] [PubMed]
25. Gonçalves, E.M.; Cruz, R.M.S.; Abreu, M.; Brandão, T.R.S.; Silva, C.L.M. Biochemical and Colour Changes of Watercress (*Nasturtium officinale* R. Br.) during Freezing and Frozen Storage. *J. Food Eng.* **2009**, *93*, 32–39. [CrossRef]
26. Khalik, K.A. Morphological Studies on Trichomes of Brassicaceae in Egypt and Taxonomic Significance. *Acta Bot. Croat.* **2005**, *64*, 57–73.
27. Lakka, A.; Grigorakis, S.; Kaltsa, O.; Karageorgou, I.; Batra, G.; Bozinou, E.; Lalas, S.; Makris, D.P. The Effect of Ultrasonication Pretreatment on the Production of Polyphenol-Enriched Extracts from *Moringa oleifera* L. (Drumstick Tree) Using a Novel Bio-Based Deep Eutectic Solvent. *Appl. Sci.* **2020**, *10*, 220. [CrossRef]
28. Paleologou, I.; Vasiliou, A.; Grigorakis, S.; Makris, D.P. Optimisation of a Green Ultrasound-Assisted Extraction Process for Potato Peel (*Solanum tuberosum*) Polyphenols Using Bio-Solvents and Response Surface Methodology. *Biomass Convers. Biorefinery* **2016**, *6*, 289–299. [CrossRef]
29. Athanasiadis, V.; Chatzimitakos, T.; Kotsou, K.; Palaiogiannis, D.; Bozinou, E.; Lalas, S.I. Optimization of the Extraction Parameters for the Isolation of Bioactive Compounds from Orange Peel Waste. *Sustainability* **2022**, *14*, 13926. [CrossRef]
30. Wardy, W.; Saalia, F.K.; Steiner-Asiedu, M.; Budu, A.S.; Sefa-Dedeh, S. A Comparison of Some Physical, Chemical and Sensory Attributes of Three Pineapple (*Ananas comosus*) Varieties Grown in Ghana. *Afr. J. Food Sci.* **2009**, *3*, 22–025.
31. Ghazzawi, H.A.; Al-Sayyed, H.F.; Al-Kurd, R.A.; Mwalla, M.M.; Arafat, T.A.; AbdelQader, S.M. Effect of Different Extraction Solvents on the Antioxidant Content and Capacity of Nine Seasonal Fruits. *Clin. Nutr. Open Sci.* **2021**, *38*, 33–42. [CrossRef]
32. Ochoa, S.; Durango-Zuleta, M.M.; Felipe Osorio-Tobón, J. Techno-Economic Evaluation of the Extraction of Anthocyanins from Purple Yam (*Dioscorea alata*) Using Ultrasound-Assisted Extraction and Conventional Extraction Processes. *Food Bioprod. Process.* **2020**, *122*, 111–123. [CrossRef]
33. Usman, M.; Nakagawa, M.; Cheng, S. Emerging Trends in Green Extraction Techniques for Bioactive Natural Products. *Processes* **2023**, *11*, 3444. [CrossRef]
34. Antony, A.; Farid, M. Effect of Temperatures on Polyphenols during Extraction. *Appl. Sci.* **2022**, *12*, 2107. [CrossRef]
35. Diamanti, A.C.; Igoumenidis, P.E.; Mourtzinos, I.; Yannakopoulou, K.; Karathanos, V.T. Green Extraction of Polyphenols from Whole Pomegranate Fruit Using Cyclodextrins. *Food Chem.* **2017**, *214*, 61–66. [CrossRef] [PubMed]
36. Zeb, A. Phenolic Profile and Antioxidant Potential of Wild Watercress (*Nasturtium officinale* L.). *Springerplus* **2015**, *4*, 714. [CrossRef] [PubMed]
37. Tan, M.-J.; Li, Y.; Zhao, S.-Q.; Yue, F.-H.; Cai, D.-J.; Wu, J.-T.; Zeng, X.-A.; Li, J.; Han, Z. Synergistic Ultrasound Pulsed Electric Field Extraction of Litchi Peel Polyphenols and Determination of Their Properties. *Int. J. Biol. Macromol.* **2024**, *260*, 129613. [CrossRef] [PubMed]
38. Manzoor, M.F.; Zeng, X.-A.; Rahaman, A.; Siddeeg, A.; Aadil, R.M.; Ahmed, Z.; Li, J.; Niu, D. Combined Impact of Pulsed Electric Field and Ultrasound on Bioactive Compounds and FT-IR Analysis of Almond Extract. *J. Food Sci. Technol.* **2019**, *56*, 2355–2364. [CrossRef]
39. Flieger, J.; Flieger, W.; Baj, J.; Maciejewski, R. Antioxidants: Classification, Natural Sources, Activity/Capacity Measurements, and Usefulness for the Synthesis of Nanoparticles. *Materials* **2021**, *14*, 4135. [CrossRef]
40. Angerhofer, C.K.; Maes, D.; Giacomoni, P.U. The Use of Natural Compounds and Botanicals in the Development of Anti-Aging Skin Care Products. In *Skin Aging Handbook*; Dayan, N., Ed.; Personal Care & Cosmetic Technology; Elsevier: Norwich, NY, USA, 2009; pp. 205–263, ISBN 978-0-8155-1584-5.
41. Lourenço, S.C.; Moldão-Martins, M.; Alves, V.D. Antioxidants of Natural Plant Origins: From Sources to Food Industry Applications. *Molecules* **2019**, *24*, 4132. [CrossRef]
42. Giallourou, N.; Oruna-Concha, M.J.; Harbourne, N. Effects of Domestic Processing Methods on the Phytochemical Content of Watercress (*Nasturtium officinale*). *Food Chem.* **2016**, *212*, 411–419. [CrossRef]
43. Calderón-Oliver, M.; Ponce-Alquicira, E. Environmentally Friendly Techniques and Their Comparison in the Extraction of Natural Antioxidants from Green Tea, Rosemary, Clove, and Oregano. *Molecules* **2021**, *26*, 1869. [CrossRef]
44. Hou, F.; Su, D.; Xu, J.; Gong, Y.; Zhang, R.; Wei, Z.; Chi, J.; Zhang, M. Enhanced Extraction of Phenolics and Antioxidant Capacity from Sorghum (*Sorghum bicolor* L. Moench) Shell Using Ultrasonic-Assisted Ethanol-Water Binary Solvent. *J. Food Process. Preserv.* **2016**, *40*, 1171–1179. [CrossRef]

45. Hikmawanti, N.P.E.; Fatmawati, S.; Asri, A.W. The Effect of Ethanol Concentrations as the Extraction Solvent on Antioxidant Activity of Katuk (*Sauropus androgynus* (L.) Merr.) Leaves Extracts. *IOP Conf. Ser. Earth Environ. Sci.* **2021**, *755*, 012060. [CrossRef]
46. Nguyen, N.Q.; Nguyen, M.T.; Nguyen, V.T.; Le, V.M.; Trieu, L.H.; Le, X.T.; Khang, T.V.; Giang, N.T.L.; Thach, N.Q.; Hung, T.T. The Effects of Different Extraction Conditions on the Polyphenol, Flavonoids Components and Antioxidant Activity of *Polyscias fruticosa* Roots. *IOP Conf. Ser. Mater. Sci. Eng.* **2020**, *736*, 022067. [CrossRef]
47. Boligon, A.A.; Janovik, V.; Boligon, A.A.; Pivetta, C.R.; Pereira, R.P.; Da Rocha, J.B.T.; Athayde, M.L. HPLC Analysis of Polyphenolic Compounds and Antioxidant Activity in *Nasturtium officinale*. *Int. J. Food Prop.* **2013**, *16*, 61–69. [CrossRef]
48. Omar, S.H. Biophenols Pharmacology against the Amyloidogenic Activity in Alzheimer's Disease. *Biomed. Pharmacother.* **2017**, *89*, 396–413. [CrossRef]
49. Singh, D.; Kumari, K.; Ahmed, S. Natural Herbal Products for Cancer Therapy. In *Understanding Cancer*; Jain, B., Pandey, S.B.T.-U.C., Eds.; Elsevier: Amsterdam, The Netherlands, 2022; pp. 257–268, ISBN 978-0-323-99883-3.
50. Safitri, E.; Purnobasuki, H.; Afifudin Al-Anshori, A.; Lestari, T.D.; Utama, S.; Yimer, N. Honey-Derived Phytochemicals: Implications for Stem Cell Activation and Health Benefits. *J. Funct. Foods* **2024**, *114*, 106058. [CrossRef]
51. Bazyar, H.; Zare Javid, A.; Ahangarpour, A.; Zaman, F.; Hosseini, S.A.; Zohoori, V.; Aghamohammadi, V.; Yazdanfar, S.; Ghasemi Deh Cheshmeh, M. The Effects of Rutin Supplement on Blood Pressure Markers, Some Serum Antioxidant Enzymes, and Quality of Life in Patients with Type 2 Diabetes Mellitus Compared with Placebo. *Front. Nutr.* **2023**, *10*, 1214420. [CrossRef]
52. Shakeri, F.; Boskabady, M.H. A Review of the Relaxant Effect of Various Medicinal Plants on Tracheal Smooth Muscle, Their Possible Mechanism(s) and Potency. *J. Ethnopharmacol.* **2015**, *175*, 528–548. [CrossRef]
53. Venigalla, M.; Sonego, S.; Gyengesi, E.; Münch, G. Curcumin and Apigenin—Novel and Promising Therapeutics against Chronic Neuroinflammation in Alzheimer′s Disease. *Neural Regen. Res.* **2015**, *10*, 1181. [CrossRef]