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Integrated Optimisation and LC-ESI-QToF-MS/MS Profiling of Phenolics Extracted from Green Tea Herbal Dust

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Abstract

The herbal tea industry has experienced substantial growth, particularly regarding green tea (*Camellia sinensis*). In the manufacturing of filter tea, fine herbal dust is generated as a residual by-product during grinding and sieving and is typically discarded as waste. This study aims to explore the application of ultrasound-assisted extraction (UAE) for secondary valorisation of green tea herbal dust by investigating the effects of various parameters on extraction efficiency. Antiradical activity of UAE extracts was determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, and the total phenolic content (TPC) was measured using Folin–Ciocalteu's assay. Furthermore, selected phenolics were quantified by HPLC and qualitatively characterised by liquid chromatography coupled with electrospray ionisation and quadrupole time-of-flight tandem mass spectrometry (LC-ESI-QToF-MS/MS). The results demonstrate that UAE parameters have a pronounced influence on the antioxidant activity, TPC, and individual polyphenolic profile of green tea herbal dust extracts. Ethanol–water mixtures at a ratio of around 40–60%, as well as moderate impulse regimes (around 60%) and extraction times (around 10 min), were the most suitable for extracting green tea polyphenols. Epigallocatechin gallate was the predominant phenolic component in most extracts, alongside epicatechin, epigallocatechin, catechin, and gallic acid. The findings highlight the UAE technique as a robust, green, and scalable method for valorising green tea by-products, thereby facilitating the development of high-value natural extracts for applications in the food, pharmaceutical, and cosmetic industries.

Keywords: ultrasound-assisted extraction; green tea; by-products; valoristion; polyphenols

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1. Introduction

Medicinal plants have been used for the prevention and treatment of various ailments since ancient times, and their therapeutic potential has been confirmed through centuries of traditional use and extensive modern scientific research. In recent decades, there has been growing global interest in medicinal herbs and products of natural origin as alternatives to synthetic drugs and conventional food supplements, largely due to concerns related to

adverse effects, misuse, and the environmental burden associated with the chemical industry [1,2]. Consequently, a significant proportion of the world's population is increasingly turning towards traditional medicine, natural products, and plant-based functional foods. Medicinal plants are typically dried under conditions appropriate for their bioactive constituents to minimise degradation of thermolabile compounds, and are then mechanically processed to ensure microbiological safety, extend shelf life, and improve extraction efficiency of bioactive compounds. Among various preparation methods, aqueous infusions are among the most widely used [3], and the resulting products are generally referred to as "herbal teas". The herbal tea industry has experienced substantial growth in recent years, driven by increasing awareness of healthy lifestyles, balanced nutrition, and preventive healthcare. In 2023, the global tea market was valued at approximately 260 billion USD and is projected to reach around 362 billion USD by 2029, confirming its strong economic relevance [4].

Green tea is the most widely consumed herbal tea worldwide and is produced from dried, non-fermented leaves of *Camellia sinensis* (L.) Kuntze, primarily cultivated in China and India [5]. According to the European Medicine Agency (EMA), green tea is indicated as a medicinal product for the relief of fatigue and sensations of weakness [6]. Its beneficial health effects are attributed to a rich and diverse phytochemical composition, including polyphenols, alkaloids, polysaccharides, saponins, free fatty acids, amino acids, vitamins, minerals, and enzymes, which often act synergistically [7,8]. Polyphenols represent the most abundant group (approximately 35%) and are mainly responsible for the characteristic colour and flavour of green tea. They include catechins and flavonols, such as kaempferol, myricetin, quercetin, chlorogenic acid, coumaroylquinic acid, and theogallin. Major catechins like (–)-epicatechin, (–)-epicatechin-3-gallate, (–)-epigallocatechin, and (–)-epigallocatechin-3-gallate are particularly well known for their strong antioxidant activity [9–11]. In addition, green tea contains purine alkaloids, primarily caffeine (2–5%), along with smaller amounts of theobromine and theophylline [8]. Beyond antioxidant activity, green tea has demonstrated antimicrobial, antitumour, antidiabetic, anti-osteoporotic, antihypertensive, and lipid-lowering effects [12].

The expanding production of green tea in the form of filter tea bags has led to a substantial increase in by-products and waste generated during industrial processing. During grinding of dried tea leaves, typically performed using hammer mills and followed by sieving, a significant fraction of the material is reduced to very fine particles with a diameter smaller than 0.315 mm. This fraction, commonly referred to as herbal tea dust, cannot be utilised further in filter tea production and is therefore separated and often discarded [13]. Depending on the processing conditions and raw material characteristics, herbal dust may constitute up to 40% of the processed plant material, representing a considerable loss of valuable biomass [14]. The accumulation of herbal dust poses both economic and environmental challenges. Poor utilisation of raw material directly reduces production efficiency, while disposal of large quantities of organic waste contributes to environmental pollution. However, herbal tea dust is not an inert waste; it predominantly originates from the leaf tissue itself—the most valuable plant part in terms of bioactive compound content. Numerous studies have demonstrated that herbal tea dust still contains significant amounts of polyphenols, flavonoids, aromatic compounds, and vitamins, confirming this by-product's high potential for secondary valorisation [13,14]. In the case of green tea, this by-product represents an especially attractive raw material due to the well-documented biological activity of its phytochemicals.

Despite its potential, the underutilisation of herbal tea dust is often linked to the limitations of conventional extraction techniques, which are typically time-consuming, solvent-intensive, and energetically inefficient. In response, increasing attention has been

directed towards the development and application of modern, green extraction technologies that reduce solvent and energy consumption while improving extraction yield and product quality. Among these, ultrasound-assisted extraction (UAE) has emerged as a particularly promising approach [15–18]. UAE relies on high-frequency ultrasound waves that induce cavitation, leading to the disruption of plant cell walls and enhanced mass transfer of bioactive compounds into the solvent. This technique significantly shortens extraction time compared to conventional methods and is particularly suitable for thermolabile compounds [19].

Building upon our previous research [13], which identified UAE as an efficient green technique for the recovery of bioactive compounds from by-products of green tea processing, the present study provides a substantially more comprehensive and systematic investigation of this approach. In contrast to earlier research, this study explores a wide range of UAE parameters in order to elucidate their individual and combined effects on extraction efficiency. Furthermore, special emphasis is placed on the detailed chemical characterisation of individual phenolic compounds using an advanced analytical technique involving liquid chromatography coupled with electrospray ionisation and quadrupole time-of-flight tandem mass spectrometry (LC-ESI-QToF MS/MS). By integrating process optimisation with in-depth phenolic profiling of green tea herbal dust, this study offers new insights into the relationship between extraction conditions and phytochemical composition, thereby significantly advancing the understanding of ultrasound-assisted valorisation of tea industry by-products.

2. Materials and Methods

2.1. Chemicals and Material

Raw green tea herbal dust, with a particle size diameter of less than 0.315 mm, was obtained from the local herbal filter tea producer Fructus (Bačka Palanka, Serbia).

Standards (gallic acid, epigallocatechin, catechin, epigallocatechin gallate, epicatechin, gallocatechin gallate, and epicatechin gallate) used for HPLC and LC-ESI-QToF-MS/MS analysis were obtained from Sigma-Aldrich (Steiheim, Germany). Chemicals 2,2-diphenyl-1-picrylhydrazyl (DPPH) and Folin–Ciocalteu's phenol reagent were purchased from Sigma-Aldrich Chemie (Steiheim, Germany). All other solvents were of analytical grade (J.T. Baker, Center Valley, PA, USA). Milli-Q pure water was used during experiments, and analytical measurements were obtained using the Milli-Q Millipore system (Burlington, MA, USA) (conductivity ≤ 0.054 μS/cm).

2.2. Ultrasound-Assisted Extraction (UAE)

UAE was performed using an ultrasonic probe (UP400St, Hielscher Utrasonics GmbH, Teltow, Germany) set at a minimum power of 400 W and a frequency of 24 kHz. In all UAE experiments, the solid–solvent ratio was maintained at 1:20 (w/v) and the amplitude was 100%. In order to ensure an environmentally friendly approach, a green solvent system based on ethanol and water in different proportions was used for the extraction process. Other extraction parameters (solvent type, time and impulse) varied according to the applied Box–Behnken design (BBD) and given data in Tables 1 and 2. The temperature was closely monitored and did not exceed 40 °C. Extraction was followed by filtration with a vacuum pump through 34 N-grade filter paper (thickness 0.20 mm). The obtained liquid extracts were stored at 4 °C prior to analysis.

Table 1. The uncoded and coded levels of independent variables used in the RSM (response surface methodology) design for the ultrasound-assisted extraction (UAE) technique applied to green tea herbal dust.

Independent Variable	Symbol	Level		
		Low (−1)	Centre (0)	High (+1)
Solvent (%)	X_1	0	48	96
Extraction time (min)	X_2	3	10	17
Impulse (%)	X_3	20	60	100

2.3. HPLC Analysis

Determination of selected phenolic compounds was performed using the HPLC method with PDA (photo-diode array detector) detection. The analysis was carried out on an Agilent 1260 Infinity II system (Agilent Technologies Inc., Santa Clara, CA, USA) equipped with a quaternary pump (G7111A), a column oven (G7116A), a fraction collector (G1364E), an autosampler (G7157A), and a PDA detector (G7115A). Separation was achieved on a Cosmosil 5C18-MS-II column (Nacalai Tesque, Inc., Kyoto, Japan) that was 250 mm long with an internal diameter of 4.6 mm and filled with 5 µm particles. Data acquisition and processing were carried out using ChemStation software (OpenLab CDS, version 2.18.18). A gradient elution was conducted with two mobile phases, a 0.1% formic acid solution in ultrapure water (Milipore Simplicity 185 System, Darmstadt, Germany) (mobile phase A) and a 0.1% formic acid solution in methanol (mobile phase B). The gradient elution was carried out with a constant flow rate of 1.0 mL/min for a total run time of 65 min with the following gradient setup: 0–8 min with 0–90% of A, 8–16 min with 90–75% of A, 16–28 min with 75–55% of A, 28–55 min with 55–20% of A, and 55–65 min with 20–10% of A. Between each run, the system was allowed 15 min to re-equilibrate to the initial conditions. The column temperature was kept constant at 50 °C, and the injection volume was set to 10 µL. Detection of polyphenols was achieved by measuring the absorbance at multiple wavelengths: 240, 250, 260, 270, 280, 330, and 360 nm. Peak purity was assessed using ChemStation software (OpenLab CDS, v. 2.18.18), where the analytes were evaluated based on the purity factor, spectral threshold, and noise threshold. Quantification was performed using the external standard method, with individual calibration curves prepared for each polyphenol over a concentration range of 1–50 mg/L. All calibration curves yielded correlation coefficients (R^2) greater than 0.99. The compounds' contents were expressed as mg/L of the analysed extract.

2.4. Determination of Antiradical Activity

Determination of antiradical activity of obtained UAE extracts of green tea herbal dust was performed using the DPPH spectrophotometric method according to Brand-Williams et al. [20] with some modifications. First, the liquid extracts were dried at a constant temperature of 45 °C and a pressure of 7.5 Torr for 24 h in a vacuum concentrator (SpeedVac SPD1030, Thermo Fisher Scientific, Waltham, MA, USA). The dry residues were then dissolved in an appropriate volume of 96% (*v*/*v*) ethanol to obtain a concentration of 0.6 mg/mL and were used for further analysis. A DPPH solution was prepared in 96% (*v*/*v*) ethanol which showed absorbance around 1, and it was used as the DPPH reagent (control). A volume of 3.0 mL of freshly prepared DPPH reagent was added to 0.2 mL of the extracts, and the reaction mixtures were kept in the dark for 15 min before measurements. The absorbances were measured at 517 nm using a UV/VIS spectrophotometer with a single-beam system (LLG-uniSPEC 2, LLG Labware, Meckenheim, Germany) using 96% (*v*/*v*)

ethanol as the blank. All experiments were conducted in triplicate, while the results were calculated using Formula (1) and expressed as the mean percentage of DPPH inhibition (%):

$$\%\ \text{DPPH inhibition} = \frac{\text{A (control)} - \text{A(sample)}}{\text{A(control)}} * 100 \quad (1)$$

2.5. Determination of Total Phenolic Content

The Folin–Ciocalteu method was employed for the determination of total phenolic content (TPC) in obtained UAE extracts of green tea herbal dust. Briefly, 100 µL of Folin–Ciocalteu reagent and 300 µL of 20% Na_2CO_3 aqueous solution were added to 20 µL of each UAE extract (3.0 mg/mL). Milli-Q water (conductivity: ≤0.055 µS/cm) was then added to the mixture to reach a total volume of 2 mL. The reaction mixtures were incubated at 40 °C for 30 min. The absorbance of each sample was measured in triplicate at a wavelength of 765 nm on a UV/VIS spectrophotometer with a single-beam system (LLG-uniSPEC 2, LLG Labware, Meckenheim, Germany), with water used as the blank. Quantification was performed with the external standard method. Calibration curve was prepared using gallic acid (Sigma-Aldrich, Steiheim, Germany) as standard compound, at concentrations ranging from 0 to 1000 mg/L (R^2 = 0.9991). The mean value and standard deviation were subsequently calculated for each sample. The obtained results were expressed as mg of gallic acid equivalent per litre of green tea extract (mg GAE/L).

2.6. Experimental Design and Process Optimisation

Experimental data generated by BBD [21] were used to derive optimal process parameters and to evaluate model adequacy for each individual response (y) obtained by UAE of the herbal dust. The extraction parameters, such as solvent (X_1), extraction time (X_2) and impulse (X_3), are independent variables each investigated for their influence on the UAE process (Table 1), more precisely on the dependent variable or response (y). Obtained data were fitted with second-order (quadratic) response models described by Equation (2):

$$y = \beta_0 + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k} \beta_{ii} X_i^2 + \sum_{\substack{i=1 \\ i<j}}^{k-1} \sum_{j=2}^{k} \beta_{ij} X_i X_j \quad (2)$$

where y represents a set of investigated responses (target individual phenolic compounds in UAE extracts, namely gallic acid, epigallocatechin, catechin, epigallocatechin gallate, epicatechin, gallocatechin gallate, epicatechin gallate, TPC, and antioxidant activity); β_0, β_i, β_{ii}, and β_{ij} parameters are designated as constant coefficients of intercept, linear, quadratic, and interaction terms, respectively; and X_i and X_j are coded (−1, 0, +1) inputs or independent variables. Statistical analysis was carried out using commercial software Design-Expert® (ver. 9, Stat-Ease Inc., Minneapolis, MN, USA)). The quality of fitted mathematical models was evaluated through an analysis of variance (ANOVA).

Table 2. Extraction design for the UAE procedure and phytochemical analysis of the extracts: DPPH inhibition [% inhibition], total phenolic content (TPC) [GAE mg/L], and HPLC-obtained content of individual polyphenols [mg/L].

Run [#]	Solvent (Ethanol–Water, *v*/*v*)	Time	Impulse	DPPH	TPC	Gallic Acid	Epigallocatechin	Catechin	Epigallocatechin Gallate	Epicatechin	Gallocatechin Gallate	Epicatechin Gallate
	%	min	%	% Inhibition	GAE mg/L	mg/L of Green Tea Extract						
1	96	10	20	30.41	67.62	16.14	185.93	53.88	776.99	45.68	9.66	81.33
2	48	10	60	64.02	221.19	110.67	103.15	34.42	3850.68	231.22	51.28	320.97
3	96	10	100	32.14	77.62	47.92	154.39	64.36	2567.13	148.37	39.34	260.92
4	48	10	60	61.82	222.14	110.44	111.13	34.07	4079.93	236.91	65.46	381.98
5	48	10	60	61.35	216.19	115.30	138.99	36.33	4370.94	250.24	79.68	449.68
6	0	3	60	24.52	65.95	101.00	35.78	11.70	1710.35	52.04	-	27.71
7	48	3	20	57.73	198.10	64.92	80.17	34.08	2695.18	153.74	49.66	284.17
8	96	17	60	57.13	164.76	57.13	169.80	47.62	3004.40	175.09	50.24	312.55
9	0	17	60	22.95	75.71	97.23	36.05	11.80	1785.05	51.95	-	31.83
10	48	17	20	60.97	220.71	96.63	138.06	55.56	3996.67	225.37	75.82	426.65
11	48	10	60	58.07	209.29	102.76	144.82	37.16	4182.43	236.18	81.28	448.27
12	0	10	20	14.32	55.71	95.79	29.24	8.66	1485.69	18.40	-	24.60
13	48	17	100	46.48	187.62	133.55	111.78	41.42	4362.03	258.35	65.09	378.12
14	0	10	100	21.41	66.19	98.36	31.17	9.09	1716.89	13.59	-	24.55
15	48	3	100	58.40	271.19	96.04	90.98	47.42	3592.91	206.73	57.85	340.19
16	96	3	60	50.87	46.67	42.52	72.51	4.89	2242.52	124.66	38.37	229.43
17	48	10	60	60.03	221.43	103.40	153.81	32.43	4151.90	236.44	81.36	446.42

[#] Constant parameters: amplitude 100% and solvent–solid ratio of 20:1 (mL/g).

2.7. High-Resolution LC-ESI-QToF-MS/MS Analysis of Extract Obtained at Optimal UAE Parameters

The UAE extract of green tea herbal dust obtained at optimal parameters was qualitatively analysed. The applied method was reported by De Luca et al. [22]. In summary, the analytical platform employed comprised an advanced ion mobility LC/QToF-MS system with an electrospray ionisation (ESI) source, consisting of a 1290 Infinity II UPLC coupled to a 6560 IM-QToF instrument (Agilent Technologies Inc., Santa Clara, CA, USA). Data acquisition and qualitative analysis of the ESI/QToF MS spectra were conducted using the MassHunter Workstation Qualitative Analysis software (version 10.0, Agilent Technologies Inc., Santa Clara, CA, USA). Metabolites were annotated using the MassHunter METLIN Metabolite PCDL database (version B.08.00, Agilent Technologies Inc., Santa Clara, CA, USA) alongside Sirius® software (version 4.7.4) for molecular formula determination and fragmentation prediction [23,24]. Furthermore, the experimental MS/MS spectra were cross-referenced with reported fragmentation patterns available in the literature and in publicly accessible mass spectral databases [25]. Finally, the identification of the compounds was confirmed by comparison of retention times and MS/MS spectra of pure standards.

3. Results and Discussion

The results presented in Table 2 demonstrate a pronounced influence of UAE parameters on the antioxidant activity, TPC, and individual polyphenolic profile of green tea herbal dust extracts. A representative HPLC chromatogram is shown in Supplementary Figure S1. The observed variability among experimental runs confirms that extraction efficiency is governed by a complex interplay between solvent composition, extraction time, and ultrasound impulse regime [26].

3.1. Influence of Extraction Parameters on Phenolic Content and Antioxidant Activity

Solvent type emerged as one of the most influential factors affecting both total phenolic yield and individual catechin content. Mixtures of ethanol and water consistently outperformed pure solvents, with the highest total phenolic contents and individual catechin concentrations obtained at intermediate ethanol concentrations (approximately 50% v/v). This observation is consistent with previous reports indicating that aqueous alcoholic mixtures provide optimal polarity for the simultaneous extraction of both hydrophilic and moderately lipophilic phenolic compounds present in green tea leaves [13].

Pure water extracts (0% ethanol) showed markedly lower DPPH inhibition values and TPC, as well as reduced concentrations of major catechins, particularly epigallocatechin gallate (EGCG). This can be attributed to the limited solubility of gallated catechins in water and the reduced penetration of aqueous solvents into the plant matrix. Conversely, a high ethanol content (96%) also resulted in suboptimal extraction efficiency, likely due to insufficient solubilisation of more polar phenolic compounds. These findings corroborate data in the literature indicating that ethanol–water mixtures at ratios of around 40–60% are most suitable for extracting green tea polyphenols using UAE [13].

Ultrasound impulse intensity significantly affected the release of phenolic compounds from green tea dust. Moderate impulse regimes (around 60%) resulted in higher TPC and catechin concentrations compared to lower (20%) or excessively high (100%) impulse levels. This suggests that moderate cavitation intensity effectively disrupts cell walls and enhances mass transfer without causing degradation of sensitive phenolic compounds [26]. At higher impulse levels, particularly when combined with longer extraction times, a decrease in antioxidant activity and certain catechin contents was observed. This phenomenon has been previously attributed to the generation of localised hotspots and free radicals

during intense cavitation, which may promote oxidative degradation of phenolic structures, especially gallated catechins such as EGCG and epicatechin gallate (ECG).

Extraction time also played a critical role. Short extraction times (3 min) were insufficient for complete diffusion of polyphenols, though prolonged extraction (17 min) did not always lead to proportional increases in phenolic yield. In some cases, extended sonication resulted in lower DPPH inhibition values, indicating possible degradation or transformation of antioxidant compounds. These findings confirm that UAE is most effective when applied for relatively short durations under optimised conditions, supporting its classification as a rapid and energy-efficient extraction technique [27]. Simić et al. [13] report UAE of green tea herbal dust with multiple amplitudes (20%, 60%, and 100%) and times (5 or 10 min). They explicitly state maximum TPC values for green tea herbal dust as 152.91 $\pm$ 0.74 mg GAE/g DE. It is important to note that the reported values may not be directly comparable due to differences in the units of measurement used. It is interesting to note, however, that the optimal solvent identified in that study corresponds to our ethanol–water (50:50, v/v) mixture. The optimal extraction time reported in the study falls within the range of 3–17 min that was investigated in our work, and is intermediate, at approximately 10 min. In contrast, it appears that the optimal amplitude differs in this case.

A strong positive correlation between TPC and DPPH radical scavenging activity was observed across most experimental runs, confirming that phenolic compounds are the primary contributors to the antioxidant potential of green tea herbal dust extracts. A strong positive correlation was observed between TPC and DPPH radical scavenging activity ($r = 0.89$, $R^2 = 0.79$, $p < 0.0001$), indicating that phenolic compounds significantly contribute to antioxidant activity. Runs exhibiting total phenolic contents above 200 mg GAE/L generally showed DPPH inhibition values exceeding 55%, highlighting the effectiveness of UAE in recovering antioxidant constituents from this industrial by-product. However, certain deviations from linearity suggest that antioxidant activity is not solely dependent on total phenolic concentration but is also influenced by the qualitative composition of individual phenolics. Previous research similarly underscores that gallated catechins, especially EGCG, exhibit the highest antioxidant potency among tea catechins [28].

HPLC analysis confirmed that EGCG was the predominant phenolic component in most extracts, consistent with the catechin profile commonly observed in green tea. Other major phenolics identified included epicatechin, epigallocatechin, catechin, and gallic acid, which are regularly reported as principal tea antioxidants [13].

Notably, the ability to modulate the individual catechin profile through controlled adjustment of UAE parameters represents a significant advantage of this technique. By selecting appropriate solvent composition and ultrasound conditions, extracts can be tailored towards a higher EGCG content or a broader catechin spectrum, depending on the intended application. Extraction yields under optimised UAE conditions are comparable to, and sometimes higher than, those reported for intact green tea leaves, highlighting the effectiveness of UAE. The yield of green tea polyphenols extracted using different extraction techniques and solvent systems typically varies within the range of 5% to over 30% of the dry weight, as reported in the extant literature. Under optimal laboratory-scale conditions, yields of 20–30% are frequently achieved. According to reports, high-efficiency methods, such as UAE, have been shown to achieve extraction yields of up to 25–30% in certain circumstances. While the final extracts may exhibit high polyphenol purity levels (80–98%), conventional industrial solvent extraction methodologies typically yield lower levels (5–10%) [28,29].

3.2. BBD Optimisation of UAE from Tea Dust

The application of RSM (response surface methodology) using a BBD enabled robust quantification of how the investigated extraction variables, namely solvent (X_1), time (X_2), and ultrasound impulse (X_3), influenced antioxidant activity and the yields of selected phenolic compounds in extracts. Across all responses (Tables S1–S9), quadratic models were statistically significant ($p \leq 0.0278$) and explained a high proportion of experimental variability (R^2 = 0.8562–0.9919), supporting the suitability of second-order polynomial modelling for these UAE systems (Tables S1–S8). Only in the case of models presented in Tables S1, S2, S4 and S9 did we notice the significant lack-of-fit values which suggest that these models may not fully capture all the variability in the experimental data. But the significant lack of fit observed for these responses can be explained by the very small experimental (pure) error obtained in the study. In RSM, the lack-of-fit test is highly sensitive under such conditions, and even small deviations between experimental and predicted values may lead to statistically significant results. In the present study, the five replicated centre points showed very good agreement, indicating a low level of pure error. However, it is important to note that the models show satisfactory overall fit, as indicated by sufficiently high R^2 values and statistically significant *p*-values, confirming that the main effects and interactions are reliably captured. Such performance aligns with previous UAE optimisation studies showing that quadratic terms frequently capture the non-linear behaviour of solubilisation, matrix disruption, and compound instability across solvent/energy domains [30].

The equations in terms of coded factors (Table S10) can be used to make predictions about the response for given levels of each factor. By default, the high levels of the factors are coded as +1 and the low levels are coded as −1. The coded equation is useful for identifying the relative impact of the factors by comparing the factor coefficients.

The final goal of response surface methodology is process optimisation where the developed model can be used for simulation of the process. By applying the desirability function method, the optimal conditions for obtaining the highest TPC and DPPH values, together with a higher content of individual phenolics (gallic acid, epigallocatechin, catechin, epigallocatechin gallate, epicatechin, gallocatechin gallate, and epicatechin gallate) in obtained extracts within the experimental range of the variables studied, were a time of 10 min and 54% and 48% ethanol solution. In this study desirability function was 0.925, which is a high value and very close to the value of one, which represents the ideal case. Under these optimal conditions, the predicted TPC value was 223.15 GAE/mgL, and the other values were 108.05 mg/L for gallic acid, 147.13 mg/L for epigallocatechin, 37.11 mg/L for catechin, 4112.13 mg/L for epigallocatechin gallate, 237.15 mg/L for epicatechin, 74.82 mg/L for gallocatechin gallate, 403.14 mg/L for epicatechin gallate and 61.01% antioxidant activity (DPPH). Predicted data were experimentally verified by performing extraction at optimal conditions, and the obtained experimental values were 228.47 GAE/mgL for TPC, 105.92 mg/L for gallic acid, 150.61 mg/L for epigallocatechin, 36.28 mg/L for catechin, 4028.44 mg/L for epigallocatechin gallate, 241.36 mg/L for epicatechin, 76.03 mg/L for gallocatechin gallate, 395.27 mg/L for epicatechin gallate and 62.44% for antioxidant activity (DPPH). Very good agreement between predicted and experimental values confirms the significance of the proposed models.

3.2.1. Antioxidant Activity: Dominance of Solvent Effects and Non-Linear Impulse Behaviour

The antioxidant activity model was highly significant (p = 0.0010) with R^2 = 0.9489 (Table S1), confirming that the chosen UAE parameters collectively explain ~95% of antioxidant activity variability. The most influential linear factor was solvent (X_1) (p = 0.0013),

whereas time (X_2) and impulse (X_3) were not significant as linear terms (p = 0.8194 and 0.7750, respectively). This indicates that, within the experimental domain, solvent polarity primarily governs the extraction of antioxidant-active constituents. It is known that solvent type controls phenolic solubility and UAE is widely reported to enhance mass transfer via cell wall disruption, but the extent to which these physical effects translate into higher antioxidant capacity remains strongly solvent-dependent [30]. Crucially, antioxidant activity exhibited a strong quadratic dependence on solvent (X_1^2, $p < 0.0001$) and a significant quadratic dependence on impulse (X_3^2, p = 0.0122). Although interaction terms (X_1X_2, X_1X_3, and X_2X_3) were not significant for antioxidant activity ($p > 0.05$), the quadratic terms indicate that solvent–energy optimisation is more critical than relying on synergistic interactions between variables in a strictly linear sense.

3.2.2. Gallic Acid

The gallic acid model was significant (p = 0.0011) with R^2 = 0.9469 (Table S2). Unlike antioxidant activity, all three linear terms significantly influenced gallic acid yield: solvent (X_1, p = 0.0001), time (X_2, p = 0.0335), and impulse (X_3, p = 0.0119). This indicates that gallic acid recovery benefits not only from appropriate solvent polarity but also from sufficient extraction time and ultrasound energy delivery. Gallic acid is highly polar, and its extraction typically increases with solvent systems that effectively solubilise polar phenolic acids while promoting matrix hydration. UAE may further enhance release of gallic acid by accelerating diffusion from disrupted tissues [31]. The quadratic effect of solvent concentration (X_1^2, p = 0.0004) indicates the presence of a well-defined optimum extraction region. Based on the response surface plots (Figure S2), it can be seen that the optimal solvent region is approximately 40–60% solvent, where the maximum gallic acid yield (≈110–125 mg L^{-1}) is predicted. At lower solvent percentages (<30%), the response increases with increasing solvent concentration, demonstrating a positive linear effect. However, beyond the optimum value (>60–70%), further increases in solvent concentration result in a negative effect, leading to a decline in gallic acid yield, as confirmed by the significant negative quadratic coefficient. Regarding the remaining variables, extraction time (X_2) shows a moderate positive effect at shorter durations, and a longer extraction time shows a negative quadratic effect, indicating slight degradation or reduced efficiency at prolonged extraction times. Similarly, ultrasound impulse (X_3) exhibits a positive effect at intermediate levels (≈50–70%), while excessively high values (>85–90%) negatively affect the response, likely due to compound degradation or cavitation-related losses.

3.2.3. Non-Gallated Catechins (EGC, Catechin)

The EGC model was significant (p = 0.0049) with R^2 = 0.9166 (Table S3). Solvent (X_1, p = 0.0002) and time (X_2, p = 0.0272) significantly affected the EGC concentration, while impulse was not significant as a linear term. Quadratic terms for solvent (X_1^2, p = 0.0350) suggest that EGC extraction increases up to an optimum, after which further increases are minimal. Since EGC is comparatively polar, solvent composition is expected to be a strong determinant of extraction efficiency, consistent with the broader UAE/polyphenol optimisation literature [30].

For catechin, the quadratic model was significant (p = 0.0278) but had the lowest R^2 among measured responses (R^2 = 0.8562) (Table S4). Solvent shows a significant linear effect (p = 0.0032), while time (p = 0.0890) and impulse were non-significant. Both solvent quadratic (X_1^2, p = 0.0352) and impulse quadratic (X_3^2, p = 0.0454) were significant, implying sensitivity to both solvent composition and ultrasound regime. The very strong lack of fit (p = 0.0006) indicates that catechin behaviour may be influenced by unmodelled chemical conversions. Catechin-type flavan-3-ols can undergo ultrasound-enhanced epimerisa-

tion/hydrolysis depending on solution conditions, which would introduce non-polynomial response patterns [32].

3.2.4. Gallated Catechins (EGCG, ECG, GCG)

The EGCG content showed a highly significant model ($p = 0.0002$; $R^2 = 0.9677$; Table S5). It was significantly affected by time ($p = 0.0153$) and ultrasound impulse ($p = 0.0087$), while the linear solvent term was not significant. However, the strong quadratic solvent effect (X_1^2, $p < 0.0001$) indicates that the EGCG content is maximised within a narrow solvent range. EGCG stability decreases with increasing temperature and pH, and its degradation kinetics depend on system conditions, as demonstrated by Xu et al. [33]. Ultrasound may further influence the EGCG content by enhancing cavitation intensity and oxygen transfer, potentially promoting oxidative degradation under high impulse conditions [34]. The significant X_1X_3 interaction ($p = 0.0463$) therefore suggests that solvent composition modulates the effect of ultrasound impulse on the EGCG content through changes in cavitation behaviour, oxygen availability, and compound stability.

The ECG model was significant ($p = 0.0011$) with a high coefficient of determination ($R^2 = 0.9475$; Table S8), indicating good agreement between the model and experimental data. Among the linear terms, only solvent concentration was significant ($p = 0.0018$), confirming it as the main factor influencing ECG extraction. Extraction time and impulse intensity were not significant within the studied ranges. The solvent quadratic term (X_1^2) was highly significant ($p < 0.0001$), indicating a pronounced curvature and the presence of a clear optimal solvent region. As shown by the response surface plots (Figure S2), the ECG yield increased with solvent concentration up to an intermediate level and then decreased at higher solvent proportions, while time and impulse showed comparatively weaker effects. This is consistent with gallated catechins having distinct solubility/stability profiles relative to non-gallated catechins.

Gallocatechin gallate (GCG) showed a significant model ($p = 0.0030$) with $R^2 = 0.9287$ (Table S7). Solvent exerted a significant linear effect ($p = 0.0059$) and a very strong quadratic effect (X_1^2, $p < 0.0001$), again emphasising solvent dominance. Since EGCG ↔ GCG epimerisation can occur depending on conditions, the observed solvent curvature may partially reflect conversion equilibria rather than extraction alone [35].

3.2.5. Epicatechin

Epicatechin was the best-modelled response, with an extremely high R^2 of 0.9919 and a highly significant model ($p < 0.0001$), as well as excellent model fit and non-significant lack of fit ($p = 0.0680$) (Table S6). All three linear factors were strongly significant (solvent $p < 0.0001$; time $p = 0.0015$; impulse $p = 0.0010$), and both solvent and impulse quadratic terms were significant (X_1^2 $p < 0.0001$; X_3^2 $p = 0.0005$). The interaction X_1X_3 was also significant ($p = 0.0030$), showing that epicatechin extraction is particularly responsive to the combined effect of solvent composition and ultrasound regime.

3.3. LC-ESI-QToF-MS/MS Qualitative Analysis of Phenolic Compounds

The UAE extracts of *C. sinensis* leaves prepared at calculated optimal extraction conditions given in Section 3.2. were used for detailed qualitative characterisation of individual phenolic compounds. Qualitative analysis of the extracts was performed using (HR) LC-ESI-QToF MS/MS in negative ionisation mode (Figure 1). Seven phenolic compounds were annotated by comparing the obtained m/z values of the molecular ion and MS/MS fragments with those found in the literature or in publicly available databases [23,25]. Additionally, identity was confirmed by comparison with the corresponding pure standard. Also, Sirius® software v. 4.7.4 was used for the tentative identification of the compounds and to predict possible MS/MS fragmentation patterns. The compounds are listed based on

their retention time in Table 3. Furthermore, m/z values of [M-H]$^-$, the molecular formula, mass error (Δppm), MS/MS data, references used for conformation of the compound identity, and identification confidence levels [36] are reported. Among the identified compounds, six of them belong to the flavonoid group, precisely catechins: (epi)-gallocatechin, catechin, (epi)-catechin, (epi)-gallocatechin-gallate, gallocatechin-gallate and (epi)-catechin gallate. Gallic acid is classified as a phenolic acid.

Compound **1** was observed at a retention time of 2.751 min and was identified as gallic acid, with a molecular formula of $C_7H_6O_5$, based on the [M-H]$^-$ m/z value of 169.0147 with only one MS/MS fragment at m/z 125.0277, corresponding to the loss of carbon dioxide. Next, compound **2** was eluted at a retention time of 5.079 min and was annotated as (epi)-gallocatechin with a molecular formula of $C_{15}H_{14}O_7$. (Epi)-gallocatechin had the [M-H]$^-$ at m/z value of 305.0674, and fragments were observed at m/z 125.0243, 137.0248 and 111.0463. Compound **3** at a retention time of 8.633 and compound **4** at a retention time of 11.443 min showed similar [M-H]$^-$ m/z values of 289.0721 and 289.0725, respectively, with the same attributed molecular formula of $C_{15}H_{14}O_6$. The former, annotated as catechin, yielded fragment ions at m/z 245.0803, 125.0238 and 123.0444, while the latter, annotated as (epi)-catechin, produced fragment ions at m/z 125.0499, 83.0146 and 202.0648. Similarly, two other stereoisomers were compounds **5** and **6** which were observed at retention times of 12.366 and 14.072 min, respectively, and were annotated as (epi)-gallocatechin gallate and gallocatechin gallate with molecular formula $C_{22}H_{18}O_{11}$. (Epi)-gallocatechin gallate produced [M-H]$^-$ ion at m/z 457.0785 and two fragments at m/z 169.0151 and 125.0247. Likewise, gallocatechin gallate produced nearly identical [M-H]$^-$ at m/z 457.0787, exhibiting a similar fragmentation pattern yielding fragments at m/z 169.0144 and 125.0246. Lastly, compound **7** was detected in a peak at a retention time of 16.504 min and annotated as (epi)-catechin gallate with a molecular formula of $C_{22}H_{18}O_{10}$. This annotation was based on the [M-H]$^-$ m/z value of 441.0838 and fragment m/z at 169.0137, 289.0718 and 125.0237. MS identification of phenolic compounds was supported by comparison of retention times and MS/MS spectra of pure standards, in addition to comparison with previously reported data [37,38].

The fragment ion at m/z 125 was consistently detected in MS/MS spectra of all identified compounds and corresponds to the gallic acid molecular ion after the loss of carbon dioxide, indicating the presence of a galloyl moiety. Furthermore, the fragment at m/z 169 corresponding to the gallic acid anion is a characteristic fragment for gallic acid derivatives. Thus, this fragment helps the differentiation of gallate esters from non-gallate derivatives such as catechin and (epi)-catechin [39].

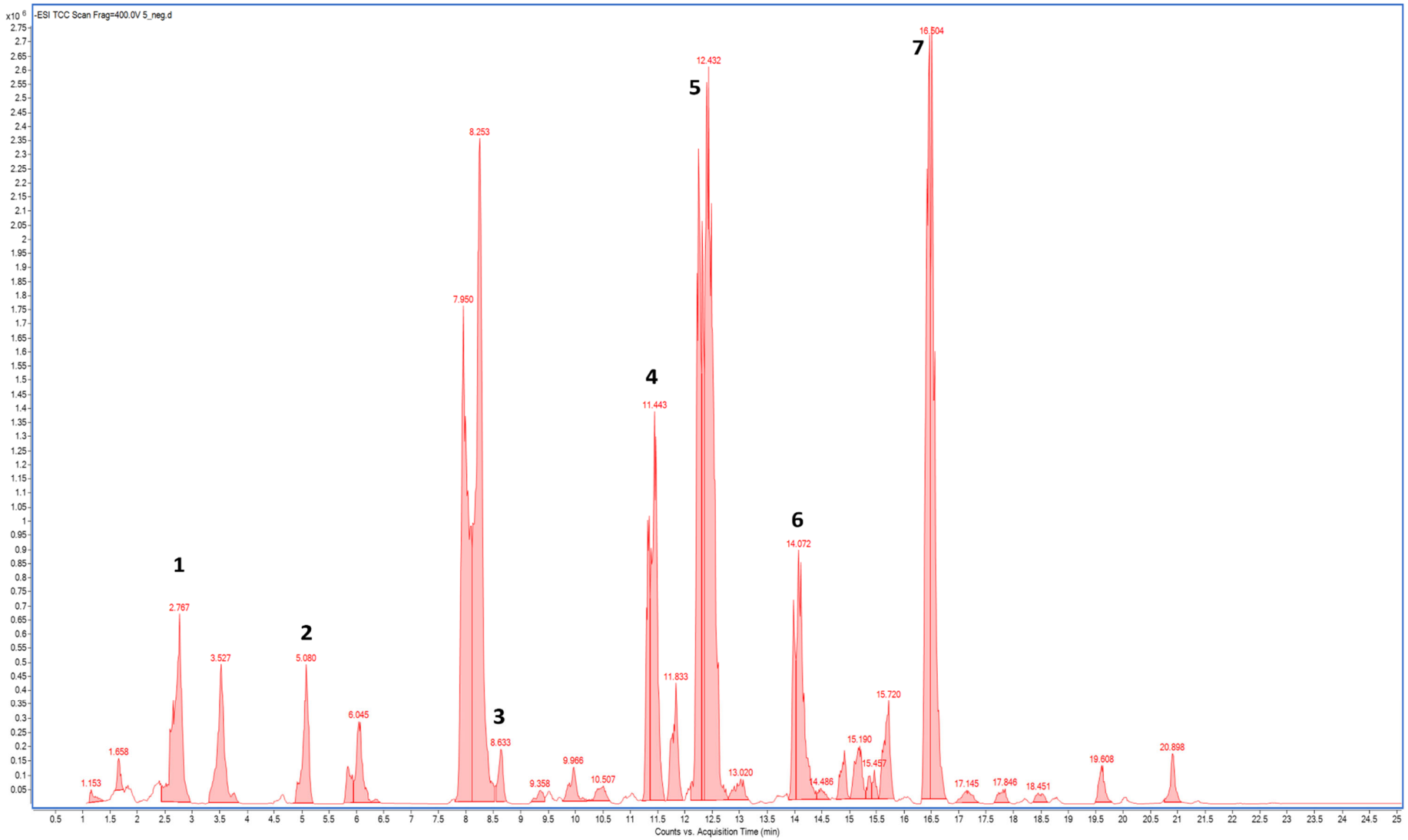

Figure 1. LC-MS/MS chromatogram obtained from *C. sinensis* UAE extract. Chromatographic peaks corresponding to the identified compounds are marked with numbers 1 to 7.

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Table 3. Phenolic compounds in herbal dust from *C. sinensis* leaves identified using (HR) LC-ESI-QToF MS/MS.

Compound n°	Rt min	Identity	[M-H]$^-$ *m/z*	Molecular Formula	Δ ppm	MS/MS * *m/z*	References	Level #
1	2.751	Gallic acid	169.0147	$C_7H_6O_5$	0.33	125.0277 (100)	[37,38]	1
2	5.079	(Epi)-gallocatechin	305.0674	$C_{15}H_{14}O_7$	0.43	125.0243 (100)/137.0248 (50)/111.0463 (36)	[37]	1
3	8.633	Catechin	289.0721	$C_{15}H_{14}O_6$	0.30	245.0803 (100)/125.0238 (77)/123.0444 (76)	[37,38]	1
4	11.443	(Epi)-catechin	289.0725	$C_{15}H_{14}O_6$	2.65	125.0499 (100)/83.0146 (98)/202.0648 (79)	[37,38]	1
5	12.366	(Epi)-gallocatechin gallate	457.0785	$C_{22}H_{18}O_{11}$	1.92	169.0151 (100)/125.0247 (46)	[37,38]	1
6	14.072	Gallocatechin gallate	457.0787	$C_{21}H_{18}O_{11}$	2.07	169.0144 (100)/125.0246 (26)	[38]	1
7	16.504	(Epi)-catechin gallate	441.0838	$C_{22}H_{18}O_{10}$	2.42	169.0137 (100)/289.0718 (35)/125.0237 (42)	[37,38]	1

* in parentheses, the relative intensity; # according to Blaženović et al. [36].

4. Conclusions

The high levels of phenolic compounds and antioxidant activity obtained in this study clearly demonstrate that green tea herbal dust is a highly valuable secondary raw material rather than a waste product. Notably, response surface modelling identified solvent composition as the dominant factor that influenced both antioxidant activity and individual phenolic yields, while the influence of extraction time and ultrasound impulse was compound-dependent.

Overall, these results confirm that UAE represents a robust, green, and scalable approach for the valorisation of by-products from green tea processing. The detailed insights into the relationship between extraction parameters and phenolic composition provided by this study significantly advance the current knowledge and support the development of high-value natural extracts for food, nutraceutical, cosmetic, and pharmaceutical applications.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/analytica7020030/s1, Table S1: Analysis of variance (ANOVA) of antioxidant activity in UAE extracts of green tea (*Camellia sinensis*); Table S2: Analysis of variance (ANOVA) for the quadratic model of the gallic acid content in green tea (*Camellia sinensis*) extracts obtained by UAE; Table S3: Analysis of variance (ANOVA) for the quadratic model of the epigallocatechin content in green tea (*Camellia sinensis*) extracts obtained by UAE; Table S4: Analysis of variance (ANOVA) for the quadratic model of the catechin content in green tea (*Camellia sinensis*) extracts obtained by UAE; Table S5: Analysis of variance (ANOVA) for the quadratic model of the epigallocatechin gallate content in green tea (*Camellia sinensis*) extracts obtained by UAE; Table S6: Analysis of variance (ANOVA) for the quadratic model of the epicatechin content in green tea (*Camellia sinensis*) extracts obtained by UAE; Table S7: Analysis of variance (ANOVA) for the quadratic model of the gallocatechin gallate content in green tea (*Camellia sinensis*) extracts obtained by UAE; Table S8: Analysis of variance (ANOVA) for the quadratic model of the epicatechin gallate content in green tea (*Camellia sinensis*) extracts obtained by UAE; Table S9: Analysis of variance (ANOVA) for the quadratic model of the TPC in green tea (*Camellia sinensis*) extracts obtained by UAE; Table S10: Polynomial equations calculated after implementation of BBD (in terms of coded factors); Figure S1: A representative HPLC chromatogram obtained from Camellia sinensis UAE extract; Figure S2: Three-dimensional plots of each response as a function of different extraction parameters.

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Abbreviations

The following abbreviations are used in this manuscript:

UAE	Ultrasound-assisted extraction
TPC	Total phenolic content
EMA	European Medicine Agency
DPPH	2,2-diphenyl-1-picrylhydrazyl
BBD	Box–Behnken design
PDA	Photo-diode array detector
GAE	Gallic acid equivalent
ANOVA	Analysis of variance
RSM	Response surface methodology
ESI	Electrospray ionisation
EGCG	Epigallocatechin gallate
ECG	Epicatechin gallate
GCG	Gallocatechin gallate

References

1. Latif, R.; Nawaz, T. Medicinal plants and human health: A comprehensive review of bioactive compounds, therapeutic effects, and applications. *Phytochem. Rev.* **2025**, *24*, 10194. [CrossRef]
2. Chakraborty, A.; Adhikary, S.; Bhattacharya, S.; Dutta, S.; Chatterjee, S.; Banerjee, D.; Ganguly, A.; Rajak, P. Pharmaceuticals and Personal Care Products as Emerging Environmental Contaminants: Prevalence, Toxicity, and Remedial Approaches. *ACS Chem. Health Saf.* **2023**, *30*, 362–388. [CrossRef]
3. Carbone, R.; Caracciolo, F.; Di Vita, G.; D'Amico, M.; Spina, D. Consumer trends in the herbal tea market: A systematic literature review. *Food Rev. Int.* **2025**, *41*, 2634–2652. [CrossRef]
4. Statista—Global: Tea Market Size 2025. Available online: https://www.statista.com/statistics/326384/global-tea-beverage-market-size/ (accessed on 10 January 2026).
5. FAOSTAT. Available online: https://www.fao.org/faostat/en/#home (accessed on 10 January 2026).
6. European Medicines Agency (EMA); Committee on Herbal Medicinal Products (HMPC). Community Herbal Monograph on *Camellia sinensis* (L.) Kuntze, Non Fermentatum Folium. Available online: https://www.ema.europa.eu (accessed on 26 January 2026).
7. Namal Senanayake, S.P.J. Green tea extract: Chemistry, antioxidant properties and food applications—A review. *J. Funct. Foods.* **2013**, *5*, 1529–1541. [CrossRef]
8. Zhao, T.; Li, C.; Wang, S.; Song, X. Green Tea (*Camellia sinensis*): A Review of Its Phytochemistry, Pharmacology, and Toxicology. *Molecules* **2022**, *27*, 3909. [CrossRef]
9. Lee, L.S.; Kim, S.H.; Kim, Y.B.; Kim, Y.C. Quantitative analysis of major constituents in green tea with different plucking periods and their antioxidant activity. *Molecules* **2014**, *19*, 9173–9186. [CrossRef]
10. Mukhtar, H.; Ahmad, N. Tea polyphenols: Prevention of cancer and optimizing health. *Am. J. Clin. Nutr.* **2000**, *71*, 1698S–1702S; discussion 1703S–1704S. [CrossRef]
11. Prasanth, M.I.; Sivamaruthi, B.S.; Chaiyasut, C.; Tencomnao, T. A Review of the Role of Green Tea (*Camellia sinensis)* in Antiphotoaging, Stress Resistance, Neuroprotection, and Autophagy. *Nutrients* **2019**, *11*, 474. [CrossRef]
12. Teixeira, A.M.; Sousa, C. A Review on the Biological Activity of *Camellia* Species. *Molecules* **2021**, *26*, 2178. [CrossRef]
13. Simić, S.; Vidović, S.; Stošić, J.L.; Filipović, K.; Aladić, K.; Jokić, S.; Gavarić, A. Utilizing Herbal Dust: Ultrasound-Assisted Extraction of Green Tea, Hibiscus, and Lemon Balm Filter Tea Industry Waste. *Processes* **2024**, *12*, 2405. [CrossRef]
14. Ivković, J.; Vladić, J.; Naffati, A.; Nastić, N.; Šavikin, K.; Tomić, M.; Vidović, S. Comparative Chemical Profiling of Underexploited Arctostaphylos uva-ursi L. Herbal Dust Extracts Obtained by Conventional, Ultrasound-Assisted and Subcritical Water Extractions. *Waste Biomass Valorization* **2022**, *13*, 4147–4155. [CrossRef]
15. Fonteles, T.; Leite, A.K.; Miguel, T.; Fernandes, F.; Pinheiro, S.; Miguel, E.; Rodrigues, S. Optimization of Sonication Parameters to Produce a Cashew Apple Bagasse Puree Rich in Superoxide Dismutase. *Foods* **2022**, *11*, 2694. [CrossRef] [PubMed]

16. Mohammadabadi, S.S.; Goli, M.; Tabasi, S.N. Optimization of Bioactive Compound Extraction from Eggplant Peel by Response Surface Methodology: Ultrasound-Assisted Solvent Qualitative and Quantitative Effect. *Foods* **2022**, *11*, 3263. [CrossRef] [PubMed]
17. Razola-Díaz, M.d.C.; Aznar-Ramos, M.J.; Guerra-Hernández, E.J.; García-Villanova, B.; Gómez-Caravaca, A.M.; Verardo, V. Establishment of a Sonotrode Ultrasound-Assisted Extraction of Phenolic Compounds from Apple Pomace. *Foods* **2022**, *11*, 3809. [CrossRef]
18. Medina-Jaramillo, C.; Gomez-Delgado, E.; López-Córdoba, A. Improvement of the Ultrasound-Assisted Extraction of Polyphenols from Welsh Onion (*Allium fistulosum*) Leaves Using Response Surface Methodology. *Foods* **2022**, *11*, 2425. [CrossRef]
19. Shen, L.; Pang, S.; Zhong, M.; Sun, Y.; Qayum, A.; Liu, Y.; Rashid, A.; Xu, B.; Liang, Q.; Ma, H.; et al. A comprehensive review of ultrasonic assisted extraction (UAE) for bioactive components: Principles, advantages, equipment, and combined technologies. *Ultrason. Sonochem.* **2023**, *101*, 106646. [CrossRef]
20. Brand-Williams, W.; Cuvelier, M.E.; Berset, C. Use of a Free Radical Method to Evaluate Antioxidant Activity. *LWT-Food Sci. Technol.* **1995**, *28*, 25–30. [CrossRef]
21. Baş, D.; Boyacı, İ.H. Modeling and optimization I: Usability of response surface methodology. *J. Food Eng.* **2007**, *78*, 836–845. [CrossRef]
22. De Luca, M.; Tuberoso, C.I.G.; Pons, R.; García, M.T.; Morán, M.d.C.; Ferino, G.; Vassallo, A.; Martelli, G.; Caddeo, C. Phenolic Fingerprint, Bioactivity and Nanoformulation of Prunus spinosa L. Fruit Extract for Skin Delivery. *Pharmaceutics* **2023**, *15*, 1063. [CrossRef]
23. Hoffmann, M.A.; Nothias, L.F.; Ludwig, M.; Fleischauer, M.; Gentry, E.C.; Witting, M.; Dorrestein, P.C.; Dührkop, K.; Böcker, S. High-confidence structural annotation of metabolites absent from spectral libraries. *Nat. Biotechnol.* **2022**, *40*, 411–421. [CrossRef]
24. Dührkop, K.; Fleischauer, M.; Ludwig, M.; Aksenov, A.A.; Melnik, A.V.; Meusel, M.; Dorrestein, P.C.; Rousu, J.; Böcker, S. SIRIUS 4: A rapid tool for turning tandem mass spectra into metabolite structure information. *Nat. Methods* **2019**, *16*, 299–302. [CrossRef] [PubMed]
25. KNApSAcKCoreSystem. Available online: http://www.knapsackfamily.com/knapsack_core/top.php (accessed on 14 May 2025).
26. Yusoff, I.M.; Mat Taher, Z.; Rahmat, Z.; Chua, L.S. A review of ultrasound-assisted extraction for plant bioactive compounds: Phenolics, flavonoids, thymols, saponins and proteins. *Food Res. Int.* **2022**, *157*, 111268. [CrossRef] [PubMed]
27. Prawira-Atmaja, M.I.; Puangpraphant, S. Effects of ultrasound-assisted bath, probe, and extraction time on bioactive compounds and antioxidant activity in white tea (*Camellia sinensis*) extracts from purple- and green-leaf cultivars. *J. Saudi Soc. Agric. Sci.* **2025**, *24*, 10. [CrossRef]
28. Luo, Q.; Zhang, J.R.; Li, H.B.; Wu, D.T.; Geng, F.; Corke, H.; Wei, X.-L.; Gan, R.-Y. Green Extraction of Antioxidant Polyphenols from Green Tea (*Camellia sinensis*). *Antioxidants* **2020**, *9*, 785. [CrossRef]
29. Athirojthanakij, W.; Rashidinejad, A. Optimizing catechin extraction from green tea waste: Comparative analysis of hot water, ultrasound-assisted, and ethanol methods for enhanced antioxidant recovery. *Food Sci. Nutr.* **2024**, *12*, 5121–5130. [CrossRef]
30. Menezes Maciel Bindes, M.; Hespanhol Miranda Reis, M.; Luiz Cardoso, V.; Boffito, D.C. Ultrasound-assisted extraction of bioactive compounds from green tea leaves and clarification with natural coagulants (chitosan and *Moringa oleífera* seeds). *Ultrason. Sonochem.* **2019**, *51*, 111–119. [CrossRef]
31. Gam, D.H.; Kim, S.Y.; Kim, J.W. Optimization of Ultrasound-Assisted Extraction Condition for Phenolic Compounds, Antioxidant Activity, and Epigallocatechin Gallate in Lipid-Extracted Microalgae. *Molecules* **2020**, *25*, 454. [CrossRef]
32. Zhu, Y.; Zhang, J.; Chen, F.; Hu, X.; Xu, D.; Cao, Y. Epimerisation and hydrolysis of catechins under ultrasonic treatment. *Int. J. Food Sci. Technol.* **2021**, *56*, 312–320. [CrossRef]
33. Xu, Y.Q.; Yu, P.; Zhou, W. Combined effect of pH and temperature on the stability and antioxidant capacity of epigallocatechin gallate (EGCG) in aqueous system. *J. Food Eng.* **2019**, *250*, 46–54. [CrossRef]
34. Xie, Y.; Zhu, Y.; Yang, J.; Zhang, G.; Tian, S.; Lian, J.; Dong, S. Effect of ultrasound on the stability of partial nitrification: Under the interference of aeration rate. *Ultrason. Sonochem.* **2023**, *100*, 106642. [CrossRef]
35. Wang, H.; Provan, G.J.; Helliwell, K. Reaction kinetics of degradation and epimerization of epigallocatechin gallate (EGCG) in aqueous system over a wide temperature range. *J. Agric. Food Chem.* **2008**, *56*, 2694–2701. [CrossRef]
36. Blaženović, I.; Kind, T.; Ji, J.; Fiehn, O. Software Tools and Approaches for Compound Identification of LC-MS/MS Data in Metabolomics. *Metabolites* **2018**, *8*, 31. [CrossRef]
37. Akhtar, N.; Thadhani, V.M.; Ul Haq, F.; Khan, M.N.; Ali, S.; Musharraf, S.G. Rapid identification and quantification of bioactive metabolites in processed *Camellia sinensis* samples by UHPLC-ESI-MS/MS and evaluation of their antioxidant activity. *J. Ind. Eng. Chem.* **2020**, *90*, 419–426. [CrossRef]

38. Jeszka-Skowron, M.; Zgoła-Grześkowiak, A.; Frankowski, R. Cistus incanus a promising herbal tea rich in bioactive compounds: LC–MS/MS determination of catechins, flavonols, phenolic acids and alkaloids—A comparison with *Camellia sinensis*, Rooibos and Hoan Ngoc herbal tea. *J. Food Compos. Anal.* **2018**, *74*, 71–81. [CrossRef]
39. Wyrepkowski, C.C.; Gomes da Costa, D.L.M.; Sinhorin, A.P.; Vilegas, W.; De Grandis, R.A.; Resende, F.A.; Varanda, E.A.; Dos Santos, L.C. Characterization and Quantification of the Compounds of the Ethanolic Extract from *Caesalpinia ferrea* Stem Bark and Evaluation of Their Mutagenic Activity. *Molecules* **2014**, *19*, 16039–16057. [CrossRef]