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Comparative optimization of traditional and ultrasound-assisted extraction methods for phenolic compounds from *Sorbus domestica* leaves: antioxidant and antidiabetic properties

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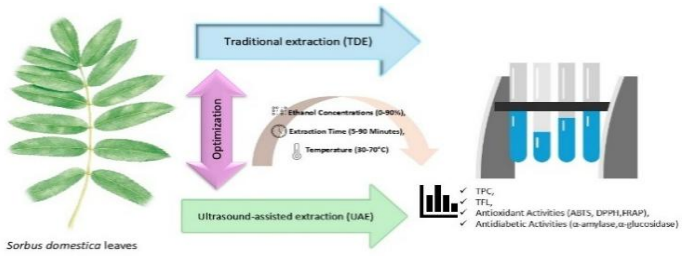
ABSTRACT

Context—Phenolic compounds from plant sources are widely recognized for their antioxidant and antidiabetic properties and are increasingly investigated for functional food and nutraceutical applications due to their potential role in the prevention of oxidative stress–related metabolic disorders. In recent years, increasing attention has been directed toward plant leaves as alternative and sustainable sources of bioactive compounds. Leaves of *Sorbus domestica* L. (service tree) represent an underutilized plant material with considerable potential bioactive value; however, information on efficient extraction strategies and functional characterization of their phenolic compounds remains limited. In particular, comparative evaluations focusing on both extraction efficiency and biological activity are still insufficient. Therefore, systematic comparisons of traditional extraction and ultrasound-assisted extraction methods are required to better understand their effectiveness in recovering phenolic compounds from *S. domestica* leaves.

Objective—The aim of this study was to compare traditional extraction (TDE) and ultrasound-assisted extraction (UAE) methods for the extraction of phenolic compounds from *S. domestica* leaves. For each method, extraction conditions were optimized, and the resulting extracts were evaluated in terms of phenolic content (TPC and TFL), antioxidant activity, and antidiabetic potential.

Method—*S. domestica* L. leaves were used as plant material in this study. Phenolic compounds were extracted using traditional extraction and UAE methods, with extraction temperature, ethanol concentration, and extraction time selected as independent variables. An experimental design approach was employed to optimize the extraction conditions for both techniques. Total phenolic content (TPC) and total flavonoid content (TFL) were quantified using spectrophotometric methods. Antioxidant activities of the extracts were evaluated using ABTS, FRAP, and DPPH assays, which are commonly applied to assess different antioxidant mechanisms. In addition, the antidiabetic potential of the extracts was assessed through α-amylase and α-glucosidase inhibition tests.



Results—The optimal conditions for TDE were 49.63% ethanol, 70.0°C, and 66.32 min, whereas UAE achieved optimal performance at 53.43% ethanol, 69.04°C, and 85.38 min. Under optimized conditions, UAE produced 94.38 mg GAE g^{-1} TPC and 89.13 mg QE g^{-1} TFL, whereas TDE yielded 73.21 mg GAE g^{-1} and 68.77 mg QE g^{-1}, respectively. This corresponds to about 29% higher TPC and 30% higher TFL in UAE compared to TDE, clearly demonstrating the superior extraction efficiency of the ultrasonic method. Antioxidant activities were also enhanced under UAE conditions. For example, UAE improved α-amylase inhibition by approximately 33% (IC_{50} reduced from 50.29 to 33.86 mg mL^{-1}) and α-glucosidase inhibition by approximately 12% compared to TDE, indicating stronger enzyme inhibitory potential. The lower IC_{50} values observed under UAE conditions suggest improved biological functionality of the extracted phenolics, which is particularly relevant for modulating postprandial hyperglycemia.

Conclusion—*S. domestica* leaves are a rich source of phenolic compounds with considerable antioxidant and antidiabetic potential. The quantitative improvements achieved by UAE indicate that extraction strategy significantly influences both yield and bioactivity. These findings highlight the potential application of UAE-derived *S. domestica* leaf extracts as functional ingredients in nutraceutical formulations, functional beverages, and glucose-regulating food systems.

Key Words—Service tree leaf, Phenolic compounds, Traditional extraction, Ultrasound-assisted extraction, Antioxidant activity

I. INTRODUCTION

The service tree (Sorbus L.) is a woody member of the Rosaceae family, classified within the subfamily Maloideae [1]. The genus comprises more than 100 species, many of which are valued for their ornamental and economic importance. In Türkiye, Sorbus is represented by 12 species and 17 naturally occurring taxa. Notable members of the Sorbus genus include *Sorbus aucuparia* (L.), *Sorbus umbellata* (Desf.) Fritsch, *Sorbus torminalis* (L.) Crantz, and *Sorbus domestica* (L.) Crantz [2].

Owing to its antioxidant, anti-inflammatory, diuretic, anti-atherogenic, and antidiabetic properties, S. domestica is widely utilized as a dietary, medicinal, and ornamental plant. The leaves, which are rich in polyphenols, have also been traditionally used in herbal medicine for treating conditions such as nephritis, diabetes, hypercholesterolemia, and prostatitis [3]. In Central Anatolia, tea made from the leaves of S. domestica is consumed as a diuretic and as a remedy for kidney stones [4],[5].

Extraction is a critical process for the isolation, identification, and utilization of phenolic compounds. The traditional method of solvent extraction, though effective, has notable disadvantages, including high solvent consumption, potential health risks, waste disposal challenges, solvent residues in extracts, extended extraction durations, and possible degradation or loss of polyphenols. In response to these limitations, increasing attention has been directed toward innovative extraction techniques such as microwave-assisted extraction, supercritical fluid extraction, and ultrasound-assisted extraction (UAE). Among these, UAE has gained particular attention due to its ability to enhance extraction efficiency through physical mechanisms. The improved performance of UAE is primarily attributed to acoustic cavitation, which involves the formation and collapse of microbubbles in the extraction medium during ultrasonic wave propagation. The implosion of these bubbles generates localized high pressure, temperature, and shear forces, resulting in cell wall disruption and enhanced solvent penetration into the plant matrix. This effect accelerates mass transfer and facilitates the release of intracellular phenolic compounds compared to conventional extraction techniques [6]. Response surface methodology (RSM) is a widely used and cost-effective tool for designing experiments in extraction studies. This approach is efficient in terms of material use, time, and cost, and has been successfully applied to optimize processes in food and natural product systems. Specifically, RSM has been instrumental in optimizing the extraction of phenolic compounds, whose bioactivity is highly influenced by extraction conditions [2].

Numerous studies have investigated the morphological and chemical properties of *S. domestica* L. fruits [1], [7]–[10]. However, relatively few studies have focused on the characteristics of *S. domestica* leaves, despite their widespread use in traditional medicine in Türkiye [3],[11],[12].

To the best of the author's knowledge, systematic optimization studies comparing TDE and UAE for phenolic recovery from *S. domestica* leaves are limited. Based on this knowledge gap, it was hypothesized that extraction method and process parameters significantly affect phenolic recovery and associated biological activities in *S. domestica* leaves. This study represents the first comprehensive investigation comparing traditional and ultrasound-assisted extraction methods for phenolic compounds from *S. domestica* leaves. Optimization processes for both methods are thoroughly discussed, and the most effective extraction conditions for maximizing phenolic content and antioxidant activity are determined. Traditional and ultrasound-assisted methods are evaluated based on their efficacy in extracting total phenolic and flavonoid content, antioxidant activities (TEAC, DPPH, FRAP), and antidiabetic properties. The findings of this study contribute significantly to understanding the methods that enhance the biological activity of *S. domestica* leaf extracts, filling a critical gap in the literature.

II. MATERIALS and METHODS

A. Materials

S. domestica leaves used as material in the study were collected at the beginning of September 2022 from wild trees in Pazar (40° 16' 38.1216'' North and 36° 17' 5.0928'' East) district of Tokat province in Türkiye. The leaves were shade-dried at ambient temperature (approximately 25^{0}C) under well-ventilated conditions for two weeks. The dried leaves were ground using a grinder and sieved through a mesh with a pore size of 0.075 mm^2. The powdered samples were then dried in an oven at 50^{0}C for 6 h to reduce residual moisture and to standardize the moisture content prior to storage and extraction. The dried powders were stored in glass jars at room temperature in a dark environment until extraction.

The following chemicals have been utilized in the analyses: gallic acid ($C_7H_6O_5$) (Sigma, Germany), ethanol (C_2H_5OH) (Tekkim, Bursa), sodium nitrite ($NaNO_2$) (Merck, Germany), sodium hydroxide (NaOH) (Merck, Germany), aluminum chloride ($AlCl_3$) (Merck, Germany), sodium carbonate (Na_2CO_3) (Isolab, Germany), Folin-Ciocalteu (Carlo Erba, France), trolox ((±)-6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) (Sigma, Germany), ABTS (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt) (Sigma, Germany), potassium peroxydisulfate ($K_2S_2O_8$) (Merck, Germany), DPPH (2,2-diphenyl-1-picrylhydrazyl) (Sigma, Germany), quercetin (BLD Pharm, China), hydrochloric acid (HCl) (Sigma, Germany), sodium acetate (CH_3COONa) (Merck, Germany), iron(III) chloride ($FeCl_3$) (Sigma, Germany), and 2,4,6-tri (2-pyridyl)-1,3,5-triazine (Tokyo Chem. Industry, Japan).

UV-VIS spectrophotometer (PG Instrument, T80+, UK), pure water device (Merck, Millipore, Germany), precision balance (Radwag, AS 220 R2, Poland), centrifuge (Nüve, Türkiye), grinder (Sinbo SCM 2934, Türkiye), vortex (Velp Scientifica, Italy), water bath (Memmert, Germany), magnetic stirrer with heater (Biosan, MSH 300, Latvia), ultrasonic bath (Elmasonic S100H, Elma, Singen, Germany) were used in different steps of the study.

B. Phenolic compounds extraction and optimization from S. domestica leaves

Optimization of extraction conditions was performed using Response Surface Methodology (RSM) with Design-Expert software (version 7.0, Stat-Ease Inc., USA). A Box–Behnken design with three independent variables at three levels was employed (Table 1). The Box–Behnken design was selected due to its efficiency in fitting second-order polynomial models while requiring fewer experimental runs. Compared to Central Composite Design (CCD), it does not include extreme axial points, thereby avoiding experiments under excessively high or low factor combinations. Although this limits exploration of the outermost experimental domain, it allows more moderate and controlled experimental conditions [13]. The range of independent variables was determined based on preliminary experiments and literature review. For each experimental run, 0.4 g of powdered *S. domestica* leaves were mixed with 10 mL of ethanol–water mixtures (0–90%, v/v). Traditional extraction (TDE) was conducted in a shaking water bath, whereas ultrasound-assisted extraction (UAE) was performed in an ultrasonic bath. Ultrasound-assisted extraction was carried out using a laboratory-scale ultrasonic bath (Elmasonic S100H, Germany) operating at 37 kHz. The stainless-steel tank had a filling volume of 9.5 L, and the maximum ultrasonic power was 600 W, corresponding to an approximate power density of 63 W/L. The bath temperature was continuously monitored and maintained at the target value (±1^{0}C) throughout the extraction

Table 1. Independent variables and their levels for the optimization of phenolic compound extraction from *S. domestica* leaves.

	Independent variables	Symbol	Level (-1)	Level (0)	Level (+1)
S. domestica Leaves	Time (min)	X_1	5	47.50	90
	Temperature (^{0}C)	X_2	30	50	70
	EtOH (%)	X_3	0	45	90

period to compensate for heat generated by ultrasonic cavitation. Samples were positioned centrally in the bath to ensure uniform acoustic exposure during treatment. The extraction experiments were conducted at varying extraction times (5–90 min) and temperatures (30–70^{0}C) according to the experimental design. After extraction, the mixtures were filtered through coarse filter paper and centrifuged at 6000 rpm for 10 min. The extracts obtained were stored at −20^{0}C until analysis.

The extraction process that will provide the highest TPC and TFL values has been optimized with the 'desirability' function approach. The model selected for the optimum conditions (OC) for phenolic compound extraction is explained by

$$Y(TPC,TFL) = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{11}X_1^2 + b_{22}X_2^2 + b_{33}X_3^2 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3. \quad (1)$$

In (1), Y $(TPC\ and\ TFL)$ represents the dependent variables, b_0 is the constant term, b_1, b_2 and b_3 are the linear coefficients, b_{11}, b_{22} and b_{33} are the quadratic coefficients, and X_1, X_2 and X_3 are the independent variables.

1. Extraction yield

Twenty grams of dried *S. domestica* leaf samples were extracted at the optimum times, temperatures, and EtOH concentrations specified in the experimental design. After evaporating the solvents to dryness, the extracts were weighed. The extraction yield was calculated according to the method reported by Akomeng and Adusei [14] using the following formula:

Yield (%) = (dry extract amount / sample amount) × 100.

2. Total phenolic compounds (TPC)

Using the Folin–Ciocalteu procedure, absorbance at 765 nm was measured to determine TPC, which was expressed as mg GAE per g of dry sample [15].

3. Total flavonoid content (TFL)

Following a modified method of Gaafar and Salama [16], absorbance at 415 nm was recorded to quantify flavonoids, and values were expressed as mg QE per g of dry sample.

4. ABTS$^{\bullet+}$ (Cation radical scavenging activity)

Using the Re et al. [17] protocol, absorbance at 734 nm was recorded to determine the antioxidant activity of extracts produced under optimized conditions, expressed as mg TE per g of extract.

5. DPPH• (Free radical scavenging activity)

Antioxidant activity of the extracts obtained under OC were tested using the Blasi et al. [18], method. Absorbance was measured at 517 nm, and results were expressed as mg TE per gram of extract.

6. FRAP (Ferric reducing antioxidant power)

The extracts obtained under OC were tested for ferric reducing antioxidant capacity using the Benzie and Strain [19] technique. In a spectrophotometer, absorbance measurements were made at 593 nm, and the FRAP samples were found as mg TE g^{-1} extract.

7. Compositional analysis of phenolic and flavonoid compounds

Determination of individual phenolics in *S. domestica* leaf extracts obtained at OC with both TDE and UAE was carried out by Topuz Türker & Bayram [20] method using LC–MS/MS (LC-MS 8050, Shimadzu). For this purpose, the extracts were filtered through a membrane filter with a pore diameter of 0.45µm and then injected into the LC–MS/MS device. Chromatographic separation was achieved on a C18 column (150 × 2.0 mm, 3 µm) at 40^{0}C using ammonium format (10 mmol/L) in water (A) and methanol (B) at a flow rate of 0.40 mL/min. Mass spectrometric detection was conducted using electrospray ionization in positive mode (ESI$^+$). Detailed LC–MS/MS operating conditions are provided in Appendix (Table A1). Results are expressed in mg per gram of dry sample.

8. Antidiabetic activity of extracts

Antidiabetic activity was evaluated based on the inhibition of α-amylase and α-glucosidase, following the approach reported by Kim et al. [21] and Temiz [22]. The antidiabetic effects were tested using acarbose solution as a positive control. The IC_{50} (mg mL^{-1}) is used to present the results.

9. Statistical analysis

The one-sample t-test in SPSS 22.0 (IBM, USA) was used to compare experimental values with model-predicted values under optimum conditions, and ANOVA confirmed that the developed models for TPC and TFL were significant ($p < 0.01$), with non-significant lack-of-fit ($p > 0.05$); detailed results are provided in Appendix (Table A2).

III. RESULTS and DISCUSSION

A. Phenolic compound extraction and optimization from S. domestica leaves

The effects of ethanol/water combination concentration (X_1: 0-90%), extraction time (X_2: 5-90 min), and extraction temperature (X_3: 30-70^{0}C) on TPC and TFL values obtained during the extraction of phenolic compounds from *S. domestica* leaves were examined in this work. Following initial trials, the solid-liquid ratio (0.4 g-10 mL) was established and maintained throughout the extraction procedure.

The traditional extraction (TDE) method was performed under 17 different experimental conditions, varying in temperature, time, and ethanol concentration. TPC values ranged from 17.01 to 73.35 mg GAE g dry^{-1} sample, while TFL values varied from 8.65 to 69.85 mg QE g^{-1} dry sample (Table 2).

The ultrasonic-assisted extraction (UAE) was similarly conducted under 17 conditions, with TPC values ranging from 37.80 to 90.35 mg GAE g^{-1} dry sample and TFL values between 31.42 and 86.50 mg QE g^{-1} dry sample (Table 3).

Based on the study's findings, it was determined that the TPC and TFL values were higher in both the traditional extraction (TDE) and ultrasound-assisted extraction (UAE) methods conducted at 45% ethanol concentration compared to those carried out with 90% ethanol concentration or pure water. Additionally, the TPC and TFL values of the extracts obtained using 90% ethanol were higher than those produced with pure water under similar conditions (Table 2 and Table 3). A comparison of the two extraction methods revealed that the TPC and TFL values of extracts obtained via UAE were consistently greater than those obtained through the TDE method. This difference can be attributed to the pore-opening and tissue-rehydrating properties of the UAE technique, which enhances the mass transfer of soluble components through osmotic and diffusion mechanisms

The quadratic models for TPC, (2) and (4), and TFL, (3) and (5) responses in TDE and UAE are shown below:

$$\text{TDE, TPC (mg GAE/g)} = -11.35 + 0.1X_1 + 0.82X_2 + 1.92X_3 + 2.45\times10^{-4}X_1X_2 + 3.08\times10^{-4}X_1X_3 - 4.15\times10^{-3}X_2X_3 - 8.45\times10^{-4}X_1^2 - 3.97\times10^{-4}X_2^2 - 0.02X_3^2 \quad (2)$$

Table 2. Experimental design and results for TDE of S. domestica leaves.

No	Time (min)	Temperature (^{0}C)	EtOH (%)	TPC (mg GAE g^{-1} dry sample)	TFL (mg QE g^{-1} dry sample)
1	90	70	45	73.35±3.84	69.00±3.39
2	47.5	30	0	17.99±1.46	8.65±0.65
3	47.5	70	0	29.41±0.90	24.58±0.84
4	47.5	50	45	66.47±1.02	65.42±1.38
5	47.5	30	90	43.45±1.03	37.88±1.71
6	90	50	90	49.68±2.45	38.54±0.53
7	47.5	50	45	70.95±2.72	69.85±1.41
8	47.5	70	90	39.93±1.20	35.69±3.39
9	90	50	0	22.89±0.85	14.23±1.50
10	5	30	45	56.08±3.39	50.73±1.40
11	5	70	45	71.68±1.76	64.73±1.17
12	5	50	90	41.45±1.37	39.31±0.89
13	47.5	50	45	65.76±2.06	58.08±2.16
14	47.5	50	45	64.26±3.42	61.85±1.51
15	90	30	45	56.91±2.17	54.69±2.39
16	47.5	50	45	70.64±3.24	65.88±2.53
17	5	50	0	17.01±0.97	8.73±0.26

Data is expressed as mean ± standard deviation (SD) ($n = 3$).

Table 3. Experimental design and results for UAE of S. domestica leaves.

No	Time (min)	Temperature (^{0}C)	EtOH (%)	TPC (mg GAE g^{-1} dry sample)	TFL (mg QE g^{-1} dry sample)
1	90	70	45	90.35±1.33	86.50±2.41
2	47.5	30	0	37.80±1.32	31.42±0.42
3	47.5	70	0	47.41±1.08	41.27±0.86
4	47.5	50	45	82.10±1.25	81.96±0.48
5	47.5	30	90	60.78±0.00	57.46±0.00
6	90	50	90	63.45±0.00	56.54±0.00
7	47.5	50	45	81.91±0.80	78.31±1.22
8	47.5	70	90	75.28±0.00	72.54±0.00
9	90	50	0	44.83±0.85	35.38±0.48
10	5	30	45	78.89±1.28	77.08±0.46
11	5	70	45	84.53±0.00	80.92±0.77
12	5	50	90	62.37±0.00	58.23±0.00
13	47.5	50	45	82.30±1.71	81.15±2.36
14	47.5	50	45	82.18±1.74	79.81±2.18
15	90	30	45	80.22±1.38	77.31±1.72
16	47.5	50	45	74.08±0.05	73.88±1.95
17	5	50	0	41.16±1.49	33.15±1.72

Data is expressed as mean ± standard deviation (SD) ($n = 3$).

$$\text{TDE, TFL (mg QE/g)} = -24.39+0.23X_1+0.85X_2+2.16X_3 +9.05\times10^{-5}X_1X_2-8.2\times10^{-4}X_1X_3-5.03\times10^{-3}X_2X_3 -1.64\times10^{-3}X_1^2-3.66\times10^{-3}X_2^2-0.02X_3^2 \quad (3)$$

$$\text{UAE TPC (mg GAE/g)} = 49.76-0.03X_1-0.54X_2+1.44X_3 +1.32\times10^{-3}X_1X_2-3.4\times10^{-4}X_1X_3+1.36\times10^{-3}X_2X_3 +1.7\times10^{-4}X_1^2+6.7\times10^{-3}X_2^2-0.014X_3^2 \quad (4)$$

$$\text{UAE TFL (mg QE/g)} = 45.23+0.053X_1-0.69X_2 +1.64X_3+1.58\times10^{-3}X_1X_2-5.1\times10^{-4}X_1X_3 +1.45\times10^{-3}\,X_2X_3-9.5\times10^{-4}X_1^2+7.84\times10^{-3}X_2^2-0.015X_3^2 \quad (5)$$

ANOVA results (Table A2 and Table A3) indicated that the developed models were statistically significant (P < 0.0001) for both traditional extraction (TDE) and ultrasound-assisted extraction (UAE). For TDE, the coefficients of determination (R2) were 0.9749 and 0.9815 for TPC and TFL, respectively, whereas for UAE, the corresponding R2 values were 0.9834 and 0.9868. The adjusted R^2 (Adj-R^2) values ranged from 0.9426 to 0.9698 across all models, demonstrating strong agreement between predicted and experimental values. Moreover, the lack-of-fit tests were not statistically significant (P > 0.05), confirming the adequacy and reliability of the second-order polynomial models.

Among the evaluated factors, temperature (X_2) and ethanol concentration (X_3) significantly influenced TPC and TFL responses in both extraction methods (P < 0.05). In addition, the quadratic term of ethanol concentration (X_3^2) exhibited a highly significant effect (P < 0.0001), indicating curvature in the response surfaces. In contrast, extraction time (X_1), its quadratic term (X_1^2), temperature quadratic term (X_2^2), and all interaction terms were not statistically significant (P > 0.05). Overall, the statistical analysis demonstrated that only X_2, X_3, and X_3^2 were significant contributors to the developed models.

Figure 1 presents 3D response surface plots illustrating the influence of temperature, ethanol concentration, and extraction time on the TPC and TFL of *S. domestica* leaf extracts obtained by TDE and UAE. Analysis of the interaction between ethanol concentration and time at a fixed temperature (50^{0}C, the midpoint of the temperature range) revealed that increasing ethanol concentration enhanced TPC and TFL values up to an optimum level, beyond which a decline was observed. This trend is consistent with the significant linear (X_3) and quadratic (X_3^2) effects observed in the model. These results indicate that ethanol–water mixtures are more effective than single-component solvents, as they facilitate the extraction of compounds soluble in both organic solvents and water. The findings further demonstrated that polyphenols, which often possess multiple hydroxyl groups (e.g., glucosides), exhibit hydrophilic properties and display higher solubility in hydroalcoholic mixtures compared to pure alcohol solvents [2]. Similarly, variations in temperature significantly affected TPC and TFL values. The positive effect of temperature (X_2) can be attributed to enhanced solvent diffusion, reduced solvent viscosity, and improved mass transfer at elevated temperatures. This behavior aligns with Fick's second law of diffusion, whereby solute transfer approaches equilibrium after sufficient contact time. In contrast, extraction time (X_1) did not show a statistically

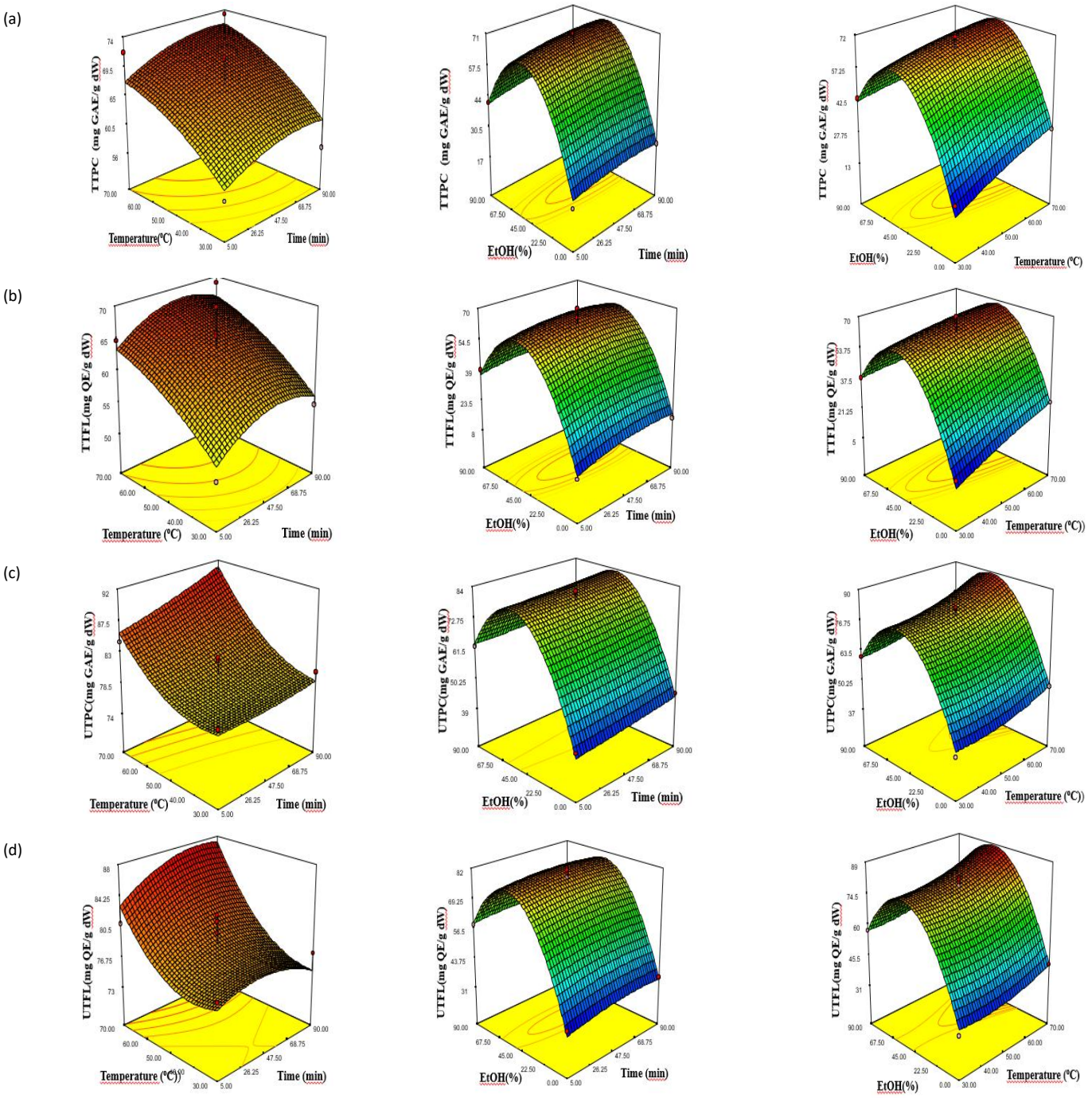


Figure 1. Response surface plots of extraction parameters for *S. domestica* leaves on (a) TTPC: Traditional Total Phenolic Content, (b) TTFL: Traditional Total Flavonoid Content, (c) UTPC: Ultrasonic Total Phenolic Content and (d) UTFL: Ultrasonic Total Flavonoid Content.

significant effect within the studied range, suggesting that near-equilibrium conditions were largely achieved during the applied extraction period. Furthermore, prolonged extraction may lead to partial reabsorption of phenolic compounds onto the plant matrix, limiting further increases in phenolic yield [23]. Consequently, extending the extraction time beyond the optimal range does not significantly enhance the recovery of phenolic compounds.

Figure 2 presents a comparison between the experimental data and the values estimated by the polynomial models generated by the program for TPC and TFL in extracts obtained through both the TDE and UAE methods. Analysis of the graphs reveals that both the experimental and estimated data closely align along the 45° line. This observation indicates that the developed models are accurate and well-suited for predicting the TPC and TFL values of the extracts.

In the optimization studies for both TDE and UAE conditions aimed at maximizing the production of phenolic and flavonoid compounds from *S. domestica* leaves, the optimal conditions for TDE were determined to be 49.63% ethanol, 70^{0}C, and 66.32 minutes. These conditions were identified as the optimal point from three different scenarios, as suggested by the "desirability" function approach in the program, which indicated that the conditions were closely aligned. Similarly, for UAE, the optimal conditions were found to be 53.43% ethanol, 69.04 ^{0}C, and 85.38 minutes, selected from three alternative conditions provided by the same program based on the "desirability" function approach, which also showed a close similarity among the suggested values.

Using traditional extraction (TDE) conditions (49.63% ethanol, 66.32 min, 70.00 ^{0}C), phenolic compounds were obtained from *S. domestica* leaves, with estimated TPC and TFL values of 72.14 mg GAE g^{-1} and 68.48 mg QE g^{-1} dry sample, respectively. To verify

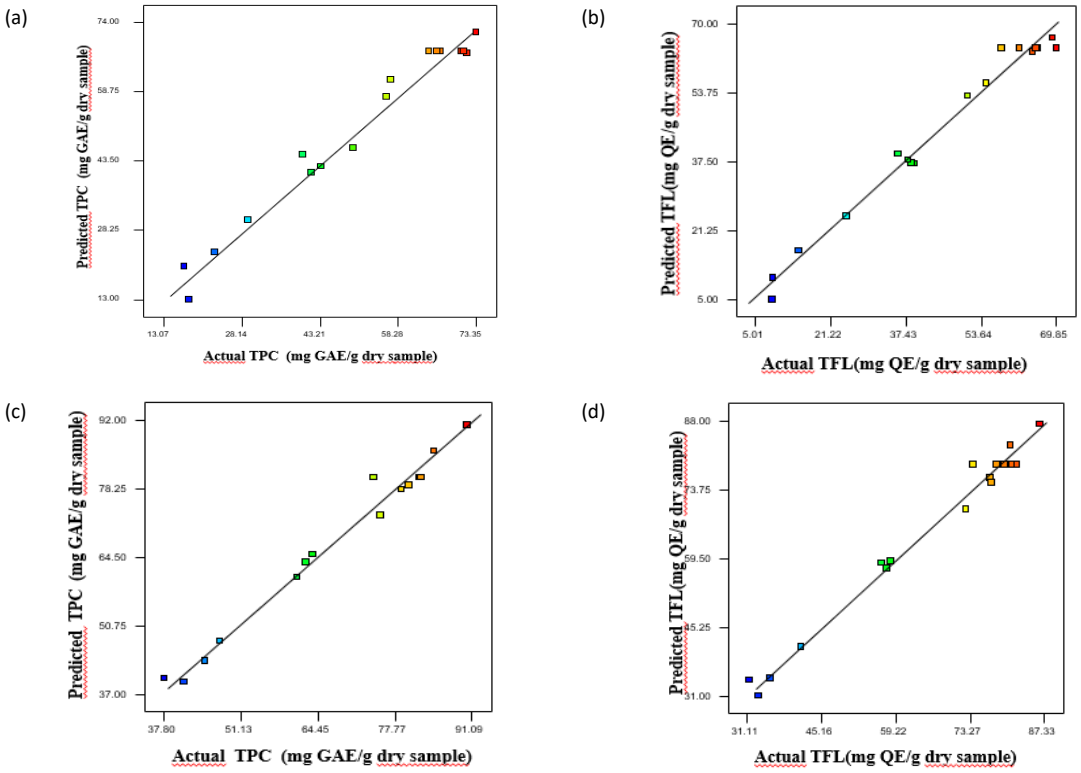


Figure 2. The relationship between experimental and predicted data with respect to the values of (a) TTPC, (b) TTFL, (c) UTPC, and (d) UTFL.

the optimal conditions, three replicate trials were conducted for TPC and TFL. The results of these verification trials were 73.21 mg GAE g^{-1} dry sample and 68.77 mg QE g^{-1} dry sample, respectively. No statistically significant difference was detected between experimental and estimated values by one-sample t-test ($P > 0.05$).

TPC and TFL values were estimated to be 90.83 mg GAE g^{-1} dry sample and 88.47 mg QE g^{-1} dry sample, respectively, in the UAE method of extracting phenolic compounds from *S. domestica* leaves (53.43% ethanol concentration, 85.38 min, 69.04 ^{0}C temperature). For TPC and TFL values, three replicate optimum point verification trials were conducted. These experiments yielded the following values: 94.38 mg GAE g^{-1} dry sample and 89.13 mg QE g^{-1} dry sample, respectively. The experimental data acquired and the estimated data were compared using a one-sample t-test, and the results showed no significant difference ($P>0.05$), statistically.

According to Lauro et al. [24], the TPC in *S. domestica* leaf extracts was reported as 66.4±10 mg GAE g^{-1}. Liashenko [25] found that the TPC in *S. domestica* leaves over a five-month period (May–September) ranged between 27.63 and 40.67 mg GAE g^{-1} dry weight. Similarly, Lykholat et al. [26] reported a value of 31.42±0.35 mg GAE g^{-1} dry weight for *S. domestica* leaves collected in Ukraine. Matczak et al. [11] indicated that the phenolic content varied significantly, ranging from 76.7 to 700.0 mg GAE g^{-1} dry weight, depending on the type of solvent used (methanol, n-butanol, water, ethyl acetate, diethyl ether). Furthermore, Rutkowska et al. [27] observed that the TPC in *S. domestica* leaves varied between 58.60 and 73.39 mg GAE g^{-1} dry weight across different months (May–October).

Liashenko [25] reported that the flavonoid content of *S. domestica* leaves over a five-month period (May–September) ranged between 9.52 and 13.04 mg Rutin g^{-1} dry weight. Additionally, The TFL of *S. domestica* leaves collected from Ukraine, extracted using 80% isopropanol, was determined to be 10.93±0.12 mg Rutin E g^{-1} dry weight [26].

The obtained data seem to correspond with some findings in the literature while showing divergence from others. This variation is thought to be linked to the climatic and geographical conditions, as well as the soil characteristics of the regions where the species grows. These environmental factors are believed to influence metabolite production in plants, thereby affecting their biological properties, including antioxidant activity.

B. Extraction yield results

The extraction efficiency obtained by TDE at OC in *S. domestica* leaves was determined as 31.99±0.41%; for UAE, the extraction efficiency was determined as 32.20±0.88%.

Tahirovic et al. [28] reported that the extraction yield of leaves from three different Sorbus species (*Sorbus austriaca, Sorbus aria* (L.) Crantz and *Sorbus aucuparia* L.) in the Bosnia region ranged from 32.60% to 36.40%. Lavanya et al. [29] found that the extraction yield for *Breynia vitis-idaea* leaf extracts using methanol was 20.50%. Similarly, Legesse et al. [30] reported that the extraction yield of *Verbascum sinaiticum* leaves varied between 19.35% and 21.60%, depending on optimization parameters like solute-to-solvent ratio, extraction temperature, and time in the ultrasound-assisted extraction process.

C. Antioxidant properties results

Out of the three conditions recommended by the program, 49.63% ethanol was chosen for TDE in the OC to produce a large amount of phenolic and flavonoid compounds. As determined by the ABTS+ technique, the extracts' antioxidant activity at OC of 70^{0}C and 66.32 minutes was 165.64±3.15 mg TE g^{-1} dry sample.

During optimization studies for UAE, among three different conditions evaluated, the optimal parameters were determined to be 53.43% ethanol, 69.04^{0}C, and 85.38 minutes. Under these OC, the antioxidant activity of the extracts, measured by $ABTS^+$ assay, was determined as 156.09±9.64 mg TE g^{-1} dry sample.

Bati et al. [31] investigated *Euclea natalensis* leaves, reporting IC_{50} values of 0.079±0.006 mg mL^{-1} and 0.128±0.012 mg mL^{-1} for methanol and water extractions, respectively. In a study of haskap berry (*Lonicera caerulea* var. kamtschatica) leaves, Sip et al. [32] observed ABTS values ranging from 0.1212 to 0.2412 mg mL^{-1} Trolox equivalent (TE) across different ethanol

concentrations. Furthermore, Tahirovic et al. [28] examined three Sorbus species from the Bosnian region, reporting ABTS values of 404.90±6.64 µmol TE g^{-1} for *Sorbus aucuparia* L., 1002.13±6.64 µmol TE g^{-1} for *Sorbus aria* (L.) Crantz, and 1236.51±20.90 µmol TE g^{-1} for *Sorbus austriaca*.

In the optimization studies of extraction conditions to produce high levels of phenolic and flavonoid compounds, the antioxidant activities of the extracts obtained under OC by TDE and UAE methods were determined as 86.18±3.33 and 83.77±10.00 mg TE g^{-1} dry sample, respectively, measured by the FRAP method.

Several studies have investigated FRAP antioxidant activity in various Sorbus species under different extraction conditions. FRAP activities varied between 22.79 to 29.67 mmol Fe^{2+} g^{-1} GAE in Matczak et al.'s [11] study of *S. domestica* leaves utilizing a variety of solvents, including water, n-butanol, methanol, ethyl acetate, and diethyl ether. Rutkowska et al. [27] determined that between May and October, the FRAP values of *S. domestica* leaves ranged from 0.398 to 0.497 mmol TE g^{-1} dry weight. The FRAP values of methanol extracts from native Sorbus species in the Artvin (Türkiye) were 6.248±0.2374 µmol $FeSO_4 \cdot 7H_2O$ g^{-1} for *S. persica*, 6.070±0.3125 µmol $FeSO_4 \cdot 7H_2O$ g^{-1} for *S. umbellata* var. cretica, and 5.322±0.1806 µmol $FeSO_4 \cdot 7H_2O$ g^{-1} for *S. subfusca*, according to Camadan et al. [33]. Tahirovic et al. [28] reported that the amount of FRAP in the leaves of 3 different Sorbus species from Bosnia varied between 815.41±0.92 µmol TE g^{-1} for *Sorbus aucuparia* L., 1516.93±0.90 µmol TE g^{-1} for *Sorbus aria* (L.) Crantz and 2169.03±91.50 µmol TE g^{-1} for *Sorbus austriaca*.

In the optimization studies of extraction conditions to produce high levels of phenolic and flavonoid compounds, the antioxidant activities of the extracts obtained under OC by TDE and UAE methods were determined as 145.55±2.41 and 136.18±6.77 mg TE g^{-1} dry sample, respectively, measured by the DPPH method.

Lykholat et al. [26] determined the level of FRAP (80% isopropanol) in *S. domestica* leaves gathered from Ukraine to be 17.11±0.95 mg Ascorbic acid E g^{-1} dry weight; Matczak et al. [11] found the DPPH activity (EC_{50}) in *S. domestica* leaves with various solvent types (water, n-butanol, methanol, ethyl acetate, diethyl ether) to be 2.53-3.28 µg GAE mL^{-1} based on the solvent type used; Rutkowska et al. [27] identified the DPPH activity in *S. domestica* leaves as 0.394-0.551 mmol TE g^{-1} dry weight during different months (May-October). Camadan et al. [33] noted the levels of DPPH (IC_{50}) in methanol-extracted leaves of various Sorbus species from the Artvin region as 0.0463 mg mL^{-1} *for S. persica*, 0.1106 mg mL^{-1} for *S. umbellata* var. cretica, and 0.1631 mg mL^{-1} for S. *subfusca*; Lauro et al. [24] indicated the amount of DPPH (EC_{50}) in *S. domestica* leaf extracts as 241±1.9 µg mL^{-1}; Tahirovic et al. [28] stated that the quantity of DPPH in leaves of three different Sorbus species from the Bosnian region ranged from 346.02±2.30 µmol TE g^{-1} for *Sorbus aucuparia* L., 645.70±2.41 µmol TE g^{-1} for *Sorbus aria* (L.) Crantz, and 842.24±16.14 µmol TE g^{-1} for *Sorbus austriaca*.

Differences observed among DPPH, ABTS, and FRAP results may be attributed to the distinct reaction mechanisms of these assays and variations in extract composition. While DPPH and ABTS evaluate radical-scavenging activity, FRAP measures reducing power; therefore, differences in phenolic structure and redox properties may lead to variable responses among the assays. Variations between the present findings and previous studies may also be associated with differences in plant genetic characteristics, environmental conditions, harvest time, and extraction parameters, all of which can influence phenolic composition and antioxidant behavior.

D. Phenolic compound compositions results

The phenolic composition of *S. domestica* leaf extracts obtained under optimized conditions (TDE and UAE) was evaluated using LC–MS/MS. Only phenolic compounds detected above their respective limits of quantification were considered for quantitative reporting. Compound identification was performed by matching retention times and precursor-to-product ion transitions (MRM) with those of authentic standards analyzed under identical LC–MS/MS conditions. The LC–MS/MS calibration and validation parameters of quercetin-3-β-D-glucoside and protocatechuic acid are presented in Table 4.

Identification of phenolic compounds in *S. domestica* leaf extracts obtained under OC was done by LC–MS/MS. Quercetin 3 beta-d-glucoside was identified at 4.51±0.18 mg g^{-1} dry sample in *S. domestica* leaf extracts obtained under OC with the TDE method and at 4.24±0.19 mg g^{-1} dry sample in extracts obtained by UAE method. Similarly, Protocatechuic acid was identified at 1.02±0.16 mg g^{-1} dry sample in *S. domestica* leaf extracts obtained under OC with the TDE method and at 1.06±0.00 mg g^{-1} dry sample in extracts obtained by UAE method.

Matczak et al. [11] determined 44 different individual phenolic substances (flavan-3-ol derivatives (catechins and proanthocyanidins, 16), flavonols (13) and phenolic acids (12)) in their study using different solvent types (water, diethyl ether, n-butanol, methanol, and ethyl acetate) from *S. domestica* leaves (UHPLC-PDA-ESI-MS), but they stated that these varied depending on the solvent type used, and only chloregenic acid was found in all solvents. According to Rutkowska et al. [27], their UHPLC-PDA-ESI-MS studies of *S. domestica* leaves in various months (May-October) stated 40 different individual phenolic substances. These included flavonoids (quercetin mono and diglycosides), flavalignan (such as cinchonine I isomer), phenolic acids (hydroxybenzoic and hydroxycinnamic acid derivatives), flavan-3-ols, and proanthocyanidins, primarily (-)-epicatechin and procyanidin B type dimers.

E. Antidiabetic activity results

The α-amylase inhibitory activity (IC_{50}) of the extracts obtained from *S. domestica* leaves under optimal conditions was 50.29 mg mL^{-1} for TDE and 33.86 mg mL^{-1} for UAE. The lower IC_{50} value observed for the UAE extract indicates stronger inhibitory activity compared to the TDE extract. In comparison, the positive control acarbose exhibited IC_{50} values of 17.73 mg mL^{-1} for α-amylase under OC.

Bouakline et al. [34] determined α-amylase activity (IC_{50}) in Pistacia lentiscus leaf extracts (aqueous acetone) as 0.266±0.01 mg mL^{-1}; Cvetkova et al. [35] determined α-amylase activity (IC_{50}) in *Vaccinium myrtillus* L. (Bilberry) leaf extracts (ethanol) as 39.33 and 63.89 mg mL^{-1} and α-amylase activity (IC_{50}) in *Vaccinium vitis-idaea* L. (Lingonberry) leaf extracts (ethanol) as 12.27 and 15.93 mg mL^{-1}; Lavanya et al. [29] determined α-amylase activity (IC_{50}) in Breynia vitis-idaea leaf extracts (methanol) as 20.42±0.01 µg mL^{-1}; Sun et al. [36] reported that α-amylase activity (IC_{50}) in coffee leaves was 7.12±0.08 mg mL^{-1}; and the same value was found to be 5.57±0.12 mg mL^{-1} in ultrasound-assisted extraction application.

In *S. domestica* leaves, the α-glucosidase inhibitory activity (IC_{50}) of the extracts obtained under optimal conditions was 26.43 mg mL^{-1} for TDE and 23.38 mg mL^{-1} for UAE. The lower IC_{50} value observed for UAE extract indicates stronger inhibitory activity compared to TDE. In comparison, the positive control acarbose offered IC_{50} values of 15.07 mg mL^{-1} for α-glucosidase under OC.

Table 4. Main validation data for phenolic compounds determined by LC-MS/MS method.

Phenolic compound	R^2	LOD	LOQ	Precursor m/z > Product m/z	Retention time (min)
Quercetin-3-β-D-glucoside	0.998587	2.322153	7.740510	462.80>301.10	6.664
Protocatechuic acid	0.999343	1.480289	4.934297	153.00>109.10	5.601

Lauro et al. [24] reported α-glucosidase activity (IC_{50}) in *S. domestica* leaf extracts as 4.3±0.8 μg mL^{-1}; Lavanya et al. [29] reported α-glucosidase activity (IC_{50}) in *Breynia vitis-idaea* leaf extracts (methanol) as 14.60 μg mL^{-1}; Liu et al. [37] reported α-glucosidase activity (IC_{50}) in *Aquilaria sinensis* leaf extracts as 6.93±1.91 μg mL^{-1} in 70% ethanol, 23.57±0.87 μg mL^{-1} in 70% methanol, and 58.54±8.97 μg mL^{-1} in water; Sun et al. [36] reported that α-glucosidase activity (IC_{50}) in coffee leaves was 92.29±1.61 μg mL^{-1}; the same value was 69.10±0.89 μg mL^{-1} in ultrasound-assisted extraction application; Wibowo et al. [38] reported that α-glucosidase activity (IC_{50}) was 3.65±0.40 mg mL^{-1} in *Aquilaria malaccensis* leaf extracts (chloroform) and 6.64±0.33 mg mL^{-1} in ethanol extraction.

The IC_{50} values for α-glucosidase were lower than those obtained for α-amylase, indicating greater sensitivity of α-glucosidase to the phenolic constituents of the extracts. This difference may be explained by structure–activity relationships of polyphenols. Compounds possessing ortho-dihydroxy groups and conjugated structures are reported to interact more effectively with the active site of α-glucosidase through hydrogen bonding and π–π interactions [39],[40]. In contrast, α-amylase generally exhibits lower binding affinity toward such structures, which may account for the weaker inhibition observed.

IV. CONCLUSION

This study shows that extraction time, ethanol concentration, and temperature play a critical role in determining phenolic extraction efficiency. These parameters were optimized using response surface methodology for *S. domestica* leaves to maximize TPC and TFL using traditional and ultrasonic extraction. Optimal conditions were 49.63% ethanol, 70 ^{0}C, 66.32 min for traditional extraction, and 53.43% ethanol, 69.04^{0}C, 85.38 min for ultrasonic extraction. From a practical perspective, UAE offers enhanced extraction efficiency, improved mass transfer, reduced solvent consumption, and shorter processing time compared to traditional extraction, making it a greener extraction alternative. However, its large-scale implementation requires careful consideration of power density distribution, energy efficiency, and reactor configuration, as well as higher initial equipment investment, to ensure economic and operational feasibility under industrial conditions.

In terms of biological relevance, the optimized extracts of *S. domestica* leaves were rich in phenolic compounds and exhibited strong antioxidant activity. Under these optimized conditions, *S. domestica* leaves also showed notable inhibitory effects against key enzymes (α-amylase and α-glucosidase) involved in glucose metabolism. The observed inhibitory activities were generally comparable to those reported for other Sorbus species and phenolic-rich leaves, while the differences can be attributed to variations in plant matrix and extraction strategy.

Overall, these results highlight the potential of *S. domestica* leaves as a source of bioactive compounds. The plant material was collected from a single harvest period and geographical location, which may affect phenolic variability. In addition, the biological activities were assessed under in vitro conditions. Future studies should examine seasonal and geographical variation and further validate the observed bioactivities under in vivo conditions.

AUTHOR STATEMENT

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CRediT Author Contribution—Conceptualization, Methodology, Validation, Formal analysis, Investigation, Resources, Data curation, Visualization, Writing, Reviewing and editing were all done by the author of the article.

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APPENDIX-A

Table A1. LC and MS Conditions.

LC Conditions (Nexera X2)		MS Conditions (LCMS-8050)	
Column	Inertsil (ODS-4), C18 column (150×2.00 mm, 3 µm)	Ionization mode	ESI (Positive)
Column oven temperature	40°C	Desolvation line temperature	250°C
Mobile phase A	10 mmol/L ammonium formate/water	Interface temperature	300°C
Mobile phase B	Methanol	Block heater temperature	400°C
Gradient program	20%B (0 min) - 70%B (3 min), 95%B (10.30 min) - 20%B (10.51-14.00 min)	Nebulizer gas flow	3 L/min.
Flow rate	0.40 mL/min	Drying gas flow.	10.0 L/min
Injection volume	5 µL	Heating gas flow	15.0 L/min.
Rinse solution	R0: 50% methanol	Dwell time	1-33 msec

Table A2. ANOVA table showing the effect of linear, quadratic and interaction terms for TPC and TFL values.

Source	Dof	Sum of squares				Average of squares			
		TTPC	TTFL	UTPC	UTFL	TTPC	TTFL	UTPC	UTFL
Model	9	6018.27	7095.01	4538.80	5772.53	668.70	788.33	504.31	641.39
X_1	1	34.46	21.00	17.69	5.03	34.46	21.00	17.69	5.03
X_2	1	199.38	220.90	198.75	180.13	199.38	220.90	198.75	180.13
X_3	1	950.66	1133.61	1028.03	1340.03	950.66	1133.61	1028.03	1340.03
X_1X_2	1	0.17	0.024	5.02	7.15	0.17	0.024	5.02	7.15
X_1X_3	1	1.39	9.83	1.67	3.85	1.39	9.83	1.67	3.85
X_2X_3	1	55.78	82.04	5.99	6.84	55.78	82.04	5.99	6.84
X_1^2	1	9.81	36.98	0.40	12.29	9.81	36.98	0.40	12.29
X_2^2	1	10.63	9.02	30.18	41.45	10.63	9.02	30.18	41.45
X_3^2	1	4678.36	5472.01	3270.42	4174.58	4678.36	5472.01	3270.42	4174.58
Residual	7	155.02	133.76	76.46	77.17	22.15	19.11	10.92	11.02
Lack of fit	3	118.78	54.51	24.58	36.46	39.59	18.17	8.19	12.15
Pure error	4	36.24	79.24	51.88	40.71	9.06	19.81	12.97	
Total	16	6173.29	7228.77	4615.27	5849.70				

Table A2. Continue…

Source	Dof	F Value				P Value			
		TTPC	TTFL	UTPC	UTFL	TTPC	TTFL	UTPC	UTFL
Model	9	30.20	41.26	46.17	58.18	< 0.0001	< 0.0001	< 0.0001	< 0.0001
X_1	1	1.56	1.10	1.62	0.46	0.2523	0.3293	0.2438	0.5209
X_2	1	9.00	11.56	18.19	16.34	0.0199	0.0114	0.0037	0.0049
X_3	1	42.93	59.33	94.11	121.55	0.0003	0.0001	< 0.0001	< 0.0001
X_1X_2	1	7.840E-003	1.239E-003	0.46	0.65	0.9319	0.9729	0.5198	0.4473
X_1X_3	1	0.063	0.51	0.15	0.35	0.8097	0.4965	0.7076	0.5732
X_2X_3	1	2.52	4.29	0.55	0.62	0.1565	0.0770	0.4830	0.4567
X_1^2	1	0.44	1.94	0.036	1.12	0.5271	0.2068	0.8541	0.3261
X_2^2	1	0.48	0.47	2.76	3.76	0.5108	0.5142	0.1405	0.0937
X_3^2	1	211.26	286.37	299.39	378.67	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Residual	7								
Lack of fit	3	4.37	0.92	0.63	1.19	0.0941	0.5086	0.6322	0.4182
Pure error	4								
Total	16								

Dof: Degree of freedom, **TTPC:** Traditional Total Phenolic Content, **TTFL:** Traditional Total Flavonoid Content, **UTPC:** Ultrasonic Total Phenolic Content, **UTFL:** Ultrasonic Total Flavonoid Content

Table A3. Statistical parameters obtained for TPC and TFL values.

Parameter	TTPC	TTFL	UTPC	UTFL
R^2	0.9749	0.9815	0.9834	0.9868
Adj-R^2	0.9426	0.9577	0.9621	0.9698
Adequate Precision	16.267	18.411	20.359	22.076
PRESS	1957.10	996.03	474.36	647.02
C.V(%)	9.33	9.68	4.80	5.12

TTPC: Traditional Total Phenolic Content, **TTFL:** Traditional Total Flavonoid Content, **UTPC:** Ultrasonic Total Phenolic Content, **UTFL:** Ultrasonic Total Flavonoid Content