Ultrasound-Assisted Optimization of Phenolic, Flavonoid, and Antioxidant Extraction from *Schefflera heptaphylla* Leaves

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This study used Response Surface Methodology, with the support of ultrasound waves, to determine the optimal extraction conditions for obtaining the total phenolic content, total flavonoid content and antioxidant activity (DPPH, ABTS) of *Schefflera heptaphylla* leaves. The experiment was arranged according to the Box-Behnken design, and a model was built to optimize each extraction process with four factors: extraction temperature (X_1), extraction time (X_2), ethanol concentration (X_3) and power level (X_4). The optimal conditions for the technological parameters of the extraction process were determined at an extraction temperature of 62 ºC, an extraction time of 49 minutes, ethanol concentration of 60 %, and power level of 309 W. We obtained a total phenolic content of 55.13 ± 0.20 mgGAE/g, total flavonoid content of 44.41 ± 0.15 mgQE/g, DPPH antioxidant capacity 158.17 ± 0.12 µg/g, and ABTS antioxidant capacity 129.33 ± 0.19 µg/g.

Keywords: *Schefflera heptaphylla*, total phenolic content, total flavonoid content, DPPH, ABTS, response surface methodology

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Schefflera heptaphylla (L.) Frodin, belonging to the *Araliaceae* family, is a medicinal plant predominantly found in tropical and subtropical regions of Asia. This species is a small to medium-sized tree, either semi-deciduous or evergreen, reaching heights of up to 25 meters with a trunk diameter of up to 80 cm. Its leaves are palmately compound, consisting of 6-8 (-11) leaflets, which vary in shape. The petiole measures 8-35 cm, while the leaflets are elliptical to ovate-elliptical, ranging from 7-20 cm in length and 3-6 cm in width, with an attenuate base, narrowly pointed apex, entire margin, and glabrous surface. The petiolules are unequal, measuring 1-5 cm [1]. Traditionally, *Schefflera heptaphylla* has been utilized in medicine to treat inflammation, rheumatism, fever, and traumatic bleeding. Pharmacological studies have demonstrated that *S. heptaphylla* possesses various biological activities, including anti-inflammatory [2], antimicrobial [3], antitumor, and antiviral properties [4].

Phenolic compounds and flavonoids are crucial in the realm of food technology due to their potent antioxidant properties [5]. These compounds, found abundantly in plants, play a significant role in neutralizing free radicals, thereby preventing oxidative stress and cellular damage. Phenolic compounds encompass a wide range of substances, including phenolic acids, flavonoids, and tannins, each contributing to the plant's defence mechanisms and overall health benefits [6].

Flavonoids, a prominent subgroup of phenolic compounds, are particularly noted for their diverse biological activities, such as anti-inflammatory, antimutagenic, and antimicrobial effects. The antioxidant capacity of these compounds is essential for maintaining food quality and extending shelf life by preventing the oxidation of lipids and other sensitive components [7].

To evaluate the antioxidant capacity of phenolic compounds and flavonoids, two widely used methods are the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical method and the 2,2′-azino-bis(3-ethylbenzthiozoline-6)-sulphonic acid (ABTS) method. The DPPH method measures the ability of antioxidants to scavenge free radicals by observing the reduction in DPPH radical concentration, which results in a colour change. This method is favoured for its simplicity and cost-effectiveness. The ABTS method involves generating ABTS radicals and measuring the antioxidant's ability to quench these radicals, leading to a decrease in colour intensity. Both methods are convenient and do not require expensive equipment, making them accessible for a wide range of applications in food technology. These methods are essential tools for ensuring the safety, quality, and nutritional value of food products [8].

Response Surface Methodology (RSM) is a widely utilized statistical technique in experimental design and optimization. It offers a systematic and

efficient approach to investigating the relationships between the input variables (factors) and the desired response variables [9]. Through its application, researchers can optimize experimental procedures, examine interactions between factors, and predict optimal conditions for the extraction, isolation, purification, and analysis of bioactive compounds. By leveraging RSM, researchers can improve the efficiency, yield, and quality of natural products, thereby advancing the field of natural product research [10].

MATERIAL AND METHODS

Material and Equipment

Material

Specimens of *Schefflera heptaphylla* were collected from Anh Son District, Nghe An Province, Vietnam, in July 2022 (18.9311°N, 105.0853°E). Taxonomic identification was conducted by the Institute of Ecology and Biological Resources under the Vietnam Academy of Science and Technology (VNLL202207). The leaf samples were processed within 24 hours post-collection. Prior to analysis, the material was uniformly cut into fragments approximately 2 mm in size, dried using a heat pump dryer, and stored in sealed packaging.

Chemicals

The chemicals used in this study included gallic acid, Folin-Ciocalteu reagent, sodium carbonate (Na_2CO_3), quercetin, aluminium chloride ($AlCl_3$), sodium nitrite ($NaNO_2$), sodium hydroxide (NaOH), Trolox, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), potassium persulfate, and quercetin reagent.

Plant Extraction (Equipment)

For each experiment, 2g of *Schefflera heptaphylla* was weighed into a 500 ml triangular flask, to which water was added at a predetermined ratio according to the optimal experimental layout method so that the solid/liquid ratio was 1/30. The flask was placed in the CYF-TES600N-4S ultrasonic device (Taiwan), and the ultrasonic power was adjusted from 200-400 W according to the experimental conditions. Extraction was performed according to a predetermined time of 30-60 minutes. The extracts were centrifuged at 10,000 x g for 15 min and the supernatants were filtered through a Whatman filter paper. The residues were extracted twice. The samples were stored at 0-4 ^oC until further use.

Methods

Total Polyphenol Content (TPC)

Total polyphenol content was determined according to the method by Quang Vinh Nguyen [11]. A gallic acid standard curve was constructed by taking 1 ml of gallic acid solution (prepared in methanol with concentrations of 0.0078, 0.0156, 0.03125, 0.0625 and 0.125 mg/ml) which was mixed with 5 ml of Folin - Ciocalteu reagent (diluted 10 times) and 4 ml of Na_2CO_3 (75 g/l) by shaking well. The absorbance of the solution was determined after 30 minutes at 765 nm using a UV-VIS spectrophotometer (V-630, Jasco, Japan). A standard curve and the corresponding correlation equation between gallic acid concentration and absorbance were established. Then, the absorbance of the extract was determined in the same order as gallic acid, replaced by 1 ml of the extract. The total polyphenol content of the extract was determined by the gallic acid equivalent (GAE) value based on the standard curve. The total polyphenol content was calculated using the formula:

$$C = c^* V/m$$

where C is the total polyphenol content (mgGAE/g), c is the absorbance value corresponding to the gallic acid standard curve (mg/ml), V is the sample volume (ml), and m is the mass of the sample solution (g).

Total Flavonoid Content (TFC)

Total flavonoid content was determined according to Vinh Nguyen [11]. A quercetin standard curve was constructed by taking 0.5 ml of quercetin solution in methanol (concentrations of 0.02, 0.04, 0.06, 0.08 and 0.1 mg/ml) which was mixed with 2.5 ml of distilled water and 0.15 ml of 5% $NaNO_2$ by shaking well. After 5 minutes, 0.3 ml of 10% $AlCl_3$ was added. After 6 minutes, 1 ml of 1 M NaOH and 0.55 ml of distilled water were added and the solution was measured at 510 nm using a UV-VIS spectrophotometer (V-630, Jasco, Japan). The standard curve and the correlation equation between quercetin concentration and absorbance were constructed. Then, the absorbance of the extract was determined as above, with 0.5 ml of the extract solution. The total flavonoid content of the extract was expressed as quercetin equivalent (quercetin equivalent – QE) based on the standard curve and determined by the formula:

$$F = f^* V/m$$

where F is the total flavonoid content (mgQE/g), f is the absorbance value corresponding to the quercetin standard curve (mg/ml), V is the sample volume (ml), and m is the sample mass (g).

DPPH Free Radical Scavenging Ability

The DPPH free radical scavenging ability was evaluated according to Tang Van Nguyen et al. [12]. A working DPPH solution was prepared by diluting the stock DPPH solution with methanol (0.24 g/l) to measure the absorbance at 515 nm. 0.1 ml of the extract was mixed with 3.9 ml of the DPPH working solution in a test tube. The mixture was incubated in the dark at ambient temperature for 30 minutes. Subsequently, its absorbance was recorded at 515 nm using a spectrophotometer (V-630, Jasco, Japan). A control sample, prepared with an equivalent volume of methanol and DPPH solution, was also analysed under the same conditions. Trolox was used as a positive control.

The DPPH free radical scavenging activity was determined using the formula:

$$\% \text{ DPPH} = [(A_1 - A_0)/A_0] \times 100\%$$

where A_1 and A_0 are the colour absorbance of the control sample (sample containing only solvent), and the colour absorbance of the reaction solution containing the extract after 30 minutes of reaction, respectively.

The ABTS Free Radical Scavenging Ability

The assessment of ABTS radical scavenging activity was carried out following the method described by Tang Van Nguyen et al. (2015) [13], with a few modifications. A working solution was prepared by combining 7.4 mM ABTS with 2.6 mM potassium persulfate and allowing the mixture to react in the dark for 12 hours to generate a stable ABTS•$^+$ solution. The resulting solution was adjusted to an absorbance of 1.1 ± 0.02 at 734 nm. For the assay, 1.8 ml of the ABTS working solution was added to 0.2 ml of the sample extract in a test tube. The mixture was shaken well and allowed to react for 2 hours in the dark, following which its absorbance was measured at 734 nm. Trolox was used as a positive control. The ABTS free radical inhibition ability was calculated as mg trolox equivalent (mg TE/g dry matter). Antioxidant activity was calculated using the formula:

$$\% \text{ Antioxidant} = [(A_B - A_A)/A_B] \times 100\%$$

where A_B and A_A are the colour absorbance of the control sample (sample containing only solvent), and the colour absorbance of the reaction solution containing the extract after 2 hours of reaction, respectively.

RESULTS AND DISCUSSION

Fitting the Response Surface Models

The Box-Behnken Design (BBD) of the Response Surface Methodology (RSM) was used to optimize the responses (TPCL, TFCL, DPPH and ABTS) of the extraction process of *S. Heptaphylla* leaves. Four independent variables were used: extraction temperature, extraction time, ethanol concentration, and power level. Preliminary trials were carried out to define the appropriate input ranges for the selected variables, as presented in Table 1.

Table 1. BBD design employed coded levels for the independent variables.

Independent variables	Units	Coded symbols	Coded variable levels		
			-1	0	1
Extraction temperature	^{o}C	X_1	50	60	70
Extraction time	min	X_2	30	45	60
Ethanol concentration	%	X_3	50	60	70
Power level	w	X_4	200	300	400

Table 2. Data obtained for the four responses based on the BBD matrix.

Run	Coded and processed variable level				Response			
	X_1	X_2	X_3	X_4	Y_1	Y_2	Y_3	Y_4
	Extraction temperature	Extraction time	Ethanol concentration	Power level	TPC (mgGAE/g)	TFC (mgQE/g)	DPPH (µg/g)	ABTS (µg/g)
1	60.00	30	60	200	43.37	39.89	152.76	99.42
2	70.00	60	60	300	50.55	41.79	157.89	113.26
3	60.00	30	60	400	52.75	29.71	158.23	117.29
4	50.00	45	70	300	43.16	38.66	151.44	103.82
5	60.00	30	70	300	43.32	34.95	153.88	107.09
6	60.00	45	50	200	54.39	33.71	156.38	120.06
7	70.00	45	50	300	54.99	35.24	156.14	122.07
8	60.00	60	50	300	56.84	31.02	157.84	126.09
9	60.00	30	50	300	47.31	32.84	152.42	106.97
10	70.00	45	60	200	42.82	44.98	154.80	103.57
11	50.00	45	50	300	46.18	27.46	155.14	104.33
12	60.00	60	60	400	55.04	39.24	158.37	120.31
13	60.00	45	70	200	42.92	47.38	150.88	103.19
14	60.00	60	70	300	46.51	44.99	152.73	108.35
15	50.00	45	60	400	45.37	33.42	155.75	106.97
16	70.00	30	60	300	47.63	39.97	156.19	105.08
17	50.00	45	60	200	45.75	37.13	155.39	107.35
18	60.00	45	60	300	55.89	45.93	160.00	130.54
19	50.00	60	60	300	48.41	38.44	156.14	109.36
20	60.00	45	50	400	56.26	30.15	156.45	117.67
21	50.00	30	60	300	39.96	31.97	153.32	90.61
22	60.00	45	60	300	55.14	45.53	159.86	129.19
23	70.00	45	60	400	56.59	35.75	160.68	122.32
24	70.00	45	70	300	44.75	40.26	155.06	101.31
25	60.00	45	70	400	51.59	32.55	157.43	123.83
26	60.00	45	60	300	56.59	46.83	161.23	132.31
27	60.00	60	60	200	51.79	42.22	156.79	119.56

Table 3. Second-order polynomial models for TPC, TFC, DPPH, and ABTS

Response	Model equations
Y_1 – TPC	$Y_1 = 55.88 + 2.38X_1 + 2.9X_2 – 3.64X_3 + 3.05X_4 – 1.38\ X_1X_2 - 1.8X_1X_3 + 3.54\ X_1X_4 – 1.68\ X_2X_3 – 1.53X_2X_4 + 1.7X_3X_4 – 5.84X_1^2 – 3.68X_2^2 - 3.09X_3^2 – 1.79X_4^2$
Y_2 – TFC	$Y_2 = 46.1 + 2.58X_1 + 2.36X_2 + 4.03X_3 – 3.71X_4 – 1.16\ X_1X_2 - 1.55X_1X_3 – 1.38\ X_1X_4 + 2.96\ X_2X_3 + 1.8X_2X_4 – 2.82X_3X_4 – 4.24\ X_1^2 – 3.99X_2^2 - 6.22X_3^2 – 4.1X_4^2$
Y_3 – DPPH	$Y_3 = 160.36 + 1.13X_1 + 1.08X_2 – 1.08X_3 + 1.64X_4 – 0.28\ X_1X_2 + 0.65X_1X_3 + 1.38\ X_1X_4 – 1.64\ X_2X_3 – 0.97X_2X_4 + 1.62X_3X_4 – 2.19X_1^2 – 2.36X_2^2 - 3.71X_3^2 – 1.45X_4^2$
Y_4 - ABTS	$Y_4 = 130.68 + 3.76X_1 + 5.87X_2 – 4.13X_3 + 4.6X_4 – 2.64X_1X_2 – 5.07X_1X_3 + 4.78\ X_1X_4 – 4.47\ X_2X_3 – 4.28X_2X_4 + 5.76X_3X_4 – 14.91X_1^2 – 10.74X_2^2 - 8.07X_3^2 – 5.98X_4^2$

In the current study, desirability functions were created for maximizing TPC, TFC, DPPH and ABTS criteria. Table 2 presents the experimental design and the corresponding four response variables.

Table 2 displays the extraction conditions and the values of the four provided ratings. The maximum values of TPC, TFC, DPPH and ABTS were 56.91 mgGAE/g, 46.29 mgQE/g, 160.77 µg/g, and 131.97 µg/g, respectively. Using multiple linear regression analysis on a dataset of 27 experimental runs, quadratic polynomial models were developed to describe the behaviour of the TPC, TFC, DPPH and ABTS responses during the extraction process, as summarized in Table 3.

The determination coefficients (R^2) for the developed models were 0.9883 (Y_1), 0.9897 (Y_2), 0.9923 (Y_3) and 0.9890 (Y_4), indicating that more than 98.8% of the variability in the experimental

data was successfully explained by the models. These high R^2 values confirmed the strong predictive capability and reliability of the polynomial models within the defined experimental range. Furthermore, the Lack of Fit F-values for Y_1, Y_2, Y_3, and Y_4 were 1.74, 2.29, 0.23, and 1.47, respectively. These relatively low values suggest that the lack of fit was not statistically significant when compared to the pure error, thereby supporting the adequacy and accuracy of the fitted models.

Response Surface Analysis of TPC, TFC, DPPH and ABTS

To visualize the interaction effects between variables, contour plots and three-dimensional response surface graphs were constructed for each fitted model. These visualizations were generated by simultaneously varying two independent factors while maintaining the remaining variables at their central (coded zero) levels. Figures 2 to 4 illustrate the resulting surfaces, which provide valuable insights into how combinations of variables influenced the response outcomes. Through the analysis of these plots, the individual and interactive effects of the variables were interpreted, offering a clearer understanding of the underlying relationships and guiding the optimization process.

Response Surface Analysis of Total Phenolic Content (TPC)

Figure 1 presents six response surface plots illustrating the influence of each pairwise combination of independent variables on total phenolic content (TPC). These visualizations provide a detailed representation of the interaction effects and support the interpretation of the response surface analysis for TPC.

Table 4: Analysis of variance (ANOVA) for evaluation of the model.

Source	Y_1 – TPC		Y_2 – TFC		Y_3 – DPPH		Y_4 – ABTS	
	F- value	p-value	F- value	p-value	F- value	p-value	F- value	p-value
Model	72.47	< 0.0001 S	82.20	< 0.0001 S	110.39	< 0.0001 S	77.03	< 0.0001 S
X_1 (Extraction temperature)	91.92	< 0.0001 S	105.86	< 0.0001 S	121.43	< 0.0001 S	62.01	< 0.0001 S
X_2 (Extraction time)	136.90	< 0.0001 S	89.14	< 0.0001 S	110.44	< 0.0001 S	150.88	< 0.0001 S
X_3 (Ethanol concentration)	216.17	< 0.0001 S	259.19	< 0.0001 S	110.73	< 0.0001 S	74.69	< 0.0001 S
X_4 (Power Level)	150.99	< 0.0001 S	219.52	< 0.0001 S	261.26	< 0.0001 S	92.72	< 0.0001 S
X_1X_2	10.33	0.0074 S	7.20	0.0199 S	2.49	0.1409 S	10.18	0.0078 S
X_1X_3	17.66	0.0012 S	12.70	0.0039 S	13.53	0.0032 S	37.41	< 0.0001 S
X_1X_4	67.90	< 0.0001 S	10.16	0.0078 S	60.27	< 0.0001 S	33.35	< 0.0001 S
X_2X_3	13.61	0.0031 S	46.72	< 0.0001 S	85.46	< 0.0001 S	29.10	0.0002 S
X_2X_4	12.70	0.0039 S	17.23	0.0013 S	29.92	0.0001 S	26.70	0.0002 S
X_3X_4	15.68	0.0019 S	42.24	< 0.0001 S	83.27	< 0.0001 S	48.34	< 0.0001 S
X_1^2	247.12	< 0.0001 S	127.36	< 0.0001 S	202.65	< 0.0001 S	432.35	< 0.0001 S
X_2^2	97.91	< 0.0001 S	113.04	< 0.0001 S	235.70	< 0.0001 S	224.41	< 0.0001 S
X_3^2	68.92	< 0.0001 S	274.34	< 0.0001 S	581.30	< 0.0001 S	126.59	< 0.0001 S
X_4^2	23.06	0.0004 S	119.30	< 0.0001 S	88.26	< 0.0001 S	69.46	< 0.0001 S
Lack of Fit	1.48	0.4689 NS	1.84	0.4037 NS	0.0672	0.9990 NS	1.14	0.5530 NS
R^2	0.9883		0.9897		0.9923		0.9890	
C.V%	1.74		2.29		0.2279		1.47	

S: significant; NS: nonsignificant

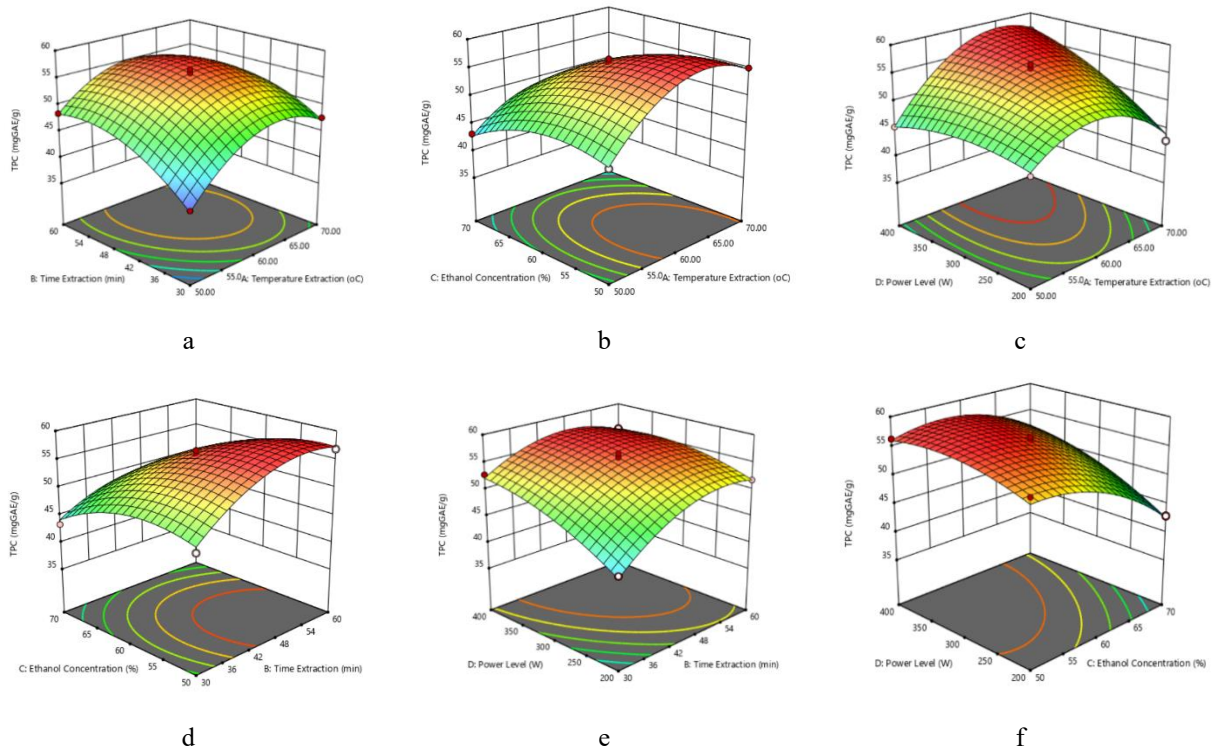

Figure 1. Response surface plots for TPC.

The response surface plots for TPC are shown in Figure 1 and Table 4. All four factors (extraction temperature, extraction time, ethanol concentration, and power level) showed a negative quadratic effect ($p < 0.0001$). The effect of ultrasonic power and temperature on TPC was clear, with the highest TPC observed at 300-400 W and temperatures around 50-60 °C [14]. The TPC value increased with increasing extraction temperature and ultrasonic power, as shown in Figure 1c. Microwave radiation causes the breaking of hydrogen bonds and the movement of soluble ions leading to the breakdown of plant tissue and the release of compounds from the raw material into the solvent. At low microwave power (100 W) the molecular diffusion rate was low, reducing the extraction efficiency. Increasing the microwave power to 300 W significantly increased the extraction efficiency. With increasing temperature and pressure, the interaction between molecules in the solvent increases, molecular movement increases, and solubility increases. Increasing temperature causes cell membrane rupture, which increases the extraction efficiency. Furthermore, at high temperature, the viscosity of the solvent decreases and the diffusion rate increases, thus increasing the extraction efficiency [15].

Response Surface Analysis of Total Flavonoid Content (TFC)

Figure 2 illustrates the response surface analysis for total flavonoid content (TFC), following the same visualization format as used for TPC.

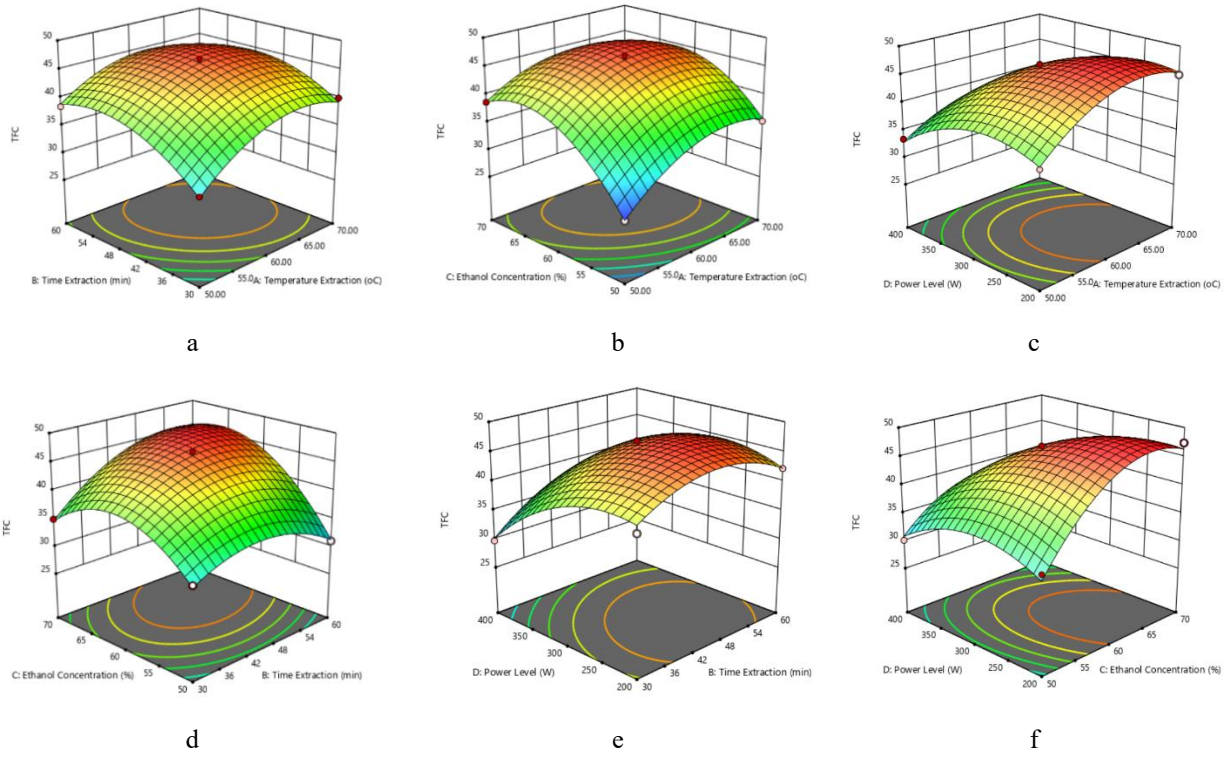

Figure 2. response surface plots for TFC.

As shown in Figure 2 and Table 4, all four factors (extraction temperature, extraction time, ethanol concentration, and power level) had a quadratic effect on TFC ($p < 0.0001$). Ultrasonic power and extraction time affected the TFC value obtained. The highest TFC value was obtained at 300 W. When the ultrasonic power was increased to a high level (400W), the TFC decreased. This can be explained by the fact that when the power increases, the diffusion rate increases rapidly, while the temperature increases, causing decomposition and thermal degradation. Each raw material will have a different extraction temperature limit. If the limit is exceeded, thermally unstable compounds disappear while some unwanted compounds are generated, reducing the content of the analytes [16]. At the same time, the TFC content was high when the extraction time was 45-50 minutes, but when the extraction time increased, the TFC content tended to decrease. This can be explained by the fact that when the extraction time exceeds a certain limit, some flavonoids are decomposed. This rule is consistent with the results of Y. Qun et al. [17] in the study of microwave-assisted extraction of polyphenols and flavonoids from *Clinacanthus nutans*.

Response Surface Analysis of DPPH

Figure 3 presents the response surface analysis for DPPH, comprising six three-dimensional plots that illustrate the interactive effects of each pair of independent variables on the DPPH radical scavenging activity. All four factors (extraction temperature, extraction time, ethanol concentration, and power level) had a quadratic effect on DPPH ($p < 0.0001$). As illustrated in Figure 3, the polyphenol content and antioxidant activity (DPPH assay) peaked at 45 minutes. If the ultrasonic extraction time is short, about 30-45 minutes, the temperature of the ultrasound waves is not enough to affect the solvent and raw materials, and only partially affects the cell membrane strength. As the extraction time increases, the temperature also increases, causing the cell membrane to break, leading to a higher content of substances in the extract [18]. In general we found that the extraction process took place for a certain period of time, the difference in concentration of the constituents to be extracted between the raw materials and the solvent decreased, and prolonging the extraction time did not increase the content of the extract.

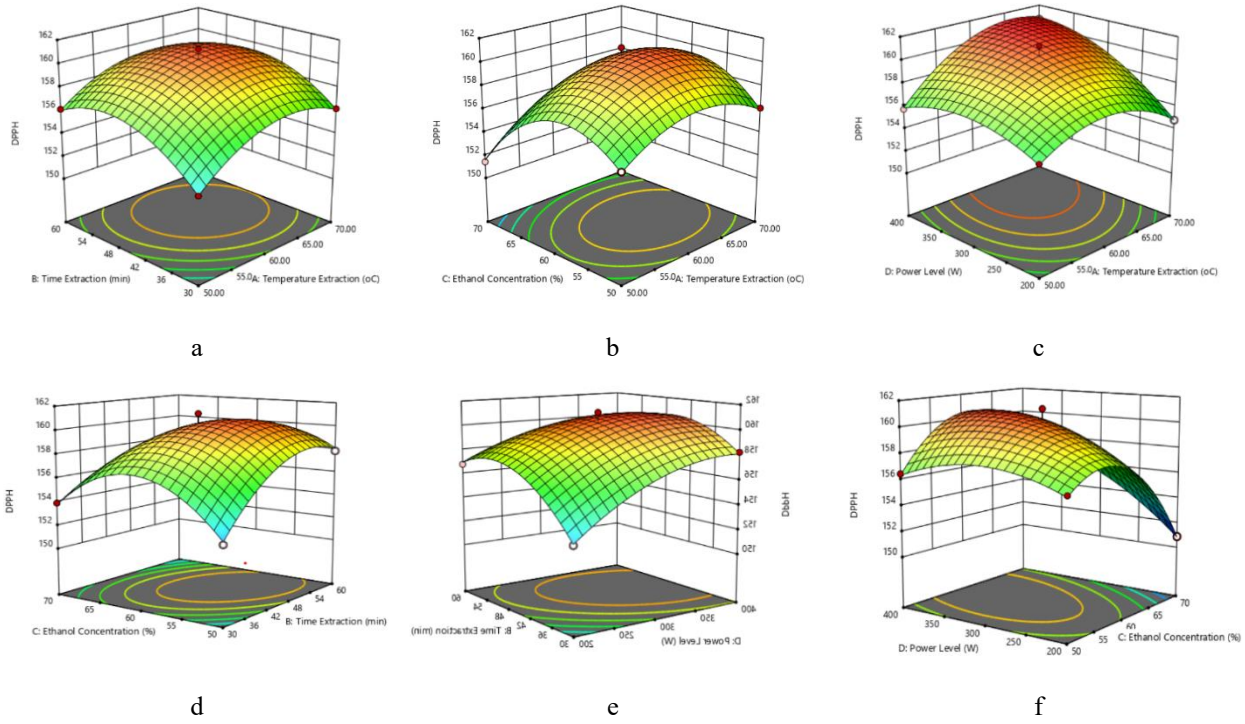

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Figure 3. Response surface plots for DPPH.

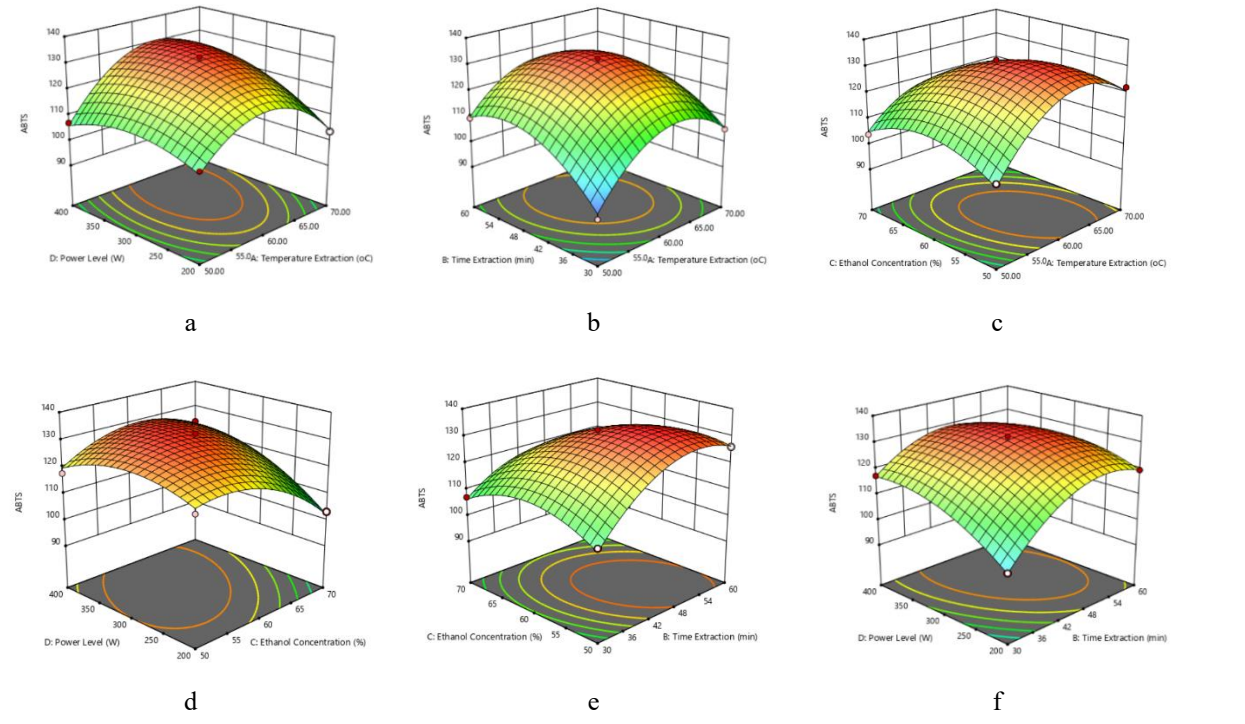

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Figure 4. Response surface plots for ABTS.

Response Surface Analysis of ABTS

Figure 4 shows the response surface analysis for ABTS, where six graphs show the effects of each pair of independent variables on ABTS. All four factors (extraction temperature, extraction time, ethanol concentration and power level) had a quadratic effect on ABTS ($p < 0.0001$). Figure 4 shows the effects of time and temperature on antioxidant activity. The antioxidant activity reached its peak at 45 minutes and 60 ºC, then decreased slightly. In the process of extracting natural compounds, too long an extraction time can lead to decomposition, reducing biological activity [19]. High temperatures can soften plant tissue, increase the rate of penetration and diffusion of solvent into the raw material, and help the extraction process take place faster [20]. However, if the extraction process takes place at too high a temperature, it will lead to denaturation, reducing the activity of natural compounds [21].

Optimization and Model Verification

The optimal conditions for extracting *S. Heptaphylla* leaves using ultrasonic methods were identified to achieve the highest values of TPC, TFC, DPPH, and ABTS. Table 5 summarizes the outcomes for the general scenario in which equal weight coefficients were applied in the final objective function.

Using the desirability function method, the simultaneous optimization process identified the optimal extraction conditions to achieve the highest levels of TPC, TFC, DPPH, and ABTS antioxidant activities. These conditions included a temperature of 62 °C, an extraction time of 49 minutes, an ethanol concentration of 60 %, and a power of 309 W. The experimental results showed that the TPC, TFC, DPPH, and ABTS antioxidant activity values during the ultrasonic extraction of *S. Heptaphylla* leaves were 55.13 ± 0.20 mgGAE/g, 44.41 ± 0.15 mgQE/g, 158.17 ± 0.12 µg/g, and 129.33 ± 0.19 µg/g, respectively. These experimental values closely matched the predicted values from the corresponding regression models (TPC = 56.91 mgGAE/g; TFC = 46.29 mgQE/g; DPPH = 160.77 µg/g; ABTS = 131.97 µg/g), with a coefficient of variation (CV) ranging from 1.6 % to 3.1 %.

CONCLUSION

Using the Box-Behnken design (BBD) response surface methodology (RSM) combined with conventional graphical methods and desirability functions, the study successfully identified the optimal region within the experimental range. The predicted values were calculated using second-order polynomial models. To achieve the maximum values for total phenolic content (TPC), total flavonoid content (TFC), DPPH antioxidant capacity, and ABTS antioxidant capacity during the extraction process of *S. Heptaphylla* leaves, the optimal conditions were determined to be an extraction temperature of 62 °C, an extraction time of 49 minutes, an ethanol concentration of 60 %, and a power level of 309 W. Under these conditions, the TPC, TFC, DPPH, and ABTS values obtained were 56.91 ± 0.20 mgGAE/g, 46.29 ± 0.15 mgQE/g, 160.77 ± 0.12 µg/g, and 131.97 ± 0.19 µg/g, respectively. Future studies should explore the bioavailability and stability of the extracted compounds under various storage and formulation conditions to enhance their practical applications in food and pharmaceutical products.

Table 5. Comparison between predicted values and experimental values for the ultrasonic extraction process of *S. Heptaphylla* leaves.

Independent variables				Dependent variables (Response)	Optimum value		% Difference (CV)
X_1 (°C)	X_2 (min)	X_3 (%)	X_4 (W)		Experimental	Predicted	
62	49	60	309	Y_1	55.13±0.20	56.91	3.1
				Y_2	44.41±0.15	46.29	1.9
				Y_3	158.17±0.12	160.77	1.6
				Y_4	129.33±0.19	131.97	2.0

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