JTE HCMUTE
ISSN: 2615-9740
JOURNAL OF TECHNICAL EDUCATION SCIENCE
Ho Chi Minh City University of Technology and Education
Website: https://jte.edu.vn
Email: jte@hcmute.edu.vn

Aqueous Ultrasound-Assisted Extraction of Phenolics and Saponins from Xao Tam Phan Plant Parts: Optimization and Comparison of Extraction Efficiency

Thao Thi Nguyen Nguyen[1,3], Tan Thanh Trinh[1], Nghia Huu Le[1], Tuyen Chan Kha[1], Hien Phuoc Phan[2], Hong Minh Xuan Nguyen[1*]

[1]*Nong Lam University, Ho Chi Minh City, Vietnam.*
[2]*Van Lang University, Ho Chi Minh City, Vietnam.*
[3]*Phu Yen University, Phu Yen Province, Vietnam.*

[*]Corresponding author. *Email: nmxhong@hcmuaf.edu.vn*

ARTICLE INFO

Received:	30/04/2024
Revised:	15/06/2024
Accepted:	24/06/2024
Published:	28/06/2024

KEYWORDS

Paramignya trimera;
Total phenolics;
Total saponins;
Ultrasound;
Xao tam phan.

ABSTRACT

Xao tam phan (XTP), a medicinal plant renowned for its health benefits, is rich in antioxidants such as saponins, phenolics, flavonoids, and proanthocyanidins. Although existing studies confirm that the presence of these beneficial phytochemicals in XTP, further research is essential to optimize their extraction yield. Ultrasound-assisted extraction (UAE), recognized for its sustainability and efficiency, presents a promising solution for maximizing these yields. This research uses UAE to optimize the extraction of beneficial phytochemicals from XTP roots, stems, and leaves. The study finds that the optimal extraction conditions vary depending on the specific plant part. For roots and stems, the highest yield was achieved using 220W ultrasonic power at 52°C for 8 min, resulting in a total phenolic content (TPC) of 7.56 and 7.83 mg GAE/g d.b., and total saponin content (TSC) of 116.24 and 117.84 mg EE/g d.b., respectively. In contrast, for leaves, using 200W ultrasonic power at 47°C for 8 min yielded higher TPC (11.60 mg GAE/g d.b.) and TSC (207.43 mg EE/g d.b.). Leaf extracts exhibited approximately double the TPC and TSC compared to other plant parts. These findings highlight the potential of XTP leaf extracts as a valuable resource for the pharmaceutical, cosmetic, and food industries.

Doi: https://doi.org/10.54644/jte.2024.1581

1. Introduction

Xao tam phan (*Paramignya trimera* (Oliv.) Guillaum) is a medicinal plant renowned for its ability to cool the liver, reduce heat, and replenish health. It's especially recognized for its potential to prevent various types of cancer, including HepG2 liver cancer, HTC116 colorectal cancer, MDA MB231 breast cancer, OVCAR-8 ovarian cancer, and cervical cancer (Nguyen et al., 2017a). Additionally, it exhibits anti-inflammatory properties [1] and α-glucosidase inhibitory activities [2]−[4]. Due to its unique antioxidant components such as saponin, phenolic, flavonoid, proanthocyanidin, and many other compounds, Xao tam phan (XTP) is often referred to as a "miracle" plant. While these studies indicate that XTP contains biologically active phytochemicals; further investigation into extraction conditions is necessary to enhance the yield of these compounds.

Extraction of natural products has been a practice since the discovery of fire. Ancient civilizations like the Mayas, Aztecs, Egyptians, Phoenicians, Jews, Arabs, Indians, Chinese, Greeks, and Romans used extracted products in perfume, cosmetics, medicine, and food. Traditional extraction methods such as infusion, decoction, digestion, maceration, and percolation have long been employed to extract bioactive compounds. However, these conventional solid-liquid extraction techniques are time-consuming and necessitate large amounts of toxic organic solvents [5]. This prompted the development of environmentally friendly (green) extraction technologies over the past two decades. Green extraction processes aim to minimize organic solvent consumption, energy usage, costs, and extraction time [6]. Ultrasound-assisted extraction (UAE) emerges as a critical technology in achieving the sustainability objectives of green extraction. The utilization of UAE in various fields including chemistry, biology, and technology has been well-documented [7]─[9].

Phytochemical and bioassay studies have confirmed the potential of XTP, leading to the exploration of various extraction techniques. However, chemical solvent methods are not appropriate for food production [10]─[12]. As a result, we adopted aqueous ultrasound-assisted extraction (UAE), a method that is eco-friendly, efficient, simple, and fast. This study focuses on optimizing parameters for the UAE with a water solvent. Utilizing response surface methodology (RSM) to maximize the extraction yield of phytochemicals from the plant's roots, stems, and leaves, particularly the total phenolic content (TPC) and total saponin content (TSC). The efficacy of UAE on different parts of XTP was evaluated by comparing the TPC and TSC in the extracts.

2. Materials and Methods

2.1. Materials

The Xao tam phan trees, aged four years, were freshly harvested from Phu Yen province, Vietnam. Upon harvesting, the plants were categorized into roots, stems, and leaves. Following a thorough washing process, the plants were allowed to drain and subsequently dried at a temperature of 40 ± 2°C, until reaching a final moisture content of approximately 10%. These dried samples were then finely ground into a powder, sieved through a 0.3 mm mesh, packed into nylon bags, and stored at -10°C for subsequent analysis. Folin-Ciocalteu reagent, gallic acid (GA), vanillin, and aescin were procured from Sigma-Aldrich Co. (USA).

2.2. Experimental methods

For each assay, 1g samples of XTP powder were utilized. These samples were placed in 250mL beakers and mixed with water at various ratios. The treatment of samples was conducted using a probe-type ultrasonic device (BSP-1200 bench-scale, USA). The sonication temperature was controlled by immersing the beakers with the samples in a manually regulated thermostatic water tank. Following sonication, the resulting mixture underwent centrifugation at 5000 rpm for 15 mins using a centrifuge (Sartorius, Switzerland). Subsequently, the supernatant was used to quantify the TPC and TSC in XTP extracts.

2.2.1. Optimization of UAE conditions for maximizing the TPC and TSC in XTP extracts

The experiments were designed using JMP version 13 Pro statistical software and Central Composite Design (CCD) to study the combined effect of independent variables on desired responses. The objective was to determine the optimal ultrasonic power (X_1, W), temperature (X_2, °C), and duration (X_3, mins) for maximizing TPC and TSC in root, stem, and leaf extracts.

Preliminary experiments, as outlined by Nguyen et al.(2023), were conducted to establish the minimum and maximum values of the dependent variables [13]. Based on these findings, the dependent variables were adjusted to define the range for the optimization study: ultrasonic power ranging from 190 to 220W, temperature ranging from 40 to 60 °C, and duration ranging from 4 to 8 mins.

Table 1 displays the levels of independent variables along with the results of 17 experiments run to determine the saponin and phenolic contents in each part of the plant extracts. Additionally, a control experiment without ultrasonication was also conducted.

Regression analysis was carried out to create quadratic models (Equation 1) [7]. The optimal value generated was then validated by comparing the predicted and experimental values.

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

In equation 1, Y_n represents the response variables, β_0 denotes the intercept, β_i signifies the linear regression coefficient for the i^{th} factor, β_{ij} and β_{ii} represent the cross-product and quadratic terms, respectively. X_i and X_j denote the input variables, and k represents the number of input variables (k = 3). The regression coefficients of individual linear, interaction, and quadratic terms are determined based on the analysis of variance (ANOVA). The magnitude of the standardized model coefficients indicates their importance in the model, with larger values among standardized coefficients being more influential. Aside from the quadratic model, the interactive effects of the factors can be illustrated in 3-D surface plots, aiding in visualizing the relationships between the variables and the response.

2.2.2. Comparison of UAE efficacy on XTP root, stem, and leaf extracts

The efficacy of recovering bioactive compounds in UAE was compared across the root, stem, and leaf extracts of XTP. To facilitate this comparison, the optimal UAE conditions for each part of the XTP plant were used to evaluate the efficacy of TPC and TSC in the extracts.

2.3. Analytical methods

TPC and TSC were measured using the spectrophotometric method, following the procedure described by Nguyen et al.(2023) [13]. The results were expressed as mg gallic acid equivalent (GAE)/g dried basis (d.b.) for TPC and mg of aescin equivalents (AE)/g d.b. for TSC.

2.4. Statistical analysis

All experiments and analytical measurements in the research were performed in triplicate. The data were statistically processed using JMP software version 13 Pro. The experimental data were presented as means and standard deviations (Mean ± SD). The differences between the mean values were evaluated using analysis of variance (ANOVA) and the least significant difference (LSD) at a 5% significant level ($p < 0.05$). The graphs of mean values and error bars were created using Excel version 2016.

3. Results and Discussion

3.1. Optimization of sonication parameters for maximizing the extraction of total phenolic compounds and saponins in XTP

The RSM of TPC and TSC from the XTP root, stem, and leaf extracts, obtained using various UAE parameters are shown in Table 2. The determination coefficient (R^2), F-values, and *p*-value from these models suggest a high model-fitting rate, indicating that 95% of the predicted values correlate with the experimental data. The results imply that the RSM of TPC and TSC of the XTP extracts is adequate and suitable for describing the effects and interactions of independent variables on the models, optimizing the UAE parameters.

3.1.1. Optimizing sonication parameters in XTP root extracts

The optimization of ultrasound parameters in XTP root extracts was conducted using the CCD-RSM. Three significant factors, namely power (X_1), temperature (X_2), and extraction time (X_3) were optimized. It was found that the highest TPC level (7.79 mg GAE/g d.b.) was achieved under conditions (210W, 6 min, and 50^{o}C), while the highest TSC level (105.61 mg AE/g d.b.) was obtained under similar conditions (Table 1).

Table 1. *The effects of UAE conditions on the TPC and TSC in each part of XTP plant extracts*

Parts of the plant	Treatment No.	Power (W)	Temperature (^{o}C)	Duration (mins)	TPC (mg GAE/g d.b.)	TSC (mg AE/g d.b.)
	1	190	45	6	7.08	75.64
	2	200	40	4	7.16	71.42
	3	200	40	8	7.37	84.86
	4	200	50	4	6.97	70.49
Roots	5	200	50	8	7.45	87.17
	6	210	37	6	7.79	89.33
	7	210	45	3	7.26	60.67
	8	210	45	6	7.61	88.55

	9	210	45	6	7.66	88.51
	10	210	45	6	7.60	88.40
	11	210	45	9	7.65	93.27
	12	210	53	6	7.52	105.61
	13	220	40	4	7.49	76.47
	14	220	40	8	7.47	84.20
	15	220	50	4	7.27	74.30
	16	220	50	8	7.46	101.39
	17	226,8	45	6	7.05	78.31
Stems	1	190	55	6	6.86	98.78
	2	200	45	4	6.65	91.33
	3	200	45	8	7.84	87.98
	4	200	65	4	7.84	77.26
	5	200	65	8	7.62	87.35
	6	210	38	6	7.26	85.57
	7	210	55	3	7.00	79.73
	8	210	55	6	7.94	85.13
	9	210	55	6	7.98	86.66
	10	210	55	6	8.00	85.28
	11	210	55	9	7.71	102.63
	12	210	72	6	7.97	72.91
	13	220	45	4	6.65	97.32
	14	220	45	8	7.61	108.00
	15	220	65	4	7.87	91.93
	16	220	65	8	7.72	97.19
	17	230	55	6	6.95	119.26
Leaves	1	190	50	6	10.99	235.00
	2	200	40	4	11.52	125.77
	3	200	40	8	11.74	173.97
	4	200	60	4	11.82	203.68
	5	200	60	8	10.90	209.45
	6	210	33	6	10.74	122.8
	7	210	50	3	11.43	141.79
	8	210	50	6	11.15	180.23
	9	210	50	6	11.14	181.29
	10	210	50	6	11.17	183.25
	11	210	50	9	11.88	194.25
	12	210	67	6	10.34	178.72
	13	220	40	4	9.73	124.76

JOURNAL OF TECHNICAL EDUCATION SCIENCE
Ho Chi Minh City University of Technology and Education
Website: https://jte.edu.vn
Email: jte@hcmute.edu.vn

14	220	40	8	11.08	148.95
15	220	60	4	10.85	191.63
16	220	60	8	10.03	178.88
17	230	50	6	10.11	168.16

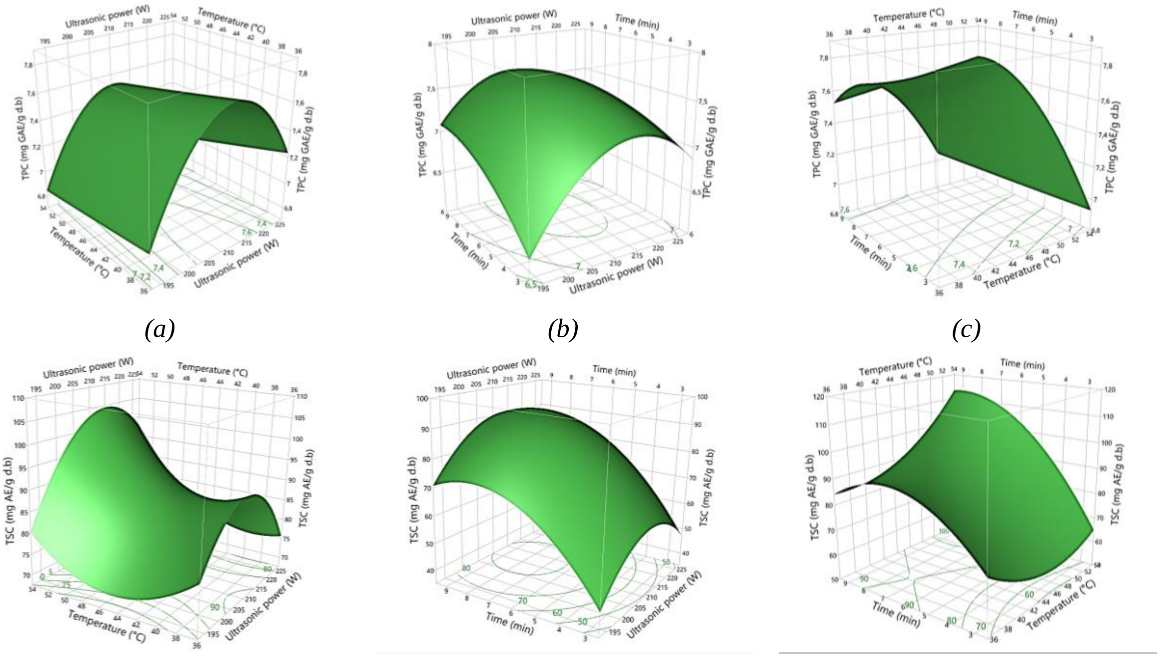
The influence of each variable (X_1, X_2, X_3) as well as the interaction between variables is determined based on Table 2. A second-order polynomial equation was derived from experimental data via multiple regression analysis, describing the efficiency of TPC (mg GAE/g d.b.) and TSC (mg AE/g d.b.):

$$Y_{TPC} = 7{,}6265 - 0{,}0562X_2 + 0{,}1110X_3 - 0{,}20831X_1^2 - 0{,}0701X_3^2 \quad (2)$$

$$Y_{TSC} = 88{,}6349 + 3{,}2064X_2 + 8{,}7732X_3 - 4{,}5747X_1^2 + 2{,}6869X_2^2 - 4{,}5764X_3^2 \quad (3)$$

Variance analysis (ANOVA) is a reliable method to assess a model's quality. It compares the variations in level combinations with the inherent randomness. The model's fit is best if it shows significant regression and an insignificant lack of fit [14]. The results indicate that the regression model for the function of TPC and TSC is statistically significant with a *p*-value <0.05, demonstrating that the probability of noise occurring in the experimental results is not more than 5%, indicating that the designed model is highly significant. The correlation coefficients $R^2_{TPC} = 0.9463$ and $R^2_{TSC} = 0.9542$ indicate that the experimental model has high compatibility with the predictive model.

The quadratic regression equation and the p-value indicated in Table 2 reveal that the primary effects of two factors, temperature and time, significantly impact both TPC and TSC. However, there is a difference in the factors affecting TPC and TSC at the quadratic level. For TPC, the effect of power is most pronounced (p<0.001), followed by time. In contrast, TSC is significantly and equally affected by all three factors. Despite this, the interaction between these factors does not statistically impact TPC and TSC (p>0.05). This data suggests that the extraction of the root is influenced by these factors in the following order: time > power > temperature.



(a) (b) (c)

(d) (e) (f)

Figure 1. *Response surface plots of (a-c) TPC and (d-f) TSC of extracts from XTP roots*

The study further examined the optimal level of each variable and the impact of their interaction on TPC and TSC using response surface plots (Figure 1). The maximum TPC and TSC can be observed most clearly in the tests illustrated in Figures 1b and 1e. The best extraction conditions yielded the highest TPC (7.67 ± 0.44 mg GAE/g d.b.) and TSC (101.86 ± 3.66 mg AE/g d.b.) using a power of 210W, a temperature of 50°C, and a duration of 8 min. The model's accuracy was confirmed by

JTE HCMUTE
ISSN: 2615-9740
JOURNAL OF TECHNICAL EDUCATION SCIENCE
Ho Chi Minh City University of Technology and Education
Website: https://jte.edu.vn
Email: jte@hcmute.edu.vn

independent replications. The practical TPC and TSC were 7.56 ± 0.35mg GAE/g d.b. and 116.24 ± 4.41 mg AE/g d.b., respectively, which were close to the predicted values. The ultrasound-treated samples showed about a 35% higher extraction yield of TPC and TSC compared to untreated samples (TPC 5.57 ± 0.10 mg GAE/g d.b., TSC 86.12 ± 0.27 mg AE/g d.b., respectively). The strong correlation between these results validates the RSM model as accurate and reliable for reflecting the optimization in the expected model.

Table 2. *The fitness parameters of the RSM best-fit model for UAE of the XTP roots, stems, and leaves*

Parts of the plant	Regression coefficients and statistical values	TPC			TSC		
		Regression coefficients	F-values	*p*-values	Regression coefficients	F-values	*p*-values
Roots	Constant						
	X_o	7.6265		<.0001*	88.6349		<.0001*
	Linear						
	X_1	0.0505	5.0200	0.0600	1.9719	3.8801	0.0895
	X_2	-0.0582	6.6483	0.0366*	3.2064	10.2594	0.0150*
	X_3	0.1110	24.2351	0.0017*	8.7732	76.8073	<.0001*
	Quadratic						
	X_{11}	-0.2083	70.2796	<.0001*	-4.5747	17.2067	0.0043*
	X_{22}	0.0007	0.0008	0.9779	2.6869	5.9358	0.0450*
	X_{33}	-0.0701	7.9683	0.0257*	-4.5764	17.2201	0.0043*
	Interaction						
	X_{12}	-0.0150	0.2593	0.6263	1.7050	1.7008	0.2334
	X_{13}	-0.0650	4.8684	0.0631	0.5875	0.2019	0.6667
	X_{23}	0.0600	4.1483	0.0811	2.8250	4.6693	0.0675
	R^2	0.9463			0.9542		
	p-values	<.0012*			<.0007*		
Stems	Constant						
	X_o	7.9589		<.0001*	85.7740		<.0001*
	Linear						
	X_1	0.0038	0.0068	0.9366	6.2213	35.6397	0.0006
	X_2	0.2558	31.4584	0.0008	-3.8216	13.4485	0.0080
	X_3	0.2178	22.7917	0.0020	4.4808	18.4875	0.0036
	Quadratic						
	X_{11}	-0.3278	42.6366	0.0003	7.9589	48.1489	0.0002
	X_{22}	-0.0768	2.3405	0.1699	-2.5699	5.0201	0.0600
	X_{33}	-0.1687	11.2948	0.0121	1.6515	2.0732	0.1931
	Interaction						
	X_{12}	0.0450	0.5701	0.4749	-0.1875	0.0190	0.8943
	X_{13}	-0.0200	0.1126	0.747	1.1500	0.7134	0.4263
	X_{23}	-0.3150	27.9341	0.0011	1.0025	0.5421	0.4855

	R^2	0.9483			0.9515		
	p-values	<.0010			<.0008		
Leaves	Constant						
	X_o	11.1494		<.0017*	181.8103		<.0036*
	Linear						
	X_1	-0.4228	35.2058	0.0006	-13.2608	17.5568	0.0041
	X_2	-0.0837	1.3799	0.2785	22.2895	49.6026	0.0002
	X_3	0.0429	0.3633	0.5657	11.2528	12.6424	0.0093
	Quadratic						
	X_{11}	-0.2007	6.5350	0.0378	6.3452	3.3120	0.1116
	X_{22}	-0.2042	6.7678	0.0353	-11.6608	11.1853	0.0123
	X_{33}	0.1909	5.9119	0.0453	-5.5454	2.5297	0.1558
	Interaction						
	X_{12}	0.0763	0.6715	0.4395	-2.0738	0.2517	0.6313
	X_{13}	0.1538	2.7302	0.1424	-5.3163	1.6544	0.2393
	X_{23}	-0.4138	19.7713	0.0030	-9.9213	5.7618	0.0474
	R^2	0.9242			0.9396		
	p-values	<.0010			<.0008		

*Statistically significant with $p < 0.05$.

3.1.2. Optimizing UAE conditions for XTP stem extracts

Table 1 presents the variations in TPC and TSC in the stem extracts. According to Table 1, the TPC value ranges from 6.65 mg GAE/g d.b. (at 200W, 45^{o}C, and 4 min) to 7.98 mg GAE/g d.b. (at 210W power, 55^{o}C temperature for 6 min). The TSC has a minimum value of 72.91 mg AE/g d.b. (at 210W, 72^{o}C, and 6 min) and a maximum value of 108 mg AE/g d.b. (at 220W, 45^{o}C, and 8 min).

The influence of each variable (X_1, X_2, X_3) as well as the interaction between variables is determined based on Table 2. A second-order polynomial equation was derived from experimental data via multiple regression analysis, describing the efficiency of TPC (mg GAE/g d.b.), and TSC (mg AE/g d.b.):

$$Y_{TPC} = 7,9589 + 0,2558X_2 + 0,2178X_3 - 0,3278X_1^2 - 0,1687X_3^2 - 0,3150X_2X_3 \quad (4)$$

$$Y_{TSC} = 85,7740 + 6,2213X_1 - 3,8216X_2 + 4,4808X_3 + 7,9589X_1^2 \quad (5)$$

The ANOVA results suggest that the regression model for TPC and TSC extracted from the XTP stem is statistically significant, as indicated by a *p*-value <0.05. High correlation coefficients, R^2_{TPC}= 0.9483 and R^2_{TSC} = 0.9515, imply a strong fit between the experimental and predictive models.

The second-order regression equation and p-value show that the TPC is significantly influenced by both temperature and time, with temperature having the most impact. For TSC, all three factors, including power, temperature, and extraction time, have a significant effect. According to the second-order value, power is the most influential factor in both TPC and TSC. While the interaction between temperature and time influences the TPC (p<0.05), TSC remains unaffected by these interactions. Thus, the extraction process for the XTP stem is impacted by factors in the sequence of power > temperature > time.

Figure 2 shows the response surface model depicting the interaction among power, temperature, and time factors affecting TPC (models a, b, and c) and TSC (models d, e, and f) in the ultrasound-assisted extraction process of the XTP stem. TPC demonstrates an increase with the rise in ultrasonic temperature from 45°C to 55°C, yet it declines beyond 55°C. Additionally, TPC exhibits an increase with the escalation of ultrasonic power from 200W to 210W, but it decreases beyond 210W. Moreover, the TPC rises with a longer extraction time. Conversely, TSC increases with an elevation in temperature from 45°C to 50°C, but it decreases exceeding 50°C. TSC steadily increases when the power exceeds 200W, and the longer the ultrasound time, the higher the TSC. Both temperature and time significantly affect the TPC ($p < 0.05$), while all three factors greatly impact the TSC.

The optimal conditions for the three factors, according to the regression equation, are an ultrasonic power of 220W, a temperature of 52°C, and a time of 8 min. Under these conditions, the maximum TPC response value reached a predicted value of 7.66 ± 0.33 mg GAE/g d.b., and the TSC reached 107.94 mg AE/g d.b. A verification experiment confirmed the accuracy of the model equations, with results closely matching the predicted values (TPC 7.83 ± 0.41 mg GAE/g d.b. and TSC 117.84 ± 4.94 mg AE/g d.b.). The ultrasound-treated samples showed a 75.6% and 193.6% higher extraction yield of TPC and TSC, respectively, compared to untreated samples (TPC 4.46 ± 0.12 mg GAE/g d.b., TSC 40.13 ± 0.35 mg AE/g d.b.), proving that the RSM model accurately and reliably reflects the expected model optimization.

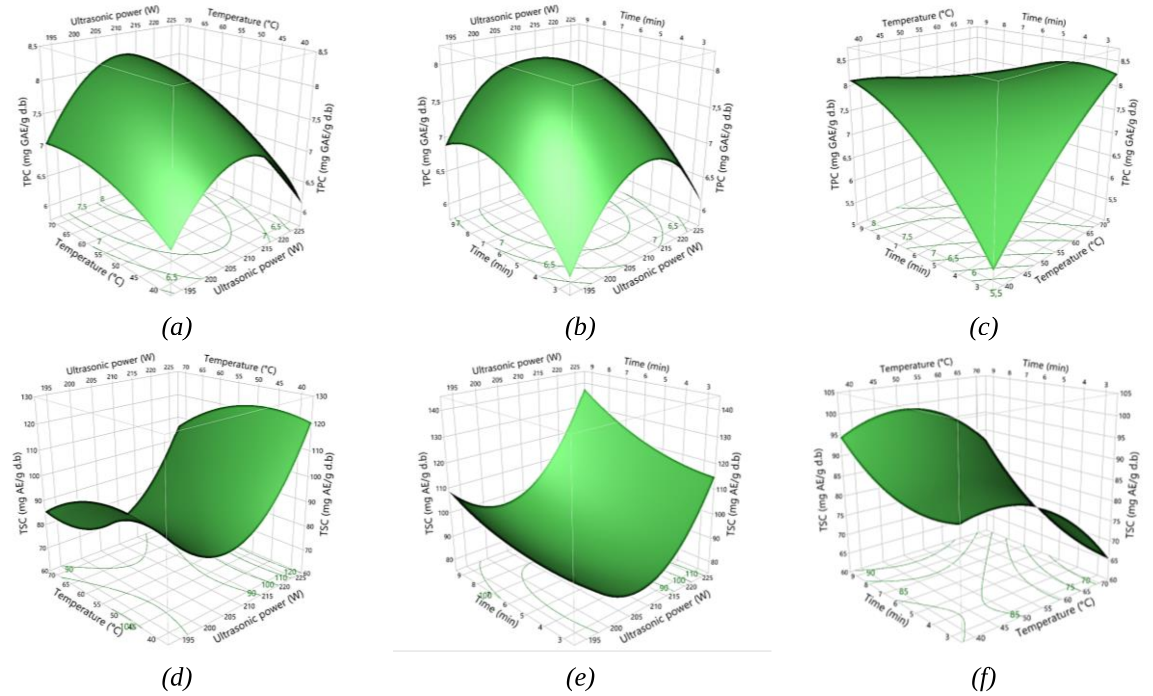


Figure 2. *Response surface plots of (a-c) TPC and (d-f) TSC of extracts from XTP stems*

3.1.3. Optimizing sonication parameters in XTP leaf extracts

Table 1 illustrates the changes in TPC and TSC in the extract from XTP leaves. These changes are influenced by factors such as power (X_1), temperature (X_2), duration (X_3), and the interaction between these factors. The lowest recorded TPC is 9.73 mg GAE/g d.b., observed under extraction conditions of 220W power, 40°C temperature, and 4 min duration. On the other hand, the highest TPC is 11.88 mg GAE/g d.b., achieved with conditions of 210W power, 50°C temperature, and 9 min duration. The lowest TSC is 122.80 mg AE/g d.b., obtained under conditions of 210W power, 33°C temperature, and 6 min duration. Meanwhile, the highest TSC is 235.00 mg AE/g d.b., with extraction conditions of 193.2W power, 50°C temperature, and 6 min duration.

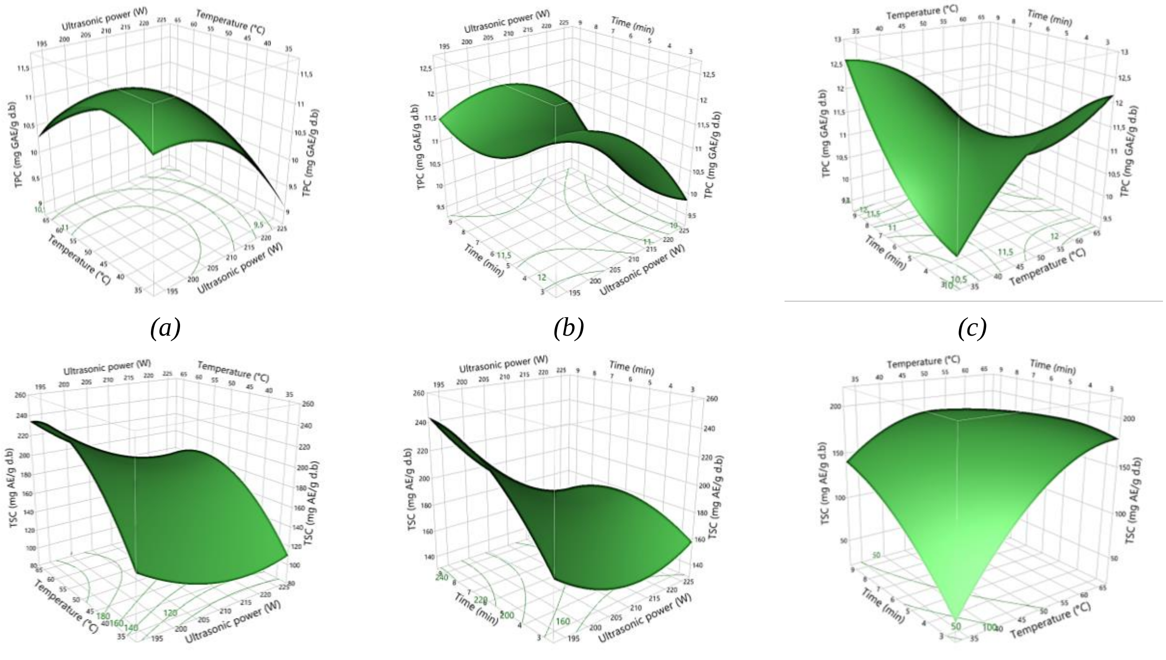
The ANOVA results in Table 2 indicate that the regression model for TPC and TSC extracted from the XTP leaves is statistically significant, with p <0.05. The correlation coefficients R^2_{TPC}= 0.9242 and R^2_{TSC} = 0.9396 imply a high correlation between the experimental and prediction models.

A second-order polynomial equation was derived from the experimental data through multiple regression analysis. This equation describes the efficiency of TPC (mg GAE/g d.b.) and TSC (mg AE/g d.b.):

$$Y_{TPC} = 11{,}1494 - 0{,}4228X_1 - 0{,}2007X_1^2 - 0{,}2042X_2^2 + 0{,}1909X_3^2 - 0{,}4138X_2X_3 \quad (6)$$

$$Y_{TSC} = 181{,}8103 - 13{,}2608X_1 + 22{,}2895X_2 + 11{,}2528X_3 - 11{,}6608X_2^2 - 9{,}9213X_{23} \quad (7)$$

The second-order regression equation and p-value highlight that the first-order value of the power factor significantly affects the TPC. Conversely, TSC is mainly influenced by power, temperature, and time, with temperature having the most substantial effect on TSC. The equation's second-order value suggests that all three factors influence TPC, while temperature impacts TSC. Moreover, the interaction between temperature and time affects both TPC and TSC, with a p <0.05. This implies that the order of factors determining the extraction of XTP leaves is temperature > power > time.



(a) *(b)* *(c)*

(d) *(e)* *(f)*

Figure 3. *Response surface plots of (a-c) TPC and (d-f) TSC of extracts from XTP leaves*

The surface response graph (Figure 3) illustrates the extraction efficiency of TPC and TSC, demonstrating an inverse correlation with three experimental factors: ultrasonic power (200-220W), temperature (45-65°C), and duration (4-8 min). TPC steadily increases as ultrasonic power rises from 200W to 210W, but begins to decrease when power surpasses 210W. Conversely, TSC decreases as power increases. At higher temperatures, TPC gradually decreases, while TSC steadily increases from 40 ^{o}C to 58^{o}C, then slightly drops after exceeding 58^{o}C. Extraction time also influences contents, as longer durations result in higher TSC and lower TPC. This suggests that the extraction factors inversely affect TPC and TSC extraction from XTP leaves.

Figure 3 shows the optimal conditions of the three factors determined by the regression equation: an ultrasonic power of 200W, a temperature of 47^{o}C, and a duration of 8 min. Under these conditions, the model predicts maximum TPC and TSC values of 11.60 ± 0.41 mg GAE/g d.b and 207.43 ± 4.16 mg AE/g d.b, respectively. A verification experiment conducted under these conditions confirmed the model's reliability, with experimental values (TPC 14.59 ± 0.50 mg GAE/g d.b. and TSC 267.67 ± 5.17 mg AE/g d.b.) consistent with the predicted value. Ultrasound-treated samples had 96.4% and 175.5% higher extraction yields of TPC and TSC, respectively, compared to untreated samples (TPC 7.43 ± 0.07 mg GAE/g d.b., TSC 97.17 ± 0.23 mg AE/g d.b.). These results affirm the RSM model's validity and accuracy in reflecting the expected model's optimization.

3.2. Comparison of UAE efficacy on TPC, TSC in the XTP root, stem, and leaf extracts

The efficacy of UAE on TPC and TSC in the XTP root, stem, and leaf extracts was compared under optimal conditions for each part of the plant. As shown in Figure 4, a significant increase in both TPC and TSC was observed when the XTP plant parts were subjected to ultrasound treatment compared to non-ultrasound-treated samples ($p < 0.05$).

For the roots, the optimal ultrasound conditions are a power of 220W, a temperature of 52°C, and a duration of 8 min. Under these conditions, the maximum TPC reached 7.56 ± 0.35 mg GAE/g d.b., and the TSC reached 116.24 ± 4.41 mg AE/g d.b. The ultrasound-treated samples exhibited a 35% higher extraction yields for both TPC and TSC compared to untreated samples (TPC 5.57 ± 0.10 mg GAE/g d.b., TSC 86.12 ± 0.27 mg AE/g d.b.).

Similarly, for stems, the optimal conditions mirror those for roots. Under these settings, the maximum TPC and TSC were 7.83 ± 0.41 mg GAE/g d.b. and 117.84 ± 4.94 mg AE/g d.b., respectively. Ultrasound-treated samples showed a 75.6% and 193.6% higher extraction yields for TPC and TSC compared to untreated samples (TPC 4.46 ± 0.12 mg GAE/g d.b., TSC 40.13 ± 0.35 mg AE/g d.b.).

For leaves, the optimal conditions involve an ultrasound power of 200W, a temperature of 47°C, and a duration of 8 min. Under these conditions, the maximum TPC and TSC values reached 11.60 mg GAE/g d.b. and 207.43 mg AE/g d.b., respectively. Ultrasound-treated samples exhibited a 96.4% and 175.5% higher extraction yields of TPC and TSC compared to untreated samples (TPC 7.43 ± 0.07 mg GAE/g d.b., TSC 97.17 ± 0.23 mg AE/g d.b.).

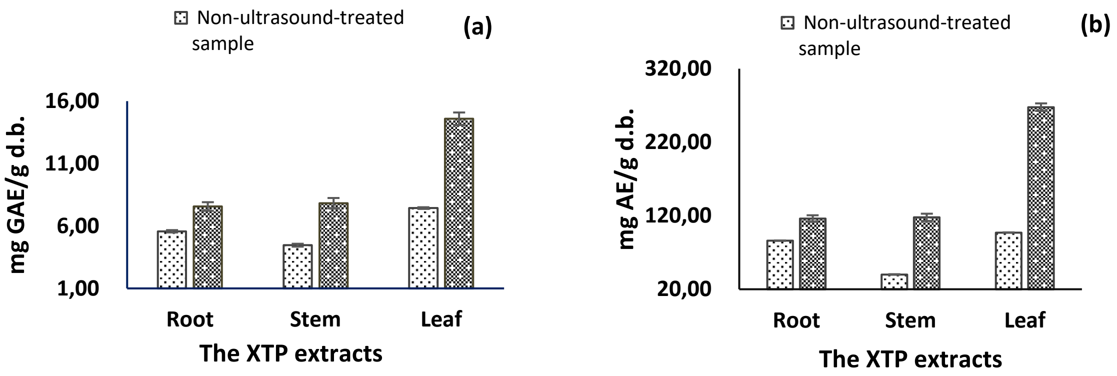


Figure 4. *TPC (a) and TSC (b) in the XTP root, stem, and leaf extracts*

The findings indicate that the optimal extraction time for all three parts of the XTP plant is 8 min. This duration effectively maximizes gas bubble phenomena and facilitates the rupture of plant cell walls, thereby enhancing the release of target components. While leaf material requires the least ultrasound energy, the stem necessitates the most to reach the maximum threshold for bubble collapse in water and to maximize solvent penetration into the plant cells. This difference can be attributed to the various compositions and cellular structures of the plant parts. Specifically, leaves are composed of cellulose, hemicellulose, and pectin, which have lower content and a more flexible structure. Consequently, cell division and rupture in leaves due to energy generated from bubble collapse are minimal. Conversely, the stem, with its stronger plant cell structure, requires higher energy levels for complete cell wall breakdown. The optimal extraction temperature for TPC and TSC from leaf material using ultrasound is the lowest, at 47°C. This temperature increases for the roots and stems. Therefore, temperature significantly influences the solvent's extraction capability and accelerates the exploitation of the biological compounds.

Under the optimal UAE conditions for each part of the XTP plant, the TPC and TSC in the leaf extracts are approximately twice as high as in the root and stem extracts. This trend contrasts with the findings of previous studies by Nguyen et al. (2017b) and Nguyen et al. (2017c) [15], [16]. The authors reported that the content of these substances in the root (238.13 mg GAE and 7731.05 mg AE) was significantly higher than in the leaves (25.4 mg GAE and 702.1 mg AE) when extracting XTP in methanol with microwave for 40 min at 360 W power. This phenomenon can be attributed to various extraction solvents and techniques, which result in different cell disruption and release mechanisms.

The study identified optimal conditions for UAE in a water environment from the roots, stems, and leaves of the XTP plant. Remarkably, the extract from the leaves exhibited the highest content of bioactive substances. This suggests its potential as a valuable source of raw materials for the pharmaceutical, cosmetic, and food industries.

4. Conclusions

This research uses ultrasound-assisted extraction to optimize the extraction of beneficial phytochemicals from Xao tam phan (XTP) roots, stems, and leaves. The optimal conditions varied with plant parts. Specifically, for roots and stems, utilizing 220W power at 52°C for 8 min yielded the highest compound extraction. Conversely, for leaves, employing 200W power at 47°C for 8 min achieved higher yields. Notably, leaf extracts contained double the compounds compared to other parts, highlighting their potential for applications in the pharmaceutical, cosmetic, and food industries.

Acknowledgments

This work belongs to the project in 2022 funded by Nong Lam University Ho Chi Minh City (NLU), Vietnam.

Conflict of Interest

The authors declare no conflict of interest.

REFERENCES

[1] H. L. T. Anh *et al.*, "Anti-inflammatory coumarins from *Paramignya trimera*," *Pharm. Bio.*, vol. 55, no.1, pp. 1195–1201, 2017.
[2] P. H. Dang *et al.*, "Two acridones and two coumarins from the roots of *Paramignya trimera*," *Tetra. Let.*, vol. *58*, no. 16, pp. 1553–1557, 2017.
[3] M. T. T. Nguyen *et al.*, "Paratrimerins G and H, two prenylated phenolic compounds from the stems of *Paramignya trimera*," *Phyto. Let*., pp. 78–82, 2018.
[4] T. Nhan, H. Phu, X. T. Ngoc, H. Tho, and T. T. Mai, "Quinoliniumolate and 2H-1, 2, 3-Triazole Derivatives from the Stems of *Paramignya trimera* and Their α-Glucosidase Inhibitory Activities: In Vitro and in Silico Studies," *J. Nat. Prod.*, vol. 80, pp. 2151−2155, 2017.
[5] L. Wang and C. L. Weller, "Recent advances in extraction of nutraceuticals from plants," *Trends in Food Sci. & Tech.*, vol. 17, no. 6, pp. 300–312, 2006.
[6] S. Armenta, S. Garrigues, and M. de la Guardia, "The role of green extraction techniques in Green Analytical Chemistry," *TrAC Trends in Ana. Chem.*, vol. 71, pp. 2–8, 2015.
[7] J. Wang, B. Sun, Y. Liu, and H. Zhang, "Optimisation of ultrasound-assisted enzymatic extraction of arabinoxylan from wheat bran," *Food Chem.*, vol 150, pp. 482–488, 2014.
[8] I. Lavilla and C. Bendicho, "Fundamentals of ultrasound-assisted extraction," *Water extraction of bioactive compounds*, Elsevier, pp. 291–316, 2017.
[9] H. Moussa, F. Dahmoune, M. M. Mahdjoub, N. Kadri, and H. Remini, "Definitive screening design and I-optimal design for optimization of ultrasound-assisted extraction of phenolic content and antioxidant capacity from *Salvia officinalis L.* leaves," *Sustainable Chem. and Phar.*, vol. 29, p. 100820, 2022.
[10] V. T. Nguyen, M. C. Bowyer, Q. V. Vuong, I. A. V. Altena, and C. J. Scarlett, "Phytochemicals and antioxidant capacity of Xao tam phan root as affected by various solvents and extraction methods," *Industrial Crops and Products*, vol. 67, pp. 192–200, 2015.
[11] V. T. Nguyen, Q. V. Vuong, M. C. Bowyer, I. A. Van Altena, and C. J. Scarlett, "Microwave-Assisted Extraction for Saponins and Antioxidant Capacity from Xao Tam Phan (*Paramignya trimera*) Root," *Journal of Food Processing and Preservation*, vol. 41, no. 2, pp. 1–11, 2017.
[12] N. T. Sang and H. K. Anh, "Study of ultrasound-assisted extraction of phenolic compounds from Xao Tam Phan (*Paramignya trimera*)," *Sustainable Global Agriculture And Food*, pp. 243–252, 2018.
[13] T. N. T. Nguyen *et al.*, "Ultrasound probe assisted extraction of *Paramignya trimera* (Oliver) Guillaum root," *IOP Conference Series: Earth and Envir. Sci.*, vol. *1155, no.* 1, p. 12021, 2023.
[14] M. A. Bezerra, R. E. Santelli, E. P. Oliveira, L. S. Villar, and L. A. Escaleira, "Response surface methodology (RSM) as a tool for optimization in analytical chemistry," *Talanta*, vol. 76, no. 5, p. 965–977, 2008.
[15] V. T. Nguyen, J. A. Sakoff, and C. J. Scarlett, "Physicochemical, antioxidant, and cytotoxic properties of Xao Tam Phan (*Paramignya trimera*) root extract and its fractions," *Chemistry & Biodiversity*, vol. 14, no. 4, p. 1600396, 2017.
[16] V. T. Nguyen, J. A. Sakoff, and C. J. Scarlett, "Physicochemical Properties, Antioxidant and Anti-proliferative Capacities of Dried Leaf and Its Extract from Xao tam phan (*Paramignya trimera*)," *Chemistry & Biodiversity*, vol. 14, no. 6, p. 1600498, 2017.

Nguyen Thi Nguyen Thao received her Master's degree in Engineering in Food Technology from the University of Technology (Vietnam National University), Ho Chi Minh City, Vietnam, in 2012. She is currently pursuing a PhD in Food Technology at Nong Lam University, Ho Chi Minh City, Vietnam. Her research focuses on the extraction and encapsulation of bioactive compounds. She has been a lecturer at Phu Yen University, Phu Yen, Vietnam, since 2008. Email: nguyenthinguyenthao@pyu.edu.vn. ORCID: https://orcid.org/0000-0001-6895-5442

Trinh Thanh Tan received his Master's degree in Food Technology at Nong Lam university, Ho Chi Minh city, Vietnam in 2024. Now, he is also a Formulation department at C.P. Vietnam corporation (a member of C.P. Group - Thailand) Email: tantrinhthanh94@gmail.com

Le Huu Nghia received his Master's degree in Food Technology at Nong Lam university, Ho Chi Minh city, Vietnam in 2024. Now, he is also a Laboratory Manager of Quality Control department at C.P. Vietnam corporation (a member of C.P. Group - Thailand) which operates integrated agro-industrial and food business according to the 3F+ model (Feed - Farm - Food). Email: andy.lhn1989@gmail.com

Kha Chan Tuyen received his degrees of Master by Research and PhD in Food Science, from the University of Newcastle, Australia. Associate Professor Tuyen Kha is currently Head of Department of Food Microbiology and Product Development and Vice Dean of the Faculty of Chemical Engineering and Food Technology, Nong Lam University, Ho Chi Minh City, Vietnam. Kha's research interest focuses on extraction and encapsulation of bioactive compounds. He is currently consulting for several food companies in Vietnam and is active in national and international collaborations on food research. Associate Professor Kha received many awards including the SERS (Scientific and Educational Research Society) Excellence Research Award - 2018 for outstanding contribution in the field of Food Technology by Scientific and Educational Research Society, Meerut, U.P., India. Since 2017, he has been appointed as a coordinator of Better Process Control School in collaboration with FDA and CBA (United States). Email: khachantuyen@hcmuaf.edu.vn

Phan Phuoc Hien received his Master's degree in Biochemistry from Natural University, Ho Chi Minh City, Vietnam. He earned his PhD in Biochemistry from a combined program between the National Centre for Natural Sciences and Technology Vietnam - Institute of Tropical Biology Vietnam and INP/ENFA/Paul Sabatier Toulouse, France. He completed his Post-doctorate in Agro-Industrial Chemistry at the National Polytechnic Institute (INP) in 2003. From 2013 to 2018, he was a lecturer at Nong Lam University in Ho Chi Minh City, Vietnam. Since 2018, he has been the director of the Institute of Applied Science at Van Lang University, HCMC, Vietnam. Hien's research focuses on Bio-Pharmaco Chemical Technology and the processing technologies of bio-products. Email: pphien@gmail.com

Nguyen Minh Xuan Hong received her Master's degree in Biotechnology from the Asian Institute of Technology (AIT) in Bangkok, Thailand. She earned her PhD in Food Science from RMIT University in Australia. Since 2003, she has been a lecturer at Nong Lam University in Ho Chi Minh City, Vietnam. Hong's research focuses on the extraction of bioactive compounds and biological coatings. Email: nmxhong@hcmuaf.edu.vn. ORCID: https://orcid.org/0000-0002-1118-4322