

Extraction of bioactive components from *Paramignya trimera* (Oliver) Guillaum stems using an optimized enzyme approach

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ARTICLE INFO

Research Paper

Received: October 02, 2024

Revised: November 22, 2024

Accepted: November 28, 2024

Keywords

Enzyme

Paramignya trimera

Total phenolics

Total saponins

Xao tam phan

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ABSTRACT

Xao tam phan (XTP) is a valuable medicinal plant native to Southeast Asia, particularly Vietnam. This plant is rich in bioactive compounds, primarily phenolics and saponins, which offers various therapeutic properties. A recent study optimized the extraction of these bioactive compounds from XTP stems using an enzyme-assisted extraction (EAE). Response surface methodology determined optimal enzyme extraction conditions at 0.8% v/w enzyme concentration, 45°C, and 105 min. Combining ultrasound with enzyme treatment (U-EAE) proved most effective, increased total phenolic content (TPC) and total saponin content (TSC) by 1.23 and 3.27 times, respectively, compared to untreated samples. The U-EAE outperformed other methods (E-UAE, UES-AE, UAE, EAE) in extracting bioactive compounds.

Cited as: Nguyen, T. T. N., Le, N. H., Trinh, T. T., Phan, H. T., Phan, H. P., & Nguyen, H. M. X. (2025). Extraction of bioactive components from *Paramignya trimera* (Oliver) Guillaum stems using an optimized enzyme approach. *The Journal of Agriculture and Development* 24(6), 133-146.

1. Introduction

Xao tam phan (*Paramignya trimera* (Oliv.) Burkill) is a medicinal plant long known as a “cure-all”. It contains flavonoids, saponins, alkaloids, and primarily coumarins and triterpenoids. Due to its diverse active ingredients with disease-preventing and treating properties, numerous studies have examined Xao tam phan’s (XTP) components and processes for extracting its valuable active ingredients as a medicinal herb source (Nguyen et al., 2015; Le et al., 2017).

The research into applying natural active ingredients to create functional foods is flourishing worldwide, including in Vietnam. This aims to harness the rich source of medical herbs to bring valuable health-promoting food products to market.

Enzyme technology offers an eco-friendly method for extracting bioactive compounds and has been widely researched and applied in agro-industrial sectors. Adding enzymes like cellulase, α -amylase, and pectinase during extraction boosts yield by breaking down cell structures and hydrolyzing polysaccharides and lipids. This process facilitates the release of bioactive compounds into the extraction solvent (Gómez-García et al., 2012; Puri et al., 2012; Swer et al., 2016).

Enzyme technology is considered an environmentally friendly alternative for extracting bioactive compounds from agro-industrial by-products (Lemes et al., 2022). However, studies on the extraction of bioactive compounds from medicinal herbs by enzymes, especially XTP, are still limited. This study aims to optimize the parameters for the enzymatic extraction of bioactive compounds from XTP with an aqueous solvent. Response surface methodology (RSM) is utilized to maximize the extraction efficiency of phenolic compounds and saponins from XTP stems.

Ultrasound-assisted extraction is one of the advanced techniques currently used to extract biological compounds from plants. The combination of ultrasound and enzymes yields superior efficiency in obtaining bioactive compounds (Tchabo et al., 2015; Li et al., 2017; Nag & Sit, 2018). Therefore, the extraction efficiency of total phenolic content (TPC) and total saponin content (TSC) from XTP stems will be compared using a combined enzyme and ultrasound approach.

2. Materials and Methods

2.1. Materials

The Xao tam phan (XTP) stems, harvested at four years of age from Phu Yen province in Vietnam, underwent a meticulous processing procedure. After thorough washing and draining, they were dried at $40 \pm 2^\circ\text{C}$ until reaching approximately 10% moisture content. The dried samples were then ground into a fine powder, passed through a 0.3 mm sieve, sealed in polyethylene bags, and stored at -10°C for further analysis.

2.2. Chemical

The chemicals used for this assay were pectinase (EC 232-885-6) and cellulase (EC 232-734-4) from Novozymes, Denmark. Analytical-grade chemicals employed in this study included Folin-Ciocalteu reagent, gallic acid (GA), vanillin, and aescin, all procured from Sigma-Aldrich Co., USA.

2.3. Experimental methods

2.3.1. Screening for key factors affecting the enzyme treatment

To determine the factors and levels influencing extraction efficiency, 5 factors were selected for experimentation: pectinase-cellulase enzyme mixture concentration (% v/w) (the ratio of the two enzymes is 1:1), treatment time (min), treatment temperature (°C), water/material ratio (v/w), and pH. The low (-1) and high (+1) levels of the 5 factors are listed in Table 1. The experiment was designed according to the Plackett-Burman

matrix with 5 factors in 12 experiments (Table 2) to screen for important factors affecting the yield of phenolics (mg GAE/g dried basis) and saponins (mg AE/g dried basis).

All treatments were performed in triplicate using a thermostatic water bath (Mettler, Germany). A control sample, without enzymatic treatment, was included in all experiments. Post-treatment, samples were heated at 90°C for 10 min to inactivate enzymolysis. The resulting mash was filtered through cloth, and the filtrate was centrifuged at 5,000 rpm for 15 min to collect the supernatant for TPC and TSC analysis.

Table 1. Variables in the Plackett-Burman matrix

Coded factors	Variables	Factor values	
		Low level (-1)	High level (+1)
A	Concentration of enzyme mixture (% v/w)	0.2	1.0
B	Treatment time (min)	60	180
C	Treatment temperature (°C)	30	70
D	Water/material ratio (v/w)	10:1	80:1
E	pH	3.0	5.0

Table 2. Plackett-Burman experimental design matrix

Pattern	Variables				
	A	B	C	D	E
0a0	0.2	60	30	80	3
---	0.2	60	70	10	3
--+	0.2	60	70	10	5
+--	0.2	180	30	10	5
+-+	0.2	180	30	80	5
a00	0.2	180	70	80	3
00a	1.0	60	30	10	5
000	1.0	60	30	80	3
000	1.0	60	70	80	5
000	1.0	180	30	10	3
00A	1.0	180	70	10	3
A00	1.0	180	70	80	5

A: concentration of enzyme mixture (% v/w); B: treatment time (min); C: treatment temperature (°C); D: water/material ratio (v/w); E: pH.

2.3.2. Optimization of parameters for extracting phenolics and saponins from XTP stems using enzyme mixture

Enzymatic treatment conditions were optimized using central composite design (CCD) and response surface methodology (RSM), based

on the results from Plackett-Burman design (PBD). These findings were used to adjust the dependent variables, defining the range for optimizing the yield of phenolics (mg GAE/g dried basis) and saponins (mg AE/g dried basis) (Table 3).

Table 3. Experimental design for optimizing enzyme extraction parameters

Survey parameters	Range of variation
Concentration of enzyme mixture (% v/w)	0.8 - 1.0
Treatment time (min)	75 - 105
Treatment temperature (°C)	45 - 55
Fixed parameters	Values
Water/material ratio (v/w)	40:1
pH	4.0

Table 4 displays the levels of independent variables along with the results of 17 experimental runs 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) (Wang et al., 2014). 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. The optimal conditions were predicted and verified experimentally.

2.3.3. Investigation of an extraction method using an enzyme mixture combined with ultrasonic treatment

Based on the optimized parameters for ultrasonic treatment of stems in an aqueous medium (220 W power, 52°C temperature, 8-min duration) (Nguyen et al., 2024), extraction methods combining enzyme treatment with ultrasonic techniques were further evaluated and determined. Through this approach, the extraction efficacy of TPC and TSC were assessed.

2.4. Analytical methods

Total phenolic content (TPC) and total saponin content (TSC) were quantified using spectrophotometric methods, as outlined by Nguyen et al. (2023).

2.4.1. Quantification of total phenolics

The TPC of XTP stem extracts was determined using the Folin-Ciocalteu method. The procedure involved diluting 0.5 mL of extract in distilled

water, mixing it with 2.5 mL of 10% (v/v) Folin-Ciocalteu reagent, and allowing it to stabilize for 6 min. Subsequently, 2 mL of 7.5% (w/v) Na_2CO_3 solution was added and the mixture was vortexed for 2 min. The resulting solution was then incubated in darkness at room temperature for 60 min. Absorbance was measured at 765 nm using an Agilent 8453 Spectrophotometer. TPC was quantified using a gallic acid standard curve (Equation 1) and expressed as mg gallic acid equivalent (GAE) per gram of dried basis (d.b.).

$$\text{TPC} = \frac{A \times V_m \times d}{m \times (100 - W_m) \times 1000} \quad A = \frac{(D_m - D_0) - b}{a} \quad (1)$$

Where:

TPC: total polyphenol content (mg GAE/g d.b.)
A: gallic acid concentration from the standard equation
 V_m : volume of extracted liquid (mL)
d: dilution factor
m: mass of the sample (g, dry matter)
 W_m : moisture content of the sample (%)

D_m : OD of sample
 D_0 : OD at the intercept point between the standard curve with the y-axis
a, b: are values of equation $y = ax + b$ using gallic acid as standard

2.4.2. Quantification of triterpenoid saponins

The total saponin content (TSC) of XTP stem extracts was determined using the following process. First, 0.5 mL of XTP sample was added to a flask and mixed with 0.5 mL of 8% (w/v) vanillin solution. Then, 5 mL of 72% (v/v) H_2SO_4 solution was added. The mixture was vortexed for

2 min, incubated at 70°C for 30 min, and rapidly cooled to room temperature using ice water. The absorbance of the mixture was measured at 560 nm using a UV-vis spectrophotometer. Aescin was used as a standard. TSC was calculated using Equation 2 and expressed as mg of aescin equivalents (AE) per g of dried basis (d.b.).

$$\text{TSC} = \frac{C_x \times V_m \times d}{m \times (100 - W_m) \times 1000} \quad (2)$$

Where:

TSC: Triterpenoid saponin content (mg EE/g d.b.)
 C_x : aescin concentration from the standard equation
d: dilution factor

V_m : volume of extracted liquid (mL)
 W_m : moisture content of the sample (%)
m: mass of the sample (g)

2.5. Statistical analysis

All experiments and analytical measurements in this study were conducted in triplicate to ensure reliability. Statistical analysis was performed using JMP software (version 13 Pro). Results are presented as mean values with standard

deviations (Mean $\pm$ SD). The analysis of variance (ANOVA) followed by least significant difference (LSD) tests was used to determine significant differences between means, with a significance level of 5% ($P < 0.05$). Graphical representations of the data, including mean values and error bars, were generated using Microsoft Excel 2016.

3. Results and Discussion

3.1. Important factors influencing the enzyme extraction

To determine the factors and levels affecting extraction efficiency, five factors were selected for experimentation: the pectinase-cellulase enzyme concentration (A), treatment time (B),

treatment temperature (°C), water/material ratio (D), and pH (E). Table 4 lists the low (-1) and high (+1) levels for each of the five factors. The experiment used the Plackett-Burman matrix, involving 5 factors across 12 experiments (Table 5). This design aimed to screen for key factors influencing the yield of TPC (mg GAE/g d.b.) and TSC (mg AE/g d.b.).

Table 4. Influence coefficients of factors in the Plackett-Burman experiment

Coded factors	Variables	Low level (-1)	High level (+1)	TPC		TSC	
				Effect coefficient	P (Prob > F)	Effect coefficient	P (Prob > F)
A	Concentration of enzyme mixture (% v/w)	0.2	1.0	9.52*	< 0.001	3.98*	0.007
B	Treatment time (min)	60	180	3.16*	0.002	3.28*	0.017
C	Treatment temperature (°C)	30	70	-5.53*	0.002	-7.42*	< 0.001
D	Water/material ratio (v/w)	10:1	80:1	-0.31	0.769	-0.02	0.984
E	pH	3.0	5.0	0.39	0.707	1.64	0.151

Significant at confidence level $\alpha = 0.05$. TPC: total phenolic content; TSC: total saponin content.

Table 4 reveals the strong impact of experimental factors on TPC and TSC. A positive effect coefficient indicates a factor's greater influence at the high level, while a negative value suggests a stronger influence at the low level. Based on the effect coefficients (considering those with larger absolute values), three factors were identified as having the strongest impact on TPC and TSC with over

95% confidence ($P < 0.05$): the concentration of pectinase-cellulase enzyme mixture (A), treatment time (B), and temperature (°C). These factors significantly affect the extraction efficiency of TPC and TSC and were included in the subsequent optimization stage. The water/material ratio (D) and pH (E) were found to have no significant effect. The modeling for TPC (Y1) and TSC (Y2) can be represented as:

$$Y_1 \text{ (mg GAE/g d.b.)} = 7.038 + 0.904A + 0.002B - 0.011C - 0.0003D + 0.015E \quad (3)$$

$$Y_2 \text{ (mg AE/g d.b.)} = 67.260 + 3.079A + 0.017B - 0.115C - 0.0002D + 0.508E \quad (4)$$

Table 5. Results of the Plackett-Burman experiment

Pattern	A	B	C	D	E	TPC (mg GAE/g d.b.)		TSC (mg AE/g d.b.)	
						Experimental value	Predicted value	Experimental value	Predicted value
0a0	0.2	60	30	80	3	6.85	7.04	66.3	67.0
---	0.2	60	70	10	3	6.68	6.65	63.8	62.4
--+	0.2	60	70	10	5	6.71	6.68	62.2	63.4
+--	0.2	180	30	10	5	7.39	7.34	69.5	70.0
++-	0.2	180	30	80	5	7.27	7.31	71.3	70.0
a00	0.2	180	70	80	3	6.97	6.86	64.2	64.4
00a	1.0	60	30	10	5	7.81	7.82	70.9	70.5
000	1.0	60	30	80	3	7.93	7.77	68.9	69.4
000	1.0	60	70	80	5	7.34	7.38	66.4	65.9
000	1.0	180	30	10	3	8.05	8.08	71.5	71.5
00A	1.0	180	70	10	3	7.47	7.61	66.8	66.9
A00	1.0	180	70	80	5	7.61	7.62	67.4	67.9

A: concentration of enzyme mixture (% v/w); B: treatment time (min); C: treatment temperature (°C);

D: water/material ratio (v/w); E: pH; TPC: total phenolic content; TSC: total saponin content.

3.2. Optimizing enzymatic parameters in XTP stem extracts

The important factors selected from PBD: the concentration of pectinase-cellulase enzyme mixture (X_1), treatment temperature (X_2), and time (X_3) were considered for further optimization using CCD. The levels for these

factors were set based on the previous PBD analysis. Each variable was studied at five coded levels ($-\alpha$, -1, 0, 1, and α), with all variables taken at the center point (coded value of zero). Table 6 presents the CCD matrix along with the experimental and predicted results.

Table 6. Matrix of experimental design encoding independent variables

Variables	Coded factors	Encoding level				
		$-\alpha$	-1	0	1	α
Concentration of enzyme mixture (% v/w)	X_1	0.73	0.80	0.90	1.00	1.07
Treatment time (min)	X_2	41.60	45.00	50.00	55.00	58.40
Treatment temperature (°C)	X_3	64.80	75.00	90.00	105.00	115.20

The optimization results for extracting from the stem are presented in Table 7.

Table 7. The effects of enzymatic conditions on the TPC and TSC in stem extracts

Run oder	Pattern	X_1	X_2	X_3	TPC (mg GAE/g d.b.)		TSC (mg AE/g d.b.)	
					Experimental	Predicted	Experimental	Predicted
					value	value	value	value
1	a00	0.73	50	90	8.48	8.49	89.3	93.7
2	---	0.8	45	75	8.51	8.54	85.2	82.1
3	---+	0.8	45	105	8.71	8.65	112.4	110.9
4	--+	0.8	55	75	8.51	8.47	85.5	83.2
5	+++	0.8	55	105	8.20	8.26	83.4	79.0
6	0a0	0.9	42	90	8.41	8.44	82.2	84.3
7	00a	0.9	50	65	8.58	8.58	81.4	84.4
8	000	0.9	50	90	8.76	8.78	78.8	81.3
9	000	0.9	50	90	8.77	8.78	81.7	81.3
10	000	0.9	50	90	8.82	8.78	84.7	81.3
11	00A	0.9	50	115	8.63	8.63	95.6	99.3
12	0A0	0.9	58	90	8.46	8.42	73.0	77.6
13	+--	1.0	45	75	8.36	8.31	77.0	76.6
14	+++	1.0	45	105	8.54	8.58	101.0	98.6
15	++-	1.0	55	75	8.61	8.68	103.8	100.6
16	+++	1.0	55	105	8.64	8.62	91.2	89.6
17	A00	1.07	50	90	8.62	8.60	95.7	98.0

TPC: total phenolic content; TSC: total saponin content.

Table 7 reveals that TPC in the extract ranges from 8.20 - 8.82 mg GAE/g d.b., while TSC varies from 73.0 - 112.4 mg AE/g d.b. TPC peaks at 8.82 mg GAE/g d.b. under conditions of 40°C, 90 min extraction time, and a 0.9% enzyme mixture concentration. TSC reaches its maximum of 112.4 mg AE/g d.b. at 45°C, with a 105-min extraction time and a 0.8% v/w enzyme mixture concentration. The impact of individual factors, pectinase-cellulase enzyme mixture concentration (X_1), temperature (X_2), and

extraction time (X_3), as well as their interactions, are analyzed based on Table 8.

The analysis of variance results in Table 8 shows that the regression model is statistically significant ($P < 0.05$), with correlation coefficients R^2 of 0.943 for TPC and 0.916 for TSC. This demonstrates a high degree of compatibility between the experimental and predictive models. The second-order polynomial equations predicting the yields of TPC (Y_1) and TSC (Y_2) are as follows:

$$Y_1 = -6.166 - 8.358X_1^2 - 0.005X_2^2 - 0.0003X_3^2 + 0.215X_1X_2 - 0.001X_2X_3 \quad (5)$$

$$Y_2 = 528.734 + 3.823X_1 + 513.900X_1^2 + 0.017X_3^2 + 11.435X_1X_2 - 0.101X_2X_3 \quad (6)$$

Where Y_1 and Y_2 represent TPC (mg GAE/g d.b.) and TSC (mg AE/g d.b.), respectively; X_1 is the concentration of pectinase-cellulase enzyme mixture (1:1) (% v/w); X_2 is the treatment temperature ($^{\circ}\text{C}$); and X_3 is the time (min).

Table 8. Parameters of the RSM-CCD model (regression coefficients, F-values, and P)

Regression coefficients and statistical values	TPC			TSC		
	Regression coefficients	F-values	P	Regression coefficients	F-values	P
Constant						
X_0	-6.166		< 0.001*	528.734		< 0.001*
Linear						
X_1	2.228	4.616	0.069	-1382.281	1.041	0.342
X_2	0.402	0.129	0.730	-0.258	2.565	0.153
X_3	0.083	0.754	0.414	3.823	12.773	0.009*
Quadratic						
X_{11}	-8.358	23.871	0.002*	513.900	14.183	0.007*
X_{22}	-0.005	52.819	< 0.001*	-0.005	0.010	0.924
X_{33}	-0.0003	14.037	0.007*	0.017	7.481	0.029*
Interaction						
X_{12}	0.215	28.102	0.001*	11.435	12.493	0.010*
X_{13}	0.027	3.891	0.089	-1.132	1.101	0.329
X_{23}	-0.001	16.551	0.005*	-0.101	25.963	0.001*
R_2	0.943			0.916		
P	0.001*			0.005*		

RSM-CCD: response surface methodology-central composite design; TPC: total phenolic content; TSC: total saponin content.

The second-order regression equation reveals that the first-order values of the pectinase-cellulase enzyme mixture concentration (X_1), temperature (X_2), and time (X_3) do not significantly affect TPC ($P > 0.05$). For TSC, only the extraction time in the first-order value of the equation has an influence ($P < 0.01$), while the other two factors do not affect the extraction efficiency. The second-order values of all three factors (enzyme concentration, temperature, and time) strongly influence the TPC extraction yield ($P < 0.05$), with the enzyme concentration having the strongest impact (regression coefficient of -8.358). Similarly, the second-order values of the enzyme concentration and treatment time

strongly affect the TSC extraction efficiency ($P < 0.05$), with the enzyme concentration having the highest influence (regression coefficient of 513.900). This indicates that the extraction is significantly influenced when factors are combined and varied, especially the enzyme mixture concentration, which greatly impacts the extraction efficiency of both TPC and TSC. The interaction between factors also affects the extraction yield from XTP stems, notably the interactions between enzyme concentration and temperature (X_{12}), and between temperature and time (X_{23}) for the extraction efficiency of TPC and TSC.

Figure 1 displays the response surface model in three-dimensional space, illustrating how these factors influence the extraction yield from XTP stems.

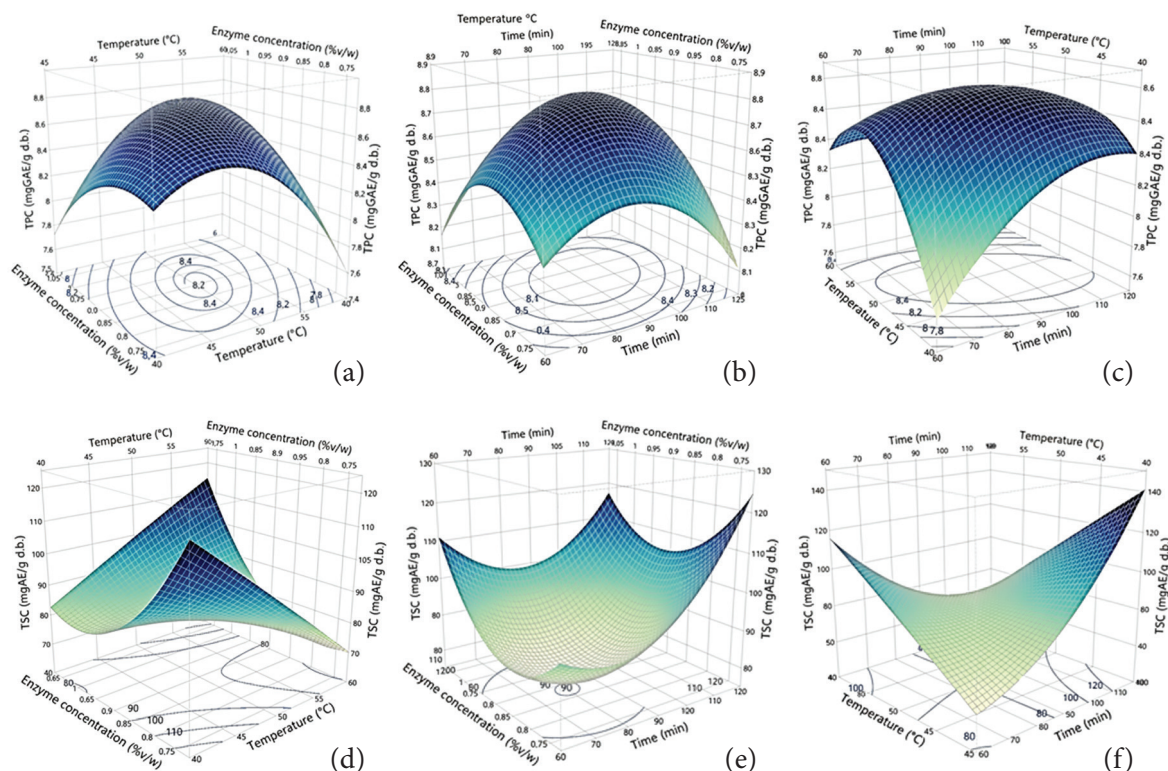


Figure 1. Response surface plots of (a-c) TPC and (d-f) TSC of extracts from XTP stems. TPC: total phenolic content; TSC: total saponin content; XTP: Xao tam phan.

The response surface diagram illustrates the correlation between any two factors according to a non-linear model. For TPC extraction (Figure 1a-c), individual factors have an insignificant influence ($P > 0.05$). However, the second-order interaction of all three factors significantly affects the extraction yield. Temperature has the highest impact ($F = 52.819$), followed by enzyme concentration ($F = 23.871$) and time ($F = 14.037$).

Observations from the response surface diagram of TPC (Figure 1 (d)-(f)) reveal that extraction time ($F = 12.773$) has a substantial influence, longer extraction times increase TSC. The interaction coefficient between extraction temperature and time (X_{23}) is negative (-0.101), indicating that simultaneously increasing heat and extending extraction time will decrease TPC.

Enzyme extraction of TPC and TSC is primarily influenced by three factors: enzyme concentration, temperature, and time. Optimizing these parameters requires considering all three factors, their interactions, and the effects on extraction efficiency. Statistical analysis using response surface methodology determined the optimal parameters, as described in Figure 2. The optimal conditions, enzyme concentration of 0.8% v/w, temperature of 45°C, and extraction time of 105 min, yielded TPC of 8.64 ± 0.11 mg GAE/g d.b. and TSC of 110.87 ± 8.85 mg AE/g d.b.

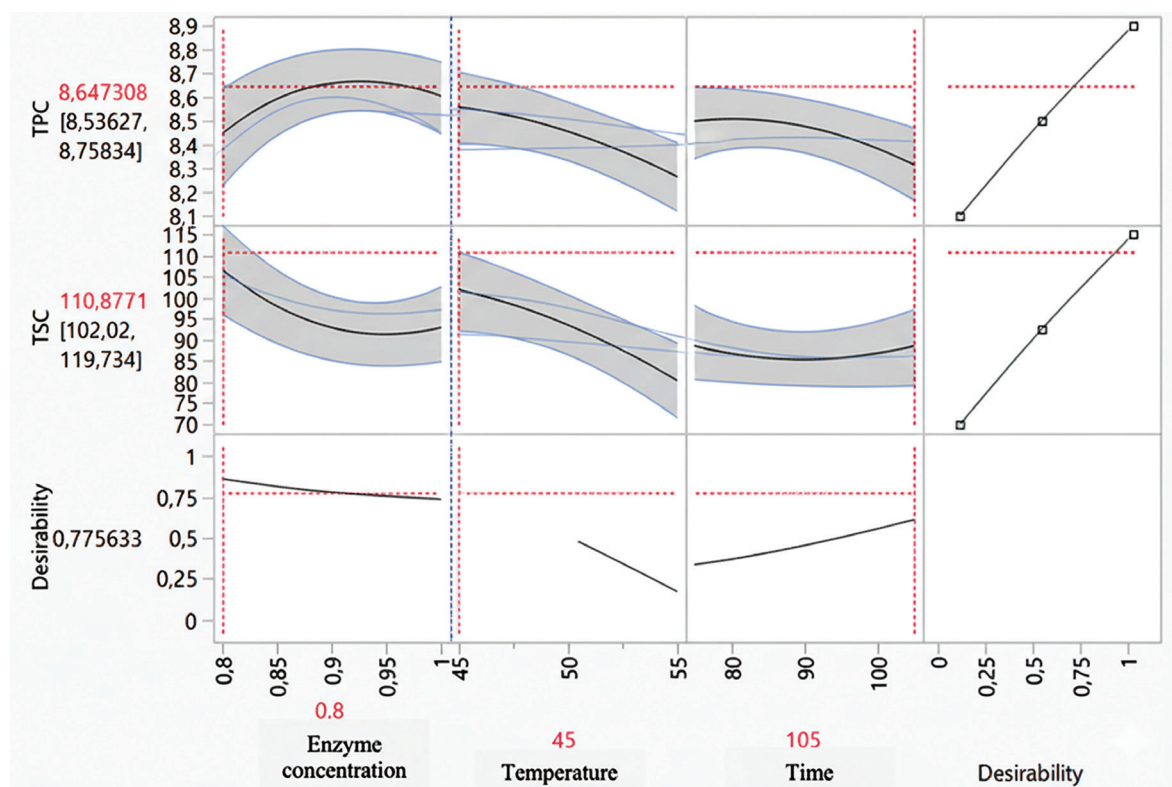


Figure 2. Optimization of factors influencing the extraction of TPC and TSC.
TPC: total phenolic content; TSC: total saponin content.

To confirm the accuracy of the model, three independent replicates were performed to measure TPC and TSC under optimal conditions. The practical TPC and TSC were 8.77 ± 0.09 mg GAE/g d.b. and 108.87 ± 2.64 mg AE/g d.b., respectively, which were similar to the predicted values of the equation (TPC 8.64 ± 0.11 mg GAE/g d.b., TSC 110.87 ± 8.85 mg AE/g d.b., respectively). Comparing extraction yields, the enzyme-treated sample exhibited an 8.8% increase in TPC and a 129.2% increase in TSC relative to the untreated sample (TPC 8.06 ± 0.38 mg GAE/g d.b., TSC 47.50 ± 2.31 mg AE/g d.b., respectively).

3.3. Comparison of extraction methods using enzyme mixtures combined with ultrasonic treatment in water

Using optimized parameters for enzyme extraction from XTP stems (0.8% v/w pectinase-cellulase enzyme concentration [1:1], 45°C treatment temperature, 105-min time) and ultrasound-assisted extraction (220 W power, 52°C temperature, 8-min duration) (Nguyen et al., 2024), TPC and TSC were extracted using various methods: enzyme-assisted extraction (EAE), ultrasound-assisted extraction (UAE), ultrasound before enzyme (U-EAE), simultaneous ultrasound and enzyme (UES-AE), enzyme before ultrasound (E-UAE), and no treatment (CONTROL). The TPC and TSC results from XTP stem extracts are displayed in Figure 3.

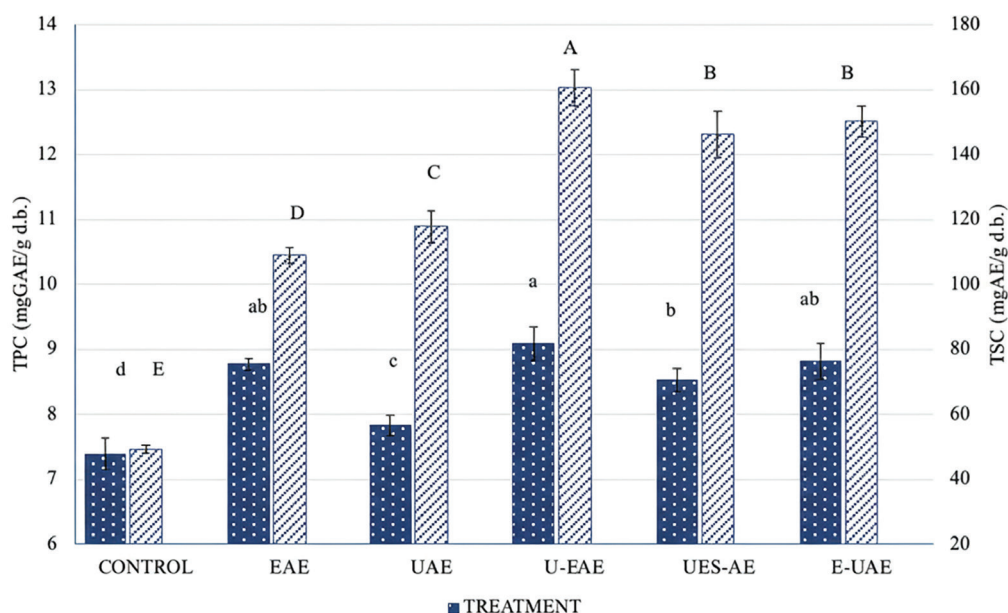


Figure 3. TPC and TSC of XTP stem extract obtained by different processing methods. The letters on the columns respectively indicate statistically significant differences at $P \leq 0.05$ of the control sample and the treated sample; $n = 3$; TPC: total phenolic content; TSC: total saponin content; CONTROL: no treatment; EAE: enzyme-assisted extraction; UAE: ultrasound-assisted extraction; U-EAE: ultrasound before enzyme; UES-AE: simultaneous ultrasound and enzyme; E-UAE: enzyme before ultrasound.

Figure 3 demonstrates that extracts from XTP stems using enzyme treatment, ultrasound, or a combination of significantly increased TSC compared to the control sample ($P < 0.05$). The U-EAE treatment method (TSC 160.55 ± 5.53 mg AE/g d.b.) yielded the highest results, followed by E-UAE (TSC 150.08 ± 4.67 mg AE/g d.b.) and UES-AE (TSC 146.14 ± 7.14 mg AE/g d.b.). These methods outperformed UAE (TSC 117.84 ± 4.94 mg AE/g d.b.) and EAE (TSC 108.87 ± 2.64 mg AE/g d.b.). For TPC, combined enzyme-ultrasound methods and enzyme-only treatments showed similar results, both surpassing the ultrasound method (TPC 7.83 ± 0.41 mg GAE/g d.b.).

According to Figure 3, the extraction methods for TPC and TSC are ranked as follows: U-EAE > E-UAE > UES-AE > UAE > EAE > CONTROL. The research on combined enzyme

and ultrasound extraction methods revealed that U-EAE was most effective for TPC and TSC extraction (TPC 9.09 ± 0.25 mg GAE/g d.b.; TSC 160.55 ± 5.53 mg AE/g d.b.). This method increased TPC and TSC by 1.23 and 3.27 times, respectively, compared to untreated samples. U-EAE's high extraction yield likely results from ultrasonic waves randomly disrupting cell walls and enzymes selectively degrading them (Lieu & Le, 2010). Moreover, combining different enzymes enhances solvent penetration, releasing more bioactive compounds and increasing extraction yield (Miron et al., 2013).

Ultrasound-assisted enzymatic extraction proves most effective for extracting TPC, TFC, and TAC compared to ultrasound-only or enzyme-only treatments (Tchabo et al., 2015). Li et al. (2017) confirmed U-EAE as an efficient alternative, improving extraction yield and

enhancing antioxidant and anticancer properties of phenolics from *Trapa quadrispinosa* stems. U-EAE is more efficient, faster, and cost-effective than other processes, aligning with previous studies (Dang et al., 2012; Nguyen et al., 2013; Nag & Sit, 2018). This effectiveness likely stems from the synergy between ultrasound and enzymes: ultrasound creates cavitation, turbulence, and shearing, breaking down compounds into smaller particles, which allows enzymes to react more efficiently (Zhao et al., 2023).

4. Conclusions

This study optimized the extraction of bioactive compounds from XTP stems using enzyme-assisted extraction (EAE). The optimal conditions were found to be 0.8% v/w enzyme concentration at 45°C for 105 min. The combining of ultrasound and enzyme treatment (U-EAE) proved the most effective, increasing total phenolic content and total saponin content by 1.23 and 3.27 times, respectively, compared to untreated samples. Ultrasound before enzyme outperformed other methods (enzyme before ultrasound, simultaneous ultrasound and enzyme, ultrasound-assisted extraction, enzyme-assisted extraction) in extracting bioactive compounds, demonstrating its potential for the extraction of valuable compounds from Xao tam phan.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

This research was funded by Nong Lam University Ho Chi Minh City, Vietnam under grant number CS-CB22-HHTP-01. We would like to thank Nong Lam University Ho Chi Minh City for the support of this study.

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