Asian Journal of Biotechnology and Bioresource Technology

Volume 9, Issue 3, Page 20-30, 2023; Article no.AJB2T.108139
ISSN: 2457-0125

Application of Response Surface Methodology for Optimizing the Extraction Conditions of Total Saponins from *Polyscias fruticosa* (L.) Harms Planted in Southern Vietnam

Hoang Le Son [a], Vo Ngoc Lam Phuong [a] and Tran Van Minh [a*]

[a] Department of Applied Biochemistry, School of Biotechnology, International University – Vietnam National University – HCMC, Vietnam.

Authors' contributions

This work was carried out in collaboration among all authors. 'All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/AJB2T/2023/v9i3185

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/108139

Original Research Article

Received: 17/08/2023
Accepted: 25/10/2023
Published: 26/10/2023

ABSTRACT

Aims: This study aimed to optimizing the extraction conditions for total saponins from *Polyscias fruticosa* using response surface methodology coupled with Box-Behnken design (RSM-BBD).
Methodology: The optimization process for extraction of saponins from the roots of *Polyscias fruticosa* was involved in three parameters including extraction temperature, time, and solvent-to-sample ratio. The three levels of independent variables were identified by screening test. The response surface methodology conjugated with Box-Behnken design was then applied to

**Corresponding author: E-mail: drminh.ptntd@yahoo.com;*

investigate the affinities between the extraction variables and experimental response (extraction yield) which was expressed in terms of mg oleanolic acid equivalents (OAE) per gram of plant extract.

Results: Based on the analysis of all integrated data, the optimal extraction conditions were predicted to be temperature of 60.91°C, extraction time of 4.57 hours and solvent/sample ratio of 10.3:1 (mL/g). The highest total saponins content value predicted by response surface methodology was 37.18 mg OAE/g of plant extract which was in close agreement with the corresponding experimental value of total saponins content (37.42 ± 0.26 mg OAE per g of plant extract) (n = 3, $p < 0.05$).

Conclusion: The strong correlation between the real and the predicted data indicated that the response model was adequate to reflect the expected optimization.

Keywords: Polyscias fruticosa; saponins; optimization; response surface methodology; box–behnken.

1. INTRODUCTION

Polyscias fruticosa (L.) Harms (*P. fruticosa*) belongs to the Araliaceae family, commonly known as the ginseng family. The plant has other names such as *Panax fruticosum*, *Panax fruticosus*, *Nothopanax fruticosum* or *Ming aralia* [1]. In Vietnam, the roots of *P. fruticosa* have long been used as a traditional medicine for treatment of various illnesses such as depression, insomnia, fatigue, rheumatoid arthritis. In addition, it is commonly believed that it might be helpful in enhancing blood circulation to brain tissues and protecting against neurodegenerative pathology such as Alzheimer and Parkinson. The main compound isolated from the roots of *P. fruticosa* is oleanolic acid saponins [2]. Saponins are secondary metabolites that are widely distributed in nature. 'Saponins' are derived from the Latin word soapwort (*Saponaria officinalis* L.), which means 'soap', because saponin molecules form soap-like foams in aqueous solutions. This property is due to its amphiphilic nature with the presence of a lipid-soluble aglycone and water-soluble chain(s) in their structure [3]. "In traditional and pharmaceutical applications, the biological activities of saponins include hemolytic, anti-inflammatory, antimicrobial, insecticidal and molluscicidal properties" [4]. "Besides that, at least 150 different types of natural saponins have been identified to possess significant anti-cancer properties" [5].

Extraction plays a crucial role in the recovery of saponins from the roots of *P. fruticosa*. The efficiency of extraction is attributed primarily to a variety of factors including extraction temperature, time [6], solvent/sample ratio [7], and particle size of the plant material [8]. Traditionally, the optimization of medicinal plant extraction has been conducted by screening the effect of one variable at a time on an experimental response [9]. This so-called one-factor-at-a-time optimization technique is involved in a large number of experiments necessary to perform the research, resulting in time-consuming processes and an increase in the consumption of materials and reagents. Moreover, the possible interaction effects among various factors can be ignored and misleading a true optimum condition. The actual response of the extraction process is influenced by the interaction between factors.

"Response surface methodology (RSM) can overcome the limitations of traditional optimization. RSM consists of a group of mathematical and statistical techniques that enables analysis of the effects of multiple factors and their interactions" [10,11,12]. "RSM is based on the fit of empirical models to the experimental data obtained in relation to experimental design, leading to the employment of polynomial functions to express the system studied and to display experimental conditions until its optimization" [13,14]. Using RSM, values for a given response at any combination of factors can also be predicted. Therefore, optimization of extraction conditions can be obtained for maximum efficacy.

Box–Behnken design (BBD) is a widely used experimental design in RSM [15,16]. The BBD for three-variable optimization with 13 to 17 experimental points (depending on the number of central points) is established. In comparison with the full one-factor-at-a-time design with $3^3 = 27$ experiments, it is noted that BBD is more economical and efficient. Therefore, RSM is more efficient not only in reducing the number of experimental runs and time needed for investigating the optimal conditions for extraction, but also improving the statistical interpretation

and demonstrating the interaction between variables.

In this study, the parameters involving the extraction of saponins from the roots of *P. fruticosa* including extraction temperature, time, and solvent/sample ratio were initially screened by single factor test to select the independent variables of major effects on the system. The RSM in conjunction with BBD was then applied to investigate the affinities between the extraction variables and experimental response (extraction yield) which was expressed in terms of mg oleanolic acid equivalents (OAE) per gram of plant extract. This present research thus aimed to optimizing the extraction conditions for total saponins from *P. fruticosa* using response surface methodology coupled with Box-Behnken design (RSM-BBD).

2. MATERIALS AND METHODS

2.1 Materials and Reagents

The fresh roots of 5-years-old *P. fruticosa* were collected in Dong Nai Province, Southern Vietnam. The plant was authenticated by The Institute of Tropical Biology Vietnam, and a voucher specimen of No. AB Bio–15–05–02 had been deposited in the herbarium of Applied Biochemistry Laboratory, Department of Applied Biochemistry, School of Biotechnology, International University Vietnam National University – Ho Chi Minh City, Vietnam. Concentrated sulfuric acid and ethanol were purchased from Merck (Darmstadt, Germany). Oleanolic acid and vanillin were purchased from Sigma-Aldrich (USA). All chemicals and reagents were stored in accordance with the most stringent regulations and freshly prepared with distilled water to the desired concentrations for experimental purposes.

2.2 Preparation of Materials

The fresh roots of *P. fruticosa* were washed thoroughly with running water to remove any possible contaminants. The roots were cut into small pieces prior to being dried in the oven at 60°C until the moisture concentration drops down to below than 10%. The dried roots were then ground into fine powder using a mechanic grinder. The fine root powder was passed through a 0.5 mm sieve for particles having size of 0.5 mm or smaller [17]. Dried root powder of *P. fruticosa* was packaged in a sealed bag and stored in a desiccator for further usage.

2.3 Methods

2.3.1 Screening test for independent variables

The optimization process for extraction of saponins from the roots of *P. fruticosa* was involved in three factors including extraction temperature, time, and solvent-to-sample (S/S) ratio. The total saponin content was then extracted using distilled water as solvent in an ultrasound water bath apparatus. Experiments were designed with variables ranges for each factor as described in Table 1 [18]. All the experiments were performed in triplicate. All the samples were collected and filtered through Whatman No.1 filter paper to remove debris. The final filtrates were concentrated under reduced pressure using a rotary evaporator until forming a brownish residue. The residue, known as total extracts, was cold stored in the refrigerator at 4^{o}C prior to further assay.

2.3.2 Determination of total saponin content

Total saponin content (TSC) was determined by Vanillin-Sulfuric acid method [19,20]. Oleanolic acid (OA) was used as standard for the calibration curve. The assay was prepared by mixing 0.5 mL of the extract with 4.5 mL of distilled water to make a 10-time diluted sample. 0.5 mL of diluted sample was then mixed with 1 mL of vanillin (8%, w/v in ethanol), followed by the addition of 5 mL of 72% sulfuric acid and cooling the reaction mixture in the ice bath. After 3 min, the mixture was transferred into 60°C water bath to warm up for 10 min. The mixture was then cooled down in ice-cold water for 3 min. The absorbance of the mixture was measured against the blank at 544 nm using UV-VIS spectrophotometer (Thermo Scientific, USA) and Biotek Synergy HT 96- well plate. The TSC of the samples was then expressed in mg of OA equivalents (OAE) per gram of extract (mg OAE/g of extract).

Table 1. Screening test for independent variables

Factor	Independent variable	Constant variable
Extraction temperature	30, 40, 50, 60 and 70˚C	10:1 S/S ratio in 3 h extraction time
Extraction time	1, 2, 3, 4, 5 and 6 hours	10:1 S/S ratio at 40˚C
Solvent-to-sample ratio	6:1, 8:1, 10:1 and 12:1 (mL/g)	40˚C in 3 h extraction time

Table 2. Box–Behnken design and observed responses

Run	Independent variable			Response* (mg OAE/g of extract)
	X1 (Temperature, ˚C)	X2 (Time, h)	X3 (S/S ratio, mL/g)	
1	50 (-1)	4 (-1)	10 (0)	32.79
2	70 (+1)	4 (-1)	10 (0)	33.78
3	50 (-1)	6 (+1)	10 (0)	29.81
4	70 (+1)	6 (+1)	10 (0)	30.23
5	50 (-1)	5 (0)	08 (-1)	28.53
6	70 (+1)	5 (0)	08 (-1)	30.27
7	50 (-1)	5 (0)	12 (+1)	30.75
8	70 (+1)	5 (0)	12 (+1)	31.40
9	60 (0)	4 (-1)	08 (-1)	31.71
10	60 (0)	6 (+1)	08 (-1)	27.88
11	60 (0)	4 (-1)	12 (+1)	34.03
12	60 (0)	6 (+1)	12 (+1)	28.97
13	60 (0)	5 (0)	10 (0)	36.60
14	60 (0)	5 (0)	10 (0)	36.54
15	60 (0)	5 (0)	10 (0)	36.94

**Average value of triplicate experiments*

2.3.3 Experimental design

Three-level Box-Behnken design (BBD) was employed for the optimization of saponins extraction from the roots of *P. fruticosa*. The three independent extraction variables were extraction temperature (X_1, ˚C), extraction time (X_2, hours) and S/S ratio (X_3, mL/g), at three levels (-1, 0, +1). The extraction yield (Y) was considered as the response. A total of 15 experiments were designed as detailed in Table 2. A second-order polynomial model was used to express the extraction as a function of the independent variables by applying the equation (1).

$$Y = \beta_0 + \sum_{i=1}^{3} \beta iXi + \sum_{i=1}^{3} \beta ii X_i^2 + \sum_{i=1}^{2} \sum_{j=i+1}^{3} \beta_{ij} X_i X_j \quad (1)$$

Where:

Y was the response variable.
β_0, β_i, β_{ii}, and β_{ij} were the constant, linear, quadratic, and interactive coefficients, respectively.
X_i and X_j were the levels of the independent variables.

2.4 Statistical Analysis

All experiments were conducted in triplicate, and the results were expressed in terms of Mean ± Standard Error of Mean (SEM). Statistical analysis was performed by SPSS and analysis of variance (ANOVA) with a *p*-value significance level of 0.05.

Minitab (Trail Version 16.2.4, Mini Tab Inc., USA) and Design-Expert (Trail Version 10.0.6, State-Ease, Inc., Minneapolis MN, USA) were used to obtain the coefficients of the quadratic polynomial model. The adequacy of the fitted model was determined by evaluating the lack of fit, the coefficient of determination (R^2), and its statistical significance was checked by an F-test from the analysis of variance (ANOVA). The significance was determined by using t-test at 0.05 levels.

3. RESULTS AND DISCUSSION

3.1 Determining Levels for Independent Variables

The three levels of the extraction temperature variable were determined based on the results of screening test carried out at 30, 40, 50, 60 and 70˚C with 10:1 S/S ratio in 3 h extraction time. A significant increase of TSC was observed in line with the increasing temperature. However, the highest TSC was recorded 32.56 ± 0.24 mg OAE/g of extract at 60˚C (Fig. 1). The extraction temperature of 50, 60 and 70˚C were then selected for the three levels of extraction temperature variable coded -1, 0, +1, respectively. The effect of different extraction time on TSC was examined for 1, 2, 3, 4, 5 and 6 hours with the 10:1 S/S ratio at the extraction temperature of 40˚C. The TSC significantly

increased with the increasing extraction time and reached the highest TSC value of 33.28 ± 0.14 mg OAE/g of extract in 5 h extraction time (Fig. 2). The three levels selected for the temperature variable were 4, 5 and 6 hours, respectively. The effect of different ratios of S/S on TSC was investigated with the ratio of 6:1, 8:1, 10:1 and 12:1 at 40˚C for 3 hours. The TSC gradually increased from 19.24 ± 0.08 (6:1 S/S ratio) to 24.86 ± 0.21 (8:1 S/S ratio), reached the highest value of 29.59 ± 0.25 (10:1 S/S ratio), and slightly decreased to 26.80 ± 0.24 mg OAE/g of extract (12:1 S/S ratio) (Fig. 3). The ratio of 8:1, 10:1 and 12:1 was then selected as the three variable levels for the S/S ratio. The three levels for independent variables were summarized in Table 3.

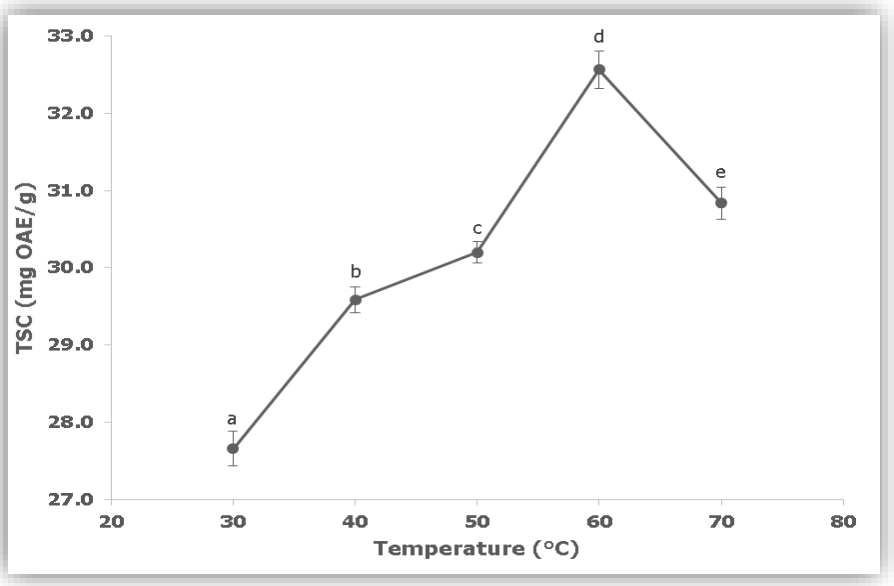

Fig. 1. Effect of temperature on TSC (n = 3). Values are presented as means ± standard deviation. [(a-e)]Values marked by different letters are significantly different ($p < 0.05$)

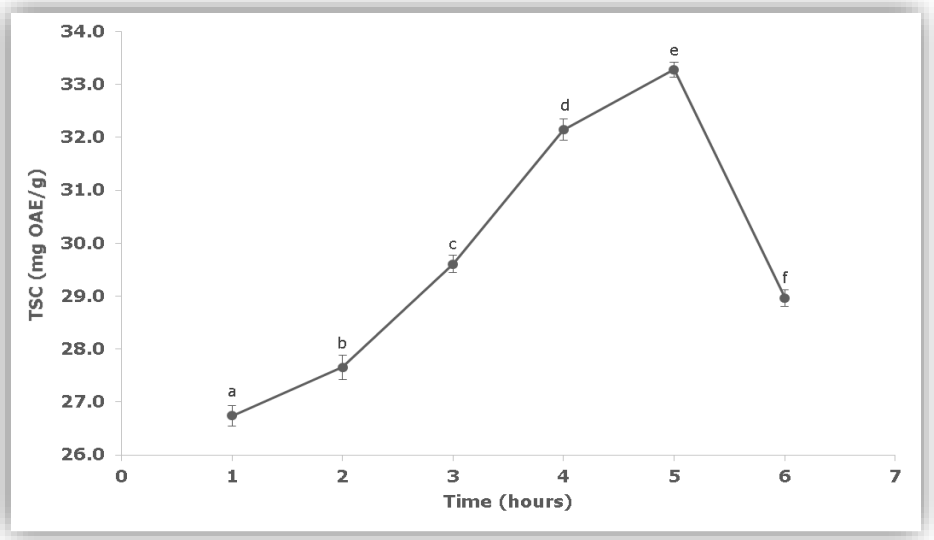

Fig. 2. Effect of time on TSC (n = 3). Values are presented as means ± standard deviation. [(a-f)]Values marked by different letters are significantly different ($p < 0.05$)

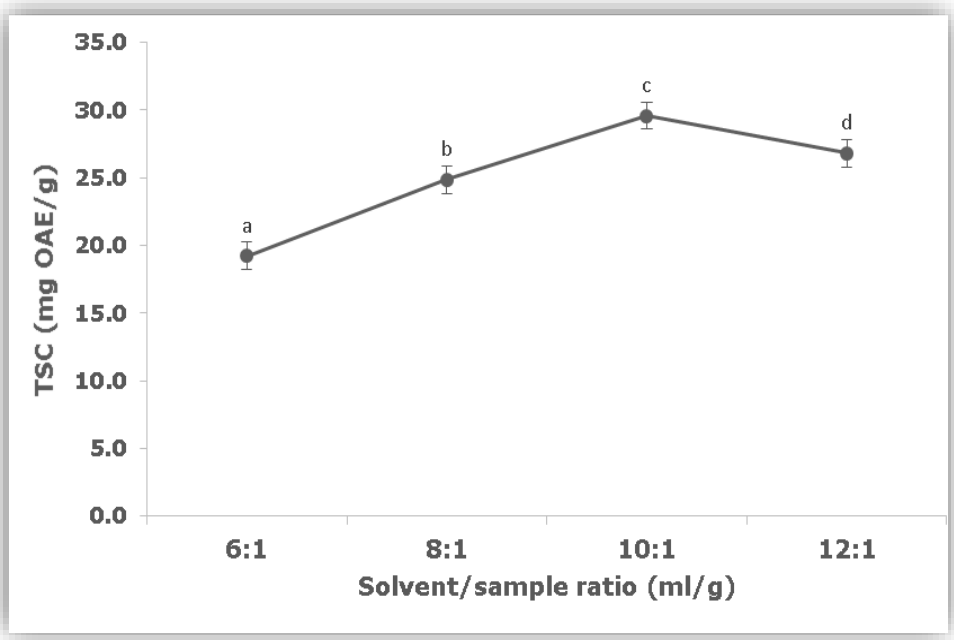

Fig. 3. Effect of solvent/sample ratio on TSC (n = 3). Values are presented as means ± standard deviation. [(a-d)]Values marked by different letters are significantly different ($p < 0.05$)

Table 3. Levels for independent variables

Independent variable	Unit	Symbol	Coded levels		
			-1	0	+1
Extraction temperature	˚C	X1	50	60	70
Extraction time	hour	X2	4	5	6
Solvent/sample ratio	mL/g	X3	8:1	10:1	12:1

Table 4. ANOVA table for the effect of temperature, time, and S/S ratio on TSC extraction

Parameter[a]	Coefficient Estimate	Standard Error	DF[b]	Sum of Squares	F-Value	*p*-value Prob > F
Intercept				**Model**		
β_0	36.69	0.25	1	124.25	75.76	< 0.0001*
β_1	0.48	0.15	1	1.81	9.91	0.0254*
β_2	-1.93	0.15	1	29.69	162.95	< 0.0001*
β_3	0.85	0.15	1	5.74	31.50	0.0025*
β_{12}	-0.14	0.21	1	0.078	0.43	0.5411
β_{13}	-0.27	0.21	1	0.30	1.64	0.2566
β_{23}	-0.31	0.21	1	0.37	2.05	0.2120
β_{11}	-2.73	0.22	1	27.43	150.51	< 0.0001*
β_{22}	-2.31	0.22	1	19.77	108.49	0.0001*
β_{33}	-3.73	0.22	1	51.36	281.82	< 0.0001*
Lack of Fit			3	0.82	5.92	0.1479
Pure Error			2	0.092		
R2	0.9927		Adj R2	0.9796		
C.V.%	1.33		PRESS	13.31		

a Coefficients refer to the general model.
b Degree of freedom.
** Significant value*

3.2 Model Fitting

Table 2 presents the extraction conditions and corresponding experimental response data of a BBD. The extraction parameters and response variables were analyzed to fit a regression equation that could predict the response within the given range of the independent variables. An analysis of variance (ANOVA) was performed for the model fitted to the experimental data, the regression coefficients of the intercept, linear, quadratic, and interaction terms of the model were calculated and presented in Table 4. Second-order polynomial equations were given as below:

$$TSC = 36.69 + 0.48X_1 - 1.93X_2 + 0.85X_3 - 2.73(X_1)^2 - 0.14X_1X_2 - 2.31(X_2)^2 - 0.27X_1X_3 - 0.31X_2X_3 - 3.73(X_3)^2 \quad (2)$$

The coefficient of determination value (R^2 = 0.9927) indicates a very strong relationship between these three variables. The second-order polynomial model was statistically significant and adequately represented the real relationship between the responses and the variables. The regression coefficients of the intercept, linear, quadratic, and interaction terms of the model were calculated and presented in Table 4. The ANOVA result indicated one linear parameter and all quadratic parameters were found to be significant ($p < 0.05$), whereas all the interaction parameters were insignificant ($p > 0.1$). The ANOVA evaluation revealed that the effect of the S/S ratio was the major contributing factor to the yield of TSC. Furthermore, results of the error analysis showed that the lack of fit was insignificant ($p > 0.05$). The coefficient of variation (C.V.%, 1.33) of less than 5% indicated that the model was reproducible. The Predicted Residual Sum of Squares (PRESS) for the model, which was a measure of how a particular model fits each point in the design, was 13.31. The model F-value was 75.76, reflecting that the model was significant.

3.3 Response Surface Analysis

To express the effect of any independent variable on the TSC extraction, the three-dimensional surface plots were generated based on the equation (2). The relationships between the extraction parameters and the response could be better understood by varying two variables within the experimental range under investigation and holding the other variable constant at its central level (0 level).

Fig. 4 shows the response surface plots for the effect of temperature and time on the extraction yield of TSC at a fixed S/S ratio of 10:1. Both temperature and time simultaneously influenced TSC extraction. The yield of TSC increased slightly with the increase of the extraction temperature and reached the peak at 60.91°C, followed by gradually declining with the further increase of the extraction temperature. Fig. 5 shows the response surface plots for the effect of time and the S/S ratio on the extraction yield of TSC at a fixed extraction temperature of 60°C. The yield of TSC steadily decreased with an increase in solvent volume. However, the highest value of TSC was noted at 4.57 hours and ratio of 10.3:1 S/S. Fig. 6 shows the response surface plots for the effect of temperature and the S/S ratio on the extraction yield of TSC at 5 h extraction time. It was evident that the extraction temperature and S/S ratio were the major contributing factors to the efficient extraction of saponins from the roots of *P. fruticosa.*

3.4 Attaining Optimum Extraction Conditions

The optimum conditions for extraction of saponins from the roots of *P. fruticosa* were obtained from software Design-Expert. Analysis of all integrated data revealed that the optimum extraction conditions were predicted to be temperature of 60.91°C, extraction time of 4.57 hours and S/S ratio of 10.3:1 (mL/g) (particle size ≤ 500 µm). The highest TSC value predicted by RSM was 37.18 mg OAE/g of plant extract. This predicted TSC value was found to be in close agreement with the corresponding experimental value of TSC (37.42 ± 0.26 mg OAE per g of plant extract) (n = 3), which demonstrated the validity of the RSM model, since there were no significant *(p* > 0.05) differences between 37.18 and 37.42 ± 0.26 mg OAE/g of plant extract. The strong correlation between the real and the predicted data indicated that the response model was adequate to reflect the expected optimization.

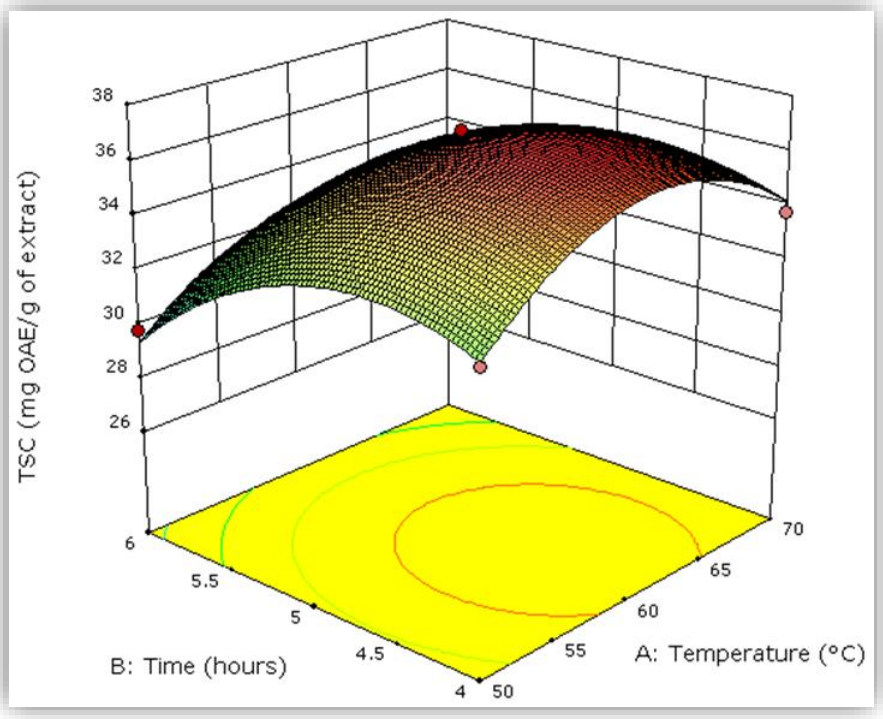

Fig. 4. The effect of temperature and time on the extraction yield of TSC at a fixed solvent/sample ratio of 10:1

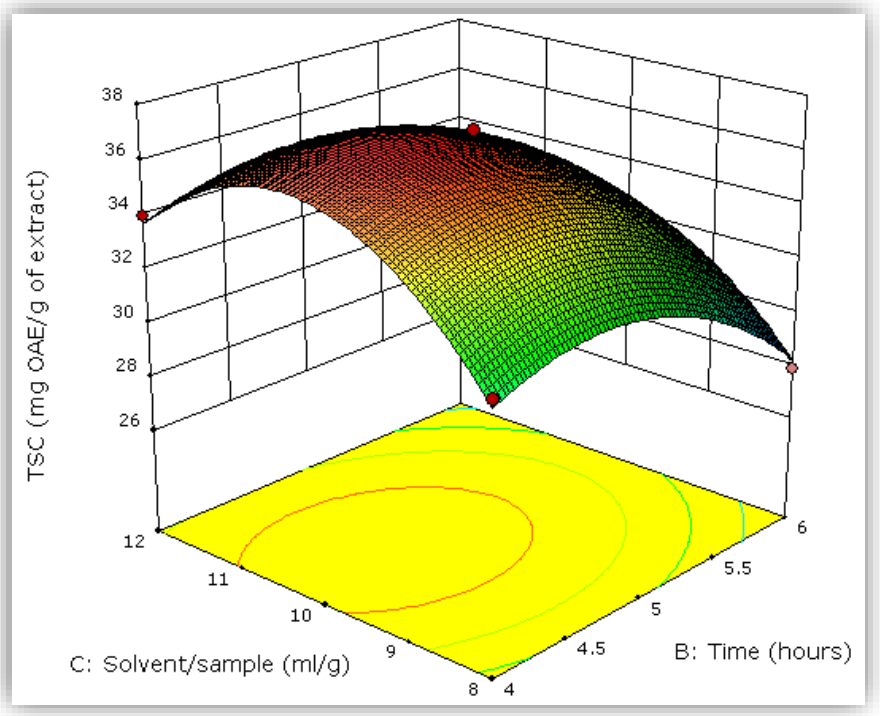

Fig. 5. The effect of time and the solvent/sample ratio on the extraction yield of TSC at a fixed extraction temperature of 60°C

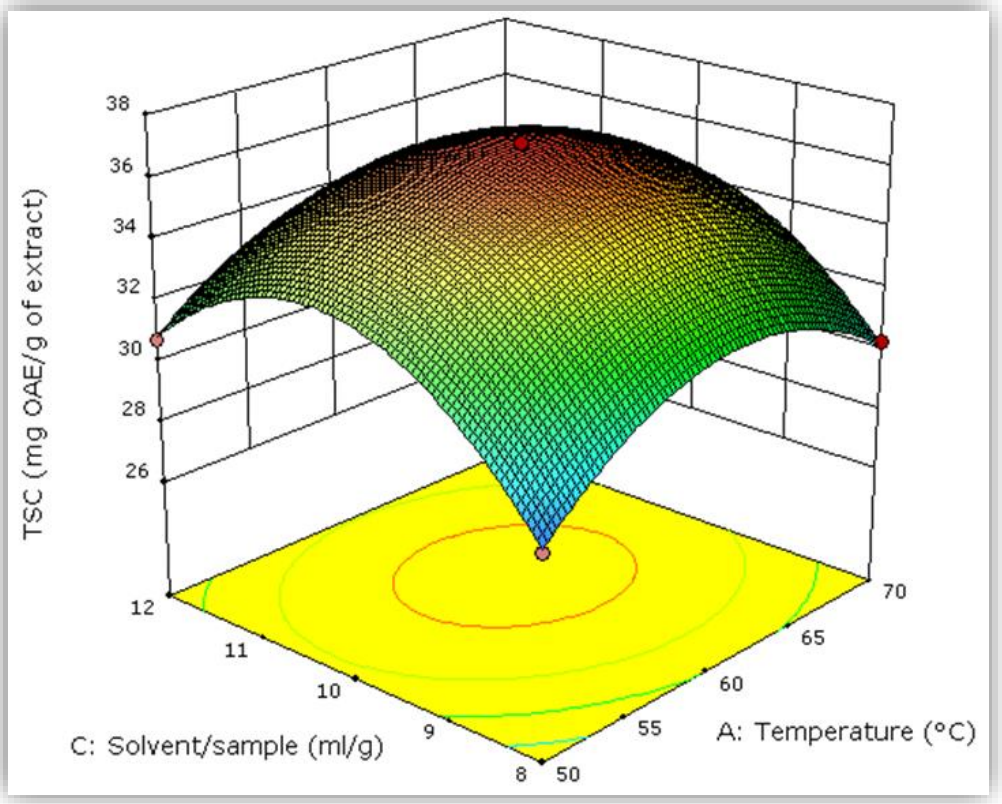

Fig. 6. The effect of temperature and the solvent/sample ratio on the extraction yield of TSC at a fixed extraction time of 5 hours

Table 5. Optimum conditions for the extraction of the highest yield of TSC

Independent variable			Predicted response (mg OAE/g of extract)	Experimental response (mg OAE/g of extract)
X1 (Temperature, ˚C)	X2 (Time, hours)	X3 (S/S ratio, mL/g)		
60.91°C	4.57	10.3:1	37.18	37.42 ± 0.26

4. CONCLUSION

In this present study, conclusively, the predicted TSC value of 37.18 mg OAE/g of plant extract was found to be in close agreement with the corresponding experimental value of TSC (37.42 ± 0.26 mg OAE per g of plant extract). The optimum extraction conditions were predicted to be temperature of 60.91°C, extraction time of 4.57 hours and S/S ratio of 10.3:1 (mL/g) (particle size ≤ 500 µm). These findings confirmed the reliability of using the response surface methodology coupled with a Box–Behnken design in optimizing the extraction conditions for TSC from the roots of *P. fruticosa.*

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Seidemann J. World spice plants: Economic usage, botany, taxonomy. published by springer berlin Heidelberg; 2010. ISBN 10: 3642060889 ISBN 13: 9783642060885.
2. Rojas-Sandoval J. Polyscias fruticosa (*Ming aralia*). CABI Compendium; 2022. Available:10.1079/cabicompendium.46036.

3. Güçlü-Ustündağ O, Mazza G. Saponins: properties, applications and processing. Crit Rev Food Sci Nutr. 2007;47(3):231-58.
DOI: 10.1080/10408390600698197. PMID: 17453922.

4. Sparg SG, Light ME, van Staden J. Biological activities and distribution of plant saponins. J Ethnopharmacol. 2004;94(2-3):219-43.
DOI: 10.1016/j.jep.2004.05.016. PMID: 15325725.

5. Man S, Gao W, Zhang Y, Huang L, Liu C. Chemical study and medical application of saponins as anti-cancer agents. Fitoterapia. 2010 Oct;81(7):703-14.
DOI: 10.1016/j.fitote.2010.06.004. Epub 2010 Jun 13. PMID: 20550961.

6. Che Sulaiman IS, Basri M, Fard Masoumi HR, et al. Effects of temperature, time, and solvent ratio on the extraction of phenolic compounds and the anti-radical activity of *Clinacanthus nutans* Lindau leaves by response surface methodology. Chemistry Central Journal. 2017;11: 54.
Available:https://doi.org/10.1186/s13065-017-0285-1

7. Monagas M, Brendler T, Brinckmann J, Dentali S, Gafner S, Giancaspro G, et al. Understanding plant to extract ratios in botanical extracts. Front. Pharmacol. 2022. 13:981978.
DOI: 10.3389/fphar.2022.981978

8. Archana RP, Dattaprasad NV, Jadhav RS. Recent advances in extraction techniques of herbals – A review. Asian J. Res. Pharm. Sci. 2020;10(4):287-292.
DOI: 10.5958/2231-5659.2020.00050.8

9. Connor JT, Alexander P, Kobi CF, Rachel G, Magda B, Thomas WC, et al. A brief introduction to chemical reaction optimization. Chemical Reviews. 2023; 123(6):3089-3126.
DOI: 10.1021/acs.chemrev.2c00798.

10. Alam MS, Damanhouri ZA, Ahmad A, Abidin L, Amir M, Aqil M, et al. Development of response surface methodology for optimization of extraction parameters and quantitative estimation of embelin from Embelia ribes Burm by high performance liquid chromatography. Pharmacogn Mag. 2015;11(Suppl 1):S166-72.
DOI: 10.4103/0973-1296.157722. PMID: 26109763; PMCID: PMC4461957.

11. Aydar AY. Utilization of response surface methodology in optimization of extraction of plant materials [Internet]. Statistical Approaches With Emphasis on Design of Experiments Applied to Chemical Processes. InTech; 2018.
Available:http://dx.doi.org/10.5772/intechopen.73690

12. Brzezińska R, Wirkowska-Wojdyła M, Piasecka I, Górska A. Application of response surface methodology to optimize the extraction process of bioactive compounds obtained from coffee silverskin. Applied Sciences. 2023;13(9): 5388.
Available:https://doi.org/10.3390/app13095388

13. Bezerra M, Santelli R, Oliveira E, Villar L, Escaleira L. Response Surface Methodology (RSM) as a tool for optimization in analytical chemistry. Talanta. 2008;76:965-77.
Available:10.1016/j.talanta.2008.05.019

14. Riswanto FDO, Rohman A, Pramono S, Martono S. Application of response surface methodology as mathematical and statistical tools in natural product research. J Appl Pharm Sci. 2019;9(10):125–133.

15. Robinson T. Box–Behnken Designs; 2008.
Available:10.1002/9780470061572.eqr029

16. Peng X, Yang G, Shi Y, et al. Box–Behnken design based statistical modeling for the extraction and physicochemical properties of pectin from sunflower heads and the comparison with commercial low-methoxyl pectin. Sci Rep. 2020;10:3595.
Available:https://doi.org/10.1038/s41598-020-60339-1

17. Methods Optimization in Accelerated Solvent Extraction in Technical note. 2013;208:1-4.
Available:https://tools.thermofisher.com/content/sfs/brochures/TN-208-Methods-Optimization-TN70742-EN.pdf.

18. Cheok CY, Abdelkarim H, Sulaiman R. Extraction and quantification of saponins: A review. Food Research International. 2014;59:16–40.
Available:10.1016/j.foodres.2014.01.057.

19. Du MH, Guo SH, Zhang J P, Hu LS, Li MZ. Quantitative analysis method of the tea

saponin. Open Journal of Forestry. 2018; 8:61-67.
Available:https://doi.org/10.4236/ojf.2018.81005

20. Li P, Liu J. Ginseng nutritional components and functional factors; 2020.
Available:10.1007/978-981-15-4688-4

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/108139