Trop J Nat Prod Res, March 2024; 8(3):6552-6557 ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Optimization of Solvent Sonication-Maceration for Enhancing Total Phenolic Content and Antioxidant Activity of *Portulaca oleracea* L. Extract Using the Simplex Centroid Design Method

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ARTICLE INFO

Article history:
Received 10 December 2023
Revised 23 February 2024
Accepted 05 March 2024
Published online 01 April 2024

ABSTRACT

The extraction of bioactive compounds from *Portulaca oleracea* L., a plant renowned for its diverse pharmacological effects, is significantly influenced by the choice of solvent. Therefore, this study aims to identify the optimal solvent or the combination of solvents for extracting *P. oleracea* using the simplex centroid design (SCD) method based on total phenolic content (TPC) and antioxidant capacity. The plant samples were extracted using a combination of sonication and maceration methods, and subjected to optimization using Design Expert 13.0. TPC was then quantified using the Folin-Ciocalteu method, while antioxidant capacity was assessed with the ferric-reducing antioxidant power (FRAP) method. The results showed that acetone-water was the solvent combination with the highest TPC, while acetone-water-methanol gave the highest antioxidant capacity. Based on a quadratic model with R^2 values of 0.9331 for TPC and 0.8074 for FRAP, the optimal solvent formulations were water (0.342), acetone (0.389), and ethanol (0.269), achieving a desirability level of 0.884. In addition, confirmation tests validated the results within the permissible interval (PI) values, indicating the reliability of the proposed model. Solvent combinations for *P. oleracea* extraction were successfully optimized through the application of SCD, yielding extract rich in phenolic content and antioxidant capacity. The results were expected to serve as a foundation for future investigations of *P. oleracea* and its pharmacological potential.

***Keywords*:** Antioxidant, optimization, *Portulaca oleracea*, total phenolic, simplex centroid design

Introduction

Purslane (*Portulaca oleracea* L.) is a common weed belonging to the family Portulacaceae and is characterized by reddish stems and alternate leaves. In addition, this resilient herbaceous plant thrives worldwide, primarily in the tropical and subtropical regions, and is recognized for its diverse culinary applications as green or yellow leafy potherbs.[1] Despite the culinary applications, purslane possesses a myriad of pharmacological effects that have garnered significant scientific interest. These effects include anti-inflammatory, wound healing, neuroprotective, anti-asthmatic, antibacterial, and antioxidant properties.[2–7]

According to previous studies, the pharmacological potency of purslane can be attributed to its rich secondary metabolite content, comprising an array of phenolic compounds. These compounds include cinnamic acid derivatives, benzoic acid derivatives, ferulic acid, caffeic acid, p-coumaric acid, syringic acid, and gallic acid.[8,9] Purslane has also been reported to contain flavonoids, alkaloids, terpenoids, vitamins, minerals, and fatty acids, all contributing to the therapeutic potential.[9,10]

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Citation: Putra RP, Aisyah SI, Kurniatin PA, Nurcholis W. Optimization of Solvent Sonication-Maceration for Enhancing Total Phenolic Content and Antioxidant Activity of *Portulaca oleracea* L. Extract Using the Simplex Centroid Design Method. Trop J Nat Prod Res. 2024; 8(3):6552-6557. https://doi.org/10.26538/tjnpr/v8i3.12

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria

In line with previous studies, the extraction of these valuable compounds from purslane is largely dependent on the selection of solvent, with the polarity of solvent being an essential factor influencing the extract quality.[11] Achieving high-quality extract is important for harnessing the full potential of inherent bioactive components. Furthermore, optimal solvent selection for secondary metabolite extraction depends on various factors, including plant variety, bioactive compound content, and the specific plant part being targeted for extraction.[12] Several studies showed that polar bioactive compounds were best dissolved in polar solvents, while non-polar compounds were more efficiently extracted using non-polar solvents.[13]

In optimizing the extraction process, the simplex centroid design (SCD) has become a valuable experimental method. In addition, this method enables the optimization of responses by varying the proportions of several variable components while maintaining a total constant (100%).[14] Several studies also successfully applied SCD to optimize the extraction process of various plant materials, including *Curcuma aeruginosa* RoxB.,[15] cardamom fruit,[16] *Justicia gendarussa* Burm.f,[17] and purple leaves (*Graptophyllum pictum*).[18]

Despite the significant pharmacological potential of purslane, there are limited reports on optimizing the extraction solvent for total phenolic content (TPC) and antioxidant capacity in the extract. This indicates that the exploration to identify the ideal solvent or combination of solvents to achieve the highest TPC, coupled with potent antioxidant capacity remains largely unexplored. Therefore, this study aims to determine the optimal extraction solvent or solvent combination for *P. oleracea* extraction using TPC and antioxidant capacity as essential parameters.

Materials and Methods

Materials

Ethanol (pro-analysis), methanol (pro-analysis), acetone (pro-analysis), Folin-Ciocalteu reagent, sodium carbonate (Na_2CO_3), and HCl were obtained from Merck (Darmstadt, Germany), while trolox and glacial acetate acid were purchased from Sigma-Aldrich (St. Louis, USA). Furthermore, gallic acid ($C_7H_6O_5.H_2O$), 2,4,6-tripyridyl-s-triazine (TPTZ), and $FeCl_3$ were obtained from Sisco Research Laboratories Pvt. Ltd. (Maharashtra, India).

Plant preparation

P. oleracea (BMK0251102016) specimens were collected from the Tropical Biopharmaca Research Center, IPB University, Bogor, West Java, Indonesia, in June 2022. Aerial parts of the samples were dried at 45°C for 60 h, followed by mashing and sieving through a 60-mesh sieve to obtain a dried powder.[19] Furthermore, the powder obtained was stored at 29°C for further use.

Experimental design and extraction

The experimental method closely followed the procedures outlined in a previous study by Makkiyah *et al*.[18] The simplex centroid design was employed using Design Expert software, comprising 4 solvent components (water, acetone, methanol, and ethanol) to optimize the extraction of *P. oleracea*. The extraction process was carried out by combining 4 grams of purslane aerial parts with 40 mL designated solvent, as shown in Table 1. Subsequently, the solution was subjected to a 30-minute sonication process using a Decon F5 Major sonicator (Decon Laboratories, US). The sonicated solution was then macerated for 180 min in a WaterBath shaker (DAIHAN WiseBath, South Korea). The mixture obtained was then filtered to yield the filtrate, which was further concentrated to a volume of 20 mL using a rotary evaporator under 200 mBar. The final concentration of the filtrate was 0.2 g/mL, facilitating subsequent analyses of TPC and antioxidant capacity.

Total phenolic content (TPC)

TPC of *P. oleracea* was determined using a modified method based on Nurcholis *et al*.[20] In a 96-well microplate (BiologiX), 10 µL sample was combined with 160 µL distilled water and 10 µL Folin-Ciocalteu reagent. In addition, the mixture was incubated in darkness for 5 minutes. After incubation, 20 µL of 10% Na_2CO_3 was added, and the test solution was further incubated for 30 minutes in the dark. The absorbance of the solution was then measured at 750 nm using a spectrophotometer. Gallic acid served as the standard at concentrations ranging from 0 to 225 ppm ($y = 0.003x + 0.0025$; $R^2 = 0.9987$).

Measurement of antioxidant capacity

To assess the antioxidant capacity through ferric reducing antioxidant power (FRAP) method, this study used the procedure outlined by Arista *et al*.[21] FRAP reagent was prepared by combining acetate buffer (pH 3.6), FeCl3 (20 mM), and 10 mM tripyridyl-s-triazine (TPTZ) (in HCl 40 mM) in a 10:1:1 (v/v/v) ratio. A 10 µL sample was then pipetted into a microplate, followed by the addition of 300 µL FRAP reagent. Subsequently, the mixture was incubated for 30 min, and the absorbance was measured at 593 nm using a spectrophotometer (SPECTROstarNano BMG LABTECH, Germany). All measurements were conducted in triplicate, with Trolox serving as the standard and concentrations ranging from 0 to 600 µM ($y = 0.0013x + 0.0101$; $R^2 = 0.9994$).

Statistical analysis

Data analysis was conducted following the method outlined in Marliani *et al*., with minor adjustments.[17] TPC and antioxidant capacity determined through FRAP assay were subjected to statistical analysis using one-way ANOVA ($\alpha = 0.05$) and Tukey's HSD test ($\alpha = 0.05$) using IBM SPSS Statistics version 25. Furthermore, the optimal results obtained from Design Expert optimization software (Stat-Ease Inc., Minneapolis, MN, USA) were selected based on the highest desirability value. Confirmation of the optimization results comprised repeating the process 3 times and validation was performed by assessing the prediction interval (PI) value.

Table 1: Simplex centroid design for *P. oleracea* optimization solvents extraction

Run	Water (A)	Acetone (B)	Methanol (C)	Ethanol (D)
1	0.00	0.00	50.00	50.00
2	50.00	0.00	50.00	0.00
3	0.00	0.00	0.00	100.00
4	0.00	50.00	0.00	50.00
5	50.00	50.00	0.00	0.00
6	0.00	0.00	100.00	0.00
7	50.00	0.00	0.00	50.00
8	0.00	33.33	33.33	33.33
9	0.00	50.00	50.00	0.00
10	25.00	25.00	25.00	25.00
11	100.00	0.00	0.00	0.00
12	33.33	33.33	33.33	0.00
13	33.33	33.33	0.00	33.33
14	0.00	100.00	0.00	0.00
15	33.33	0.00	33.33	33.33

Results and Discussion

Optimization of extraction by simplex centroid design (SCD)

Purslane optimization of the extraction solvent was aimed at enhancing the extraction of phenolic compounds and antioxidant capacity by evaluating the potential interaction effects between solvents. Furthermore, solvents were mixed to determine whether the combined use could yield responses surpassing those achieved with individual solvents, ultimately determining the optimal composition. The results showed that the use of SCD comprising 4 solvent components, including water, acetone, methanol, and ethanol led to 15 unique formulas. Each combination in this experiment had an equivalent proportion of 1 or 100%.[22] As shown in Table 2, varying solvent composition exerted discernible effects on TPC and antioxidant capacity of purslane aerial parts extract. TPC and antioxidant capacity ranged from 0.1992 to 0.5098 mg GAE/g DW and 7.17 to 43.80 µmol

TE/g DW, respectively. The results indicated the significance of solvent selection and composition in optimizing the extraction of bioactive compounds from purslanes.

Fitting models

The response variables were subjected to analysis of variance (ANOVA) to assess the model's performance with 95% confidence intervals, as shown in Table 3. An R^2 coefficient exceeding 70% was considered indicative of a well-suited regression model, with values approaching one signifying further model improvement. In this study, a quadratic model was generated, explaining 93% ($p < 0.05$) of the variability in TPC and 80% ($p > 0.05$) of the variability in antioxidant capacity (FRAP). Furthermore, the adjusted R^2 values, reflecting the experimental results against the theoretical values, were 0.8127 and 0.4608 for TPC and FRAP, respectively. The adequacy precision value, which compared the predicted value range with the average prediction error at design points, exceeded the ideal threshold of 4, affirming the reliability of the proposed model.[23]

Effect of solvent system on TPC

The highest TPC of 0.5098 mg GAE/g DW was achieved using a water-acetone solvent in purslane extraction process. The results were consistent with Irfan *et al*.[24], demonstrating elevated TPC in the 50% (v/v) acetone extract of *Cymbopogon citratus leaves*. Similar results were also obtained by Nasr *et al*.[25], where the acetone-water extract of *Eucalyptus camaldulensis* exhibited the highest TPC among various solvent combinations. Although acetone alone was inefficient as solvent,[26] the addition significantly reduced the polarity of water, thereby enhancing the extraction efficiency. This phenomenon was attributed to the broader solubility of phenolic compounds in solvents with lower polarity than water. Consequently, it emphasized the effectiveness of organic-water solvent mixtures in maximizing phenolic compound extraction.[27,28] The observed lower TPC in purslane acetone extract compared to the water extract (0.2295 mg GAE/g DW) further showed the importance of solvent selection and composition in optimizing the extraction processes. The addition of acetone effectively decreased the polarity of water, thereby enhancing the extraction efficiency. According to previous studies, the extraction efficiency of phenolic compounds experienced a significant increase when employing a mixture of water and organic solvents, such as acetone, methanol, and ethanol compared to pure solvents, which tended to produce lower extraction yields (Figure 1).[29–31]

Table 2: Total phenolic contents and antioxidant capacity of *P. Oleracea*

	Solvent				Responses variable	
Run	**Water (A)**	**Aceton (B)**	**Methanol (C)**	**Ethanol (D)**	**TPC (mg GAE/g DW)**	**FRAP (µmol TE/g DW)**
1	0.00	0.00	50.00	50.00	0.4245 ± 0.02^{a}	18.63 ± 1.00^{de}
2	50.00	0.00	50.00	0.00	0.3856 ± 0.06^{a}	27.10 ± 1.04^{c}
3	0.00	0.00	0.00	100.00	0.4194 ± 0.02^{a}	18.25 ± 0.22^{de}
4	0.00	50.00	0.00	50.00	0.4462 ± 0.02^{a}	23.81 ± 0.10^{cd}
5	50.00	50.00	0.00	0.00	0.5098 ± 0.02^{a}	27.57 ± 0.93^{bc}
6	0.00	0.00	100.00	0.00	0.4351 ± 0.02^{a}	13.33 ± 1.55^{ef}
7	50.00	0.00	0.00	50.00	0.4341 ± 0.02^{a}	27.72 ± 0.60^{bc}
8	0.00	33.33	33.33	33.33	0.4715 ± 0.02^{a}	24.22 ± 1.32^{cd}
9	0.00	50.00	50.00	0.00	0.3856 ± 0.05^{a}	14.25 ± 0.73^{e}
10	25.00	25.00	25.00	25.00	0.5053 ± 0.01^{a}	33.88 ± 2.44^{b}
11	100.00	0.00	0.00	0.00	0.1992 ± 0.02^{b}	7.17 ± 0.09^{f}
12	33.33	33.33	33.33	0.00	0.4119 ± 0.04^{a}	43.80 ± 0.98^{a}
13	33.33	33.33	0.00	33.33	0.4896 ± 0.04^{a}	41.11 ± 0.85^{a}
14	0.00	100.00	0.00	0.00	0.2295 ± 0.01^{b}	23.95 ± 3.05^{cd}
15	33.33	0.00	33.33	33.33	0.4801 ± 0.02^{a}	28.02 ± 0.42^{bc}

Note: Numbers in the same column followed by the same letter indicate results that are not significantly different ($p > 0.05$). TPC

The water extract of purslane yielded the lowest TPC at 0.1992 mg GAE/g DW, suggesting that semipolar compounds were predominant among the phenolic compounds in purslane sample. Given that water was highly polar compared to solvents, such as methanol, ethanol, and acetone, the extraction capacity for polar components, including carbohydrates and other proximates could lead to lower TPC per gram of the sample. This observation showed the influence of solvent polarity on the extraction efficiency of phenolic compounds. The quadratic model describing the interaction between water, acetone, methanol, and ethanol solvents on TPC of purslane revealed the significance of solvent composition (Equation 1). Furthermore, the acetone-water combination exhibited the highest coefficient for TPC, suggesting a synergistic effect between these solvents in enhancing phenolic compound extraction.[32]

$$TPC = 0.2057A + 0.2342B + 0.4362C + 0.4158D + 1.02AB + 0.4743AC + 0.4905AD + 0.1504BC + 0.5097BD + 0.0763CD \quad ...(1)$$

Effect of solvent system on antioxidant capacity

The antioxidant capacity of purslane extract varied significantly depending on solvent composition, as shown in Table 2. The extract using the ternary solvent water-acetone-methanol exhibited the highest antioxidant capacity at 43.80 µmol TE/g DW, followed by the water-acetone-ethanol extract at 41.11 µmol TE/g DM, and the water-acetone-methanol-ethanol extract at 33.88 µmol TE/g DW. This observation was consistent with Lv *et al*.,[33] which revealed the efficacy of a water-acetone-methanol mixture in extracting phenolic acids, renowned for the potent antioxidant properties. The ability of methanol to neutralize polyphenol oxidase capacity facilitated the extraction of polyphenols bound to cell walls, thereby inhibiting the degradation in plants, as elucidated by Silva *et al*.[34] Furthermore, the addition of water enhanced solvation capacity of acetone, leading to an increase in the efficiency in extracting antioxidant compounds, as reported by Alothman *et al*.[26, 35]

The antioxidant capacity of the water extract from purslane was found to be the lowest among the tested solvents, with a value of 7.17 µmol TE/g DW. This observation was consistent with a previous study that antioxidant capacity tended to correlate with TPC of the extract. Nurcholis *et al*.[16] also reported similar results in the study of cardamom fruit samples, where the water extract exhibited the highest antioxidant capacity using FRAP method.[16] The disparity suggested that the

metabolites extracted from the aqueous extract of purslane could have inherently lower antioxidant capacity when evaluated using FRAP method. In addition, the variations in the content of secondary metabolites, which were known for the antioxidant properties, could also have contributed to these differences. According to Chen *et al*.[36] secondary metabolites in plants exhibited varying levels of biological activity, depending on solvent polarity. This indicated that it was essential to use solvents with diverse polarities during the extraction process to ensure a comprehensive evaluation of the secondary metabolites produced.

To explore the antioxidant dynamics in purslane, a quadratic model encapsulated by Equation 2, which delineated the interplay among various solvents, namely water, acetone, methanol, and ethanol, and the collective impact on the plant's antioxidant properties as gauged by FRAP assay was constructed. The coefficients determined for each solvent, namely 5.57 (water), 21.92 (acetone), 12.22 (methanol), and 17.67 (ethanol) indicated the contributions to antioxidant capacity, with the methanol-water pairing being the most significant. This binary solvent synergy showed the complex nature of solvent interactions in modulating FRAP value, a proxy for antioxidant potential.[33] Furthermore, visual representation through a contour plot augmented understanding (Figure 2), revealing that solvent mixtures enhanced antioxidant activity, a phenomenon depicted by the transition of colors across the plot.

$$FRAP = 5.57A + 21.92B + 12.22C + 17.67D + 84.67AB + 87.31AC + 70.49AD + 10.21BC + 29.13BD + 12.95CD \quad ...(2)$$

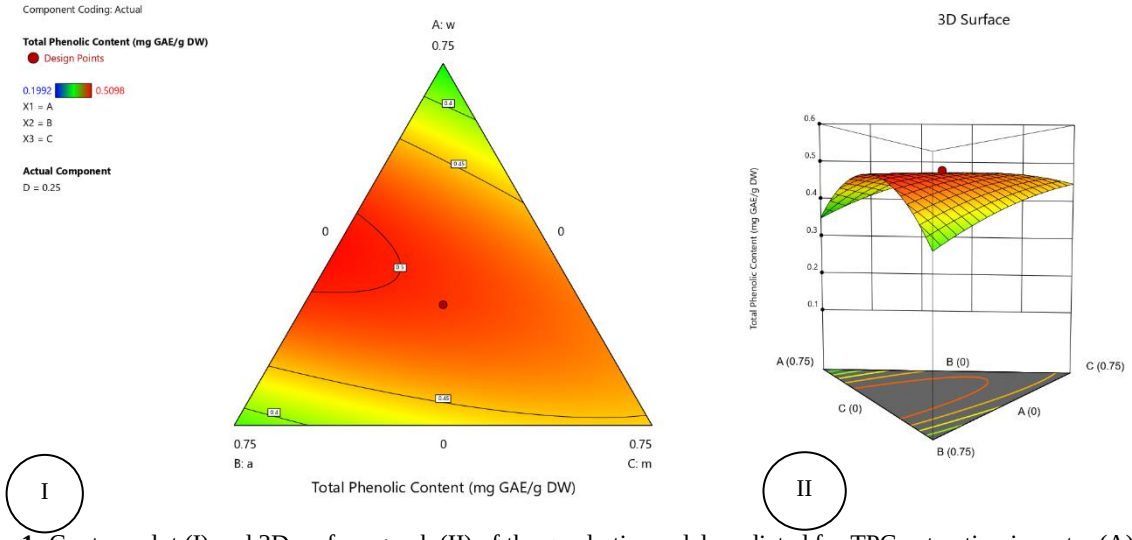

Figure 1: Contour plot (I) and 3D surface graph (II) of the quadratic model predicted for TPC extraction in water (A), acetone (B), methanol (C), and ethanol (D).

Optimum formulation and confirmation

Optimization of the extraction parameters using Design Expert 13.0 led to the identification of an optimum formulation, as presented in Table 4. Desirability was employed as a method to optimize the response components, with values ranging from 0 to 1, where higher values indicated more favorable responses.[37] In this context, a desirability value close to 1 suggested the optimal combination for maximizing both TPC and antioxidant capacity. The best formulation had a desirability value of 0.884, predicting TPC of 0.508 mg GAE/g DW and antioxidant capacity of 35.984 µmol TE/g DW. This optimal formulation comprised a blend of 3 solvents with ratios of 0.342, 0.389, and 0.269 in water, acetone, and ethanol, respectively.

The optimized formulation was confirmed using predicted interval (PI) values, which provided a range in which the actual response value was expected to fall with a certain level of confidence.[38] The percentage of PI (%PI) represented the width of the interval relative to the predicted response value, with 95% PI indicating a 95% probability that the actual response value lied in the calculated interval. The optimum formulation yielded TPC of 0.5098 mg GAE/g DW and antioxidant capacity of 29.0786 µmol TE/g DW. The prediction interval generated by the optimization model ranged from 0.4249 to 0.5901 mg GAE/g DW for TPC and 20.6095 to 51.3609 µmol TE/g DW for antioxidant capacity (Table 5). The results demonstrated that the values obtained from the optimized formulation were in the range of PI values, indicating the reliability of the model. The confirmation showed the robustness of the optimization method employed to maximize the extraction efficiency of bioactive compounds, providing valuable insights for future studies and industrial applications in natural product extraction.

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Conclusion

In conclusion, SCD proved effective in optimizing the extraction solvent for purslane based on total phenolic levels and antioxidant capacity. The results revealed that extract using the water-acetone solvent mixture exhibited the highest TPC, while a combination of water-acetone-methanol solvents demonstrated superior antioxidant capacity. Furthermore, the optimal solvent formulation, with a desirability value of 0.884, comprised a blend of water (0.342), acetone (0.388), and ethanol (0.270). The confirmation analyses revealed that the optimum formulation fell in PI values, lending credence to the reliability of the model used. Further investigations into the primary phenolic compounds responsible for the antioxidant capacity of purslane are needed, offering promising avenues for deeper insights into the therapeutic potential and industrial applications.

Table 3: Results of analysis of variance (ANOVA) response variables on optimizing the extraction solvent of *P. oleracea*

Parameters	Total phenolic content	Ferric reducing antioxidant power
	Quadratic models	
F	7.75	2.33
p	0.0182	0.1824
R^2	0.9331	0.8074
Adjusted R^2	0.8127	0.4608
Adeq precision	9.49	5.04

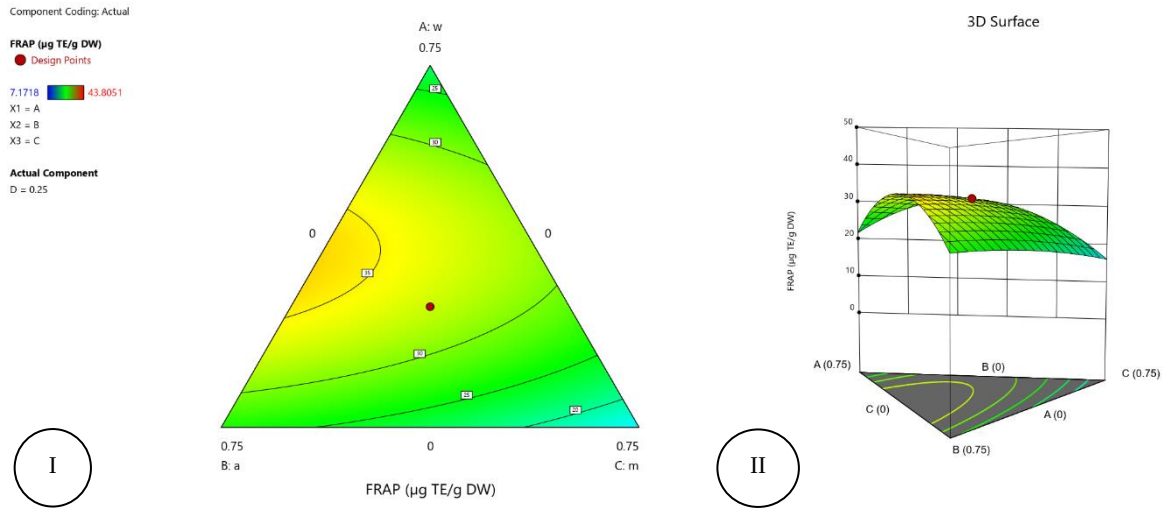

Figure 2: Contour plot (I) and 3D surface graph (II) of the quadratic model predicted for antioxidant capacity in water (A), acetone (B), methanol (C), and ethanol (D).

Table 4: The results of optimization of *P. oleracea* extraction solvents

No	Water (A)	Acetone (B)	Methanol (C)	Ethanol (D)	TPC (mg GAE/g)	FRAP (µmol TE/g)	*Desirability*	
1	0.342	0.389	0.000	0.269	0.508	35.984	0.884	*Selected*
2	0.443	0.526	0.031	0.000	0.475	35.476	0.828	
3	0.450	0.450	0.099	0.000	0.477	35.130	0.826	
4	0.358	0.000	0.370	0.272	0.466	31.057	0.749	
5	0.395	0.000	0.605	0.000	0.458	30.462	0.729	

Note: TPC, GAE, FRAP, and TE are total phenolic content, gallic acid equivalent, ferric reducing antioxidant power, trolox equivalent, respectively.

Table 5: Confirmation of *P. oleracea* extraction solvent optimization results

Responses variable	Predicted Mean	Predicted Median	n	SE Pred	95% PI low	Data Mean	95% PI high
TPC	0.5080	0.5078	3.00	0.0323	0.4250	0.5098	0.5908
FRAP	35.985	35.985	3.00	5.9814	20.6095	29.0786	51.3609

Note: TPC, FRAP, SE, and PI are total phenolic content (mg GAE/g DW), ferric reducing antioxidant power (µmol TE/g), standard error, and predicted interval, respectively.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgments

This study was fully funded by the PDUPT research program (15887/IT3.D10/PT.01.02/P/T/2023) from the Ministry of Research, Technology, and Higher Education of Indonesia.

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