

APPLICATION OF MIXTURE DESIGN TO ENHANCE FLAVONOID CONTENT AND IDENTIFY SECONDARY METABOLITES IN *PORTULACA OLERACEA* L.

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Abstract

Flavonoids are secondary plant metabolites with numerous pharmacological benefits. However, selecting an appropriate extraction solvent is crucial for effectively extracting flavonoids from plants. *Portulaca oleracea* L., a wild plant rich in flavonoids, offers significant potential for development through solvent extraction techniques. Therefore, this study aims to enhance the total flavonoid content (TFC) of *P. oleracea* extracts using a simplex centroid mixture design (SCMD) and to identify the secondary metabolites present. SCMD was formulated using Design Expert v.13 software. Various extraction solvents, including water, acetone, methanol, and ethanol, served as independent variables, while the response variable was the TFC, measured through the AlCl₃ colorimetric method. LC-HR/MS analysis was performed to identify the secondary metabolites of the optimal extract. The results showed that an acetone–ethanol mixture produced the highest TFC (2.07 mg QE/g DW), but not significantly different from 100% methanol (1.95 mg QE/g DW) and acetone–methanol (1.88 mg QE/g DW) combinations. A linear model ($p < 0.05$) was generated from these response formulations. The optimal extraction solvent formula identified was a 50:50 acetone–methanol mixture, with a desirability value of 0.81. Model validation confirmed the reliability of this formula (RSE < 10%). Additionally, the *P. oleracea* extract showed significant antioxidant capacity, with reducing power against ABTS radicals (21.16 $\mu\text{mol TE/g DW}$), Fe³⁺ (12.33 $\mu\text{mol TE/g DW}$), and Cu²⁺ (38.57 $\mu\text{mol TE/g DW}$). Secondary metabolite analysis also showed the presence of 9 phenols, 8 flavonoids, 5 alkaloids, 5 indoles, and 3 quinolines. These results provide a valuable reference for further studies on the extraction of flavonoid from *P. oleracea*.

Keywords: Antioxidant; LC-HR/MS; Simplex Centroid Mixture Design; Total Flavonoid Content

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Introduction

Flavonoid are secondary metabolites of plants characterized by a benzo- γ -pyrone framework within the structure. These compounds are synthesized through various pathways, including phenylpropanoid, shikimate, and flavonoid biosynthesis. Flavonoid have diverse biochemical and antioxidant effects, relevant in the context of multiple diseases such as cancer, Alzheimer's disease, and atherosclerosis. Due to antioxidant, anti-inflammatory, antimutagenic, and anticarcinogenic properties, as well as the ability to modulate cellular enzyme function, flavonoid are widely used in pharmaceutical, medicinal, and cosmetic applications (Pandey & Rizvi, 2009; Cavalcante *et al.*, 2018; Liga *et al.*, 2023). The biological activity and bioavailability are influenced by the chemical structure. Depending on variations in the primary structure, flavonoid are classified into several subclasses, including flavanones, flavones, isoflavones, flavonols, anthocyanins, and chalcones (Panche *et al.*, 2016; Dias *et al.*, 2021). However, the application in the medical, pharmaceutical, food, and cosmetic industries is often limited by challenges in extraction, isolation, and purification from crude plant extracts, which can affect the yield obtained (Sajid *et al.*, 2021).

Purslane (*Portulaca oleracea* L.) is a wild plant rich in flavonoids and has been used as a potherb. This plant, widespread in tropical and subtropical regions, is rich in active compounds that have pharmacological effects (Putra *et al.*, 2024). Sri Lanka has used purslane in treating ulcers and burns. The plant is also used in the treatment of worms in Dominica and western India (Wang *et al.*, 2023). Previous studies have reported that various extracts of purslane from Xichang, Taiwan, contain flavonoid (27.21 - 437.38 mg quercetin/g extract) acting as antioxidant, α -glucosidase, and tyrosinase inhibitors (Chen *et al.*, 2022). Sicari *et al.* (2018) successfully identified several flavonoid compounds such as apigenin, kaempferol, luteolin, quercetin, isorhamnetin, kaempferol-3-O-glucoside, and rutin from an 80% methanol extract of *P. oleracea*.

The selection of extraction solvents is one factor affecting the success of flavonoid extraction. The polarity of the solvent affects the type and number of molecules extracted as well as the extraction yield. Solvents with various relative polarities, such as water (1.00), ethanol (0.65), methanol (0.76), and acetone (0.65), have been used to extract multiple bioactive compounds. The selection of solvent depends on the solubility of the targeted substance (Suhaimi *et al.*, 2019; Chen *et al.*, 2022; Ponphaiboon *et al.*, 2023).

Mixture design is a combination of mathematical and statistical methods in modeling and analyzing the effects of each component to produce an optimal formula (Hwangdee *et al.*, 2022). Makkiyah *et al.* (2023) successfully applied a simplex centroid mixture design (SCMD) to select flavonoid extraction solvents for purple leaves (*Graptophyllum pictum* (L.) Griff.). On the other hand, studies applying mixture design in the formulation of flavonoid extraction solvents for *P. oleracea* have not been widely conducted. Therefore, this study aims to enhance the total flavonoid content (TFC) in *P. oleracea* extract through the application of a SCMD and evaluate antioxidant capacity of the optimized extract. The secondary metabolites obtained through the optimized extraction process were also evaluated.

Materials and Methods

Chemicals and reagents

The chemicals and reagents used include pro-analytical grade solvents (acetone, methanol, and ethanol), hydrochloric acid (HCl), ammonium acetate, copper (II) chloride dihydrate ($\text{CuCl}_2 \cdot 6\text{H}_2\text{O}$), neocuproine, potassium persulfate ($\text{K}_2\text{S}_2\text{O}_8$), Folin-Ciocalteu reagent, sodium carbonate (Na_2CO_3), aluminum chloride (AlCl_3), and quercetin obtained from Merck-Millipore (Darmstadt, Germany). Additionally, 2,4,6-tripyridyl-s-triazine (TPTZ) and gallic acid were sourced from SRL Ltd. (Maharashtra, India), while Trolox, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), and glacial acetic acid were obtained from Sigma-Aldrich (St. Louis, USA). Distilled water (aquadest) was also used.

Plant material and sample preparation

This study used the aerial parts of *Portulaca oleracea* L. (voucher number: BMK0251102016), obtained from the Tropical Biopharmaca Research Center, IPB University, West Java, Indonesia (6°35'15.7"S 106°48'05.0"E). The plant material was dried in an oven at 45°C for 60 hours. According to Putra *et al.* (2023), heating raw material at high temperature can trigger the Maillard reaction, which decomposes phenolic compounds and produces new product. Therefore, this condition was selected to avoid the degradation of secondary metabolites of the material. After drying, the purslane was ground, sifted through an 80-mesh sieve, and stored at room temperature.

Experimental design and extraction

SCMD was developed using Design Expert 13.0 software (Stat-Ease Inc., Minneapolis, USA). The independent variables were four solvents (water, acetone, methanol, and ethanol), and the dependent variable (response) was TFC (Table 1).

The extraction process used a combination of sonication and maceration methods. Four grams of aerial parts of purslane were dissolved in 40 mL of solvent (1:10) according to the SCMD (Table 1). The solution was subjected to sonication using a Decon F5 Major sonicator (Decon Laboratories, USA) for 30 minutes. Following sonication, the mixture was macerated for 180 minutes in a WiseBath shaker (DAIHAN WiseBath, South Korea) at room temperature. The macerated mixture was then filtered to obtain the filtrate, which was evaporated using a rotary evaporator under 200 mBar (LabTech Ltd.). The final concentration of the filtrate at 0.2 g/mL was prepared for total flavonoid testing.

Table 1. Mixture design of *P. oleracea*

Run	A (%)	B (%)	C (%)	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

A: water ; B: acetone ; C: methanol ; D: ethanol

TFC

Total flavonoid was assessed using the aluminum chloride colorimetric assay, following the protocol by Makkiyah *et al.* (2023). A 96-well microplate was set up, with each containing 120 μ L of distilled water, 10 μ L of the sample, 10 μ L of 10% aluminum chloride (AlCl_3), 10 μ L of glacial acetic acid, and 50 μ L of ethanol. The mixture was incubated in the dark at room temperature for 30 minutes. Absorbance was measured at 415 nm using a nanophotometer (BMG Labtech, Germany). Subsequently, calibration was performed using quercetin standards at concentrations of 25 to 550 ppm. Each measurement was conducted in triplicate, and the results were reported as milligrams of quercetin equivalent per gram of dried weight (mg QE/g DW).

Antioxidant capacity

The verified optimal solvent composition was subsequently analyzed for antioxidant capacity using the 2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), ferric reducing antioxidant power (FRAP), and cupric ion-reducing antioxidant capacity (CUPRAC) methods. Trolox was used as the standard for all assays, and results were expressed as micromoles of Trolox equivalent per gram of dried weight ($\mu\text{mol TE/g DW}$) following the method described by Nurcholis *et al.* (2022). Each assay was performed in triplicate.

For the ABTS assay, the reagent was prepared by mixing 7.7 mM ABTS with 2.4 mM potassium persulfate in a 2:1 ratio (v/v), followed by incubation for 16 hours. The ABTS blank was adjusted to an absorbance of 0.7 ± 0.2 at 734 nm. To measure antioxidant capacity, 20 μ L of the sample was combined with 180 μ L of ABTS reagent in a 96-well microplate. The mixture was incubated in the dark for 6 minutes, and absorbance was measured at 734 nm using a nanophotometer. The assay was conducted in triplicate. The Trolox standard series included concentrations of 50, 100, 150, 200, 250, 300, 350, 400, 450, and 500 μM .

For the FRAP assay, 10 μ L of sample was mixed with 300 μ L of FRAP reagent in a 96-well microplate. After 30 minutes of incubation, absorbance was measured at 593 nm using a nanophotometer. The assay was repeated in triplicate. The Trolox standard series included concentrations of 50, 100, 150, 200, 250, 300, 450, 500, and 550 μM .

For the CUPRAC assay, a solution was prepared using $\text{CuCl}_2 \cdot 6\text{H}_2\text{O}$ (10^{-2} M), neocuproine (7.5×10^{-3} M), and ammonium acetate buffer (pH 7). In a 96-well microplate, 50 μ L of sample was mixed with 50 μ L of $\text{CuCl}_2 \cdot 6\text{H}_2\text{O}$, 50 μ L of neocuproine, and 50 μ L of ammonium acetate buffer (pH 7). The mixture was incubated for 30 minutes in the dark before measuring absorbance at 450 nm. The assay was conducted in triplicate. The Trolox standard series included concentrations of 50, 100, 150, 200, 250, 300, 350, 400, 500, 550, and 600 μM .

LC-HR/MS profiling secondary metabolites

Profiling of secondary metabolites was performed using Liquid Chromatography coupled with High-Resolution Tandem Mass Spectrometry (LC-HR/MS), following the method described by Maulana *et al.* (2022). The analysis was conducted with a UHPLC Vanquish Tandem Q Exactive Plus Orbitrap HRMS (ThermoScientific). A 5 mg sample extract was dissolved in 1 mL of LC-MS grade methanol, sonicated for 30 minutes, and then filtered through a 0.2 μm PTFE membrane filter. A

2.0 µL aliquot of the prepared sample was injected into the LC-HR/MS system using a syringe. The mobile phase consisted of water with 0.1% formic acid (solvent A) and 0.1% formic acid in acetonitrile (solvent B), with a flow rate of 0.2 mL/min. The gradient elution method was as follows: 0-1 min (5% B), 1-25 min (5%-95% B), 25-28 min (95% B), and 28-30 min (5% B). The stationary phase was an Accucore C18 column (100 × 2.1 mm, 1.5 µm) (ThermoScientific) maintained at 30°C. Mass detection was performed in negative ionization mode, with a mass range of 100-1,500 m/z. Data from LC-MS/MS analysis were processed using online databases (mzCloud, ChemSpider, and PubChem) to determine the predicted chemical structures of the metabolites.

Statistical analysis

Data on TFC and antioxidant capacity were analyzed using one-way ANOVA, with statistical significance determined at a p-value < 0.05. Tukey's post hoc test was performed to identify significant differences between groups, using IBM SPSS Statistics 25.0. SCMD was used for experiment design, model creation, contour plotting, and optimization through Design Expert Version 13.0. The optimal formulation was selected based on the highest desirability value, with the objective settings to simultaneously maximize TFC and antioxidant capacity. Verification of the optimization results (triplicate) was conducted by calculating the percentage residual standard error (%RSE).

Results and Discussion

Response of mixture design

The SCMD is an optimization method that operates on the principle of optimizing responses generated by multiple independent variables in varying proportions while keeping the total concentration constant (Patel *et al.*, 2017). It is a Design of Experiment (DoE) model specifically based on mixture design principles. The number of design points in SCMD is calculated using the equation $2q - 1$, where q represents the number of independent variables (Patel *et al.*, 2017).

Table 2 shows the observed responses based on solvent proportions formulated using SCMD. The TFC of the *P. oleracea* extract in this study ranged from 0.65 to 2.07 mg QE/g DW. The acetone-ethanol mixture yielded the highest TFC, while the water-methanol-ethanol mixture resulted in the lowest. Post hoc Tukey test analysis showed that using either a single solvent or a binary mixture of acetone and methanol, or binary mixture of acetone-ethanol was more effective in extracting

total flavonoid from *P. oleracea* compared to water as a single, binary, or ternary solvent. This efficiency is probably due to the lower polarity of flavonoid compounds in *P. oleracea*, making solvents such as methanol and acetone more effective at binding these compounds.

A study by Thao *et al.* (2023) on *P. oleracea* from Soc Trang, Vietnam, using n-hexane, ethyl acetate, methanol, and water extracts, reported higher TFC, ranging from 0.97 to 44.78 mg QE/g. Similarly, Rohman *et al.* (2023) found that *P. oleracea* extracts obtained through 72 hours of maceration with ethanol, ethyl acetate, and n-hexane had total flavonoid values ranging from 3.26 to 11.56 µg QE/g, with the highest levels observed in ethyl acetate extracts. Variations in growing conditions, extraction methods, and instrumentation presumably contributed to differences in flavonoid content (Putra *et al.*, 2023). Instrumental calibration and sensitivity may account for additional variability. Studies conducted using HPLC generally report higher accuracy and specificity in flavonoid quantification than spectrophotometric methods, which are more prone to interference from other phenolic compounds.

Table 2. Total flavonoid of *P. oleracea* based on SCMD

Run	Total flavonoid content (mg QE/g DW)
1	1.67 ± 0.11 ^b
2	0.74 ± 0.04 ^d
3	1.17 ± 0.05 ^c
4	2.07 ± 0.07 ^a
5	0.65 ± 0.02 ^d
6	1.95 ± 0.09 ^{ab}
7	0.78 ± 0.04 ^d
8	1.78 ± 0.05 ^{ab}
9	1.88 ± 0.10 ^{ab}
10	0.70 ± 0.01 ^d
11	0.53 ± 0.01 ^d
12	0.74 ± 0.01 ^d
13	0.73 ± 0.04 ^d
14	1.98 ± 0.08 ^{ab}
15	0.65 ± 0.05 ^d

Numbers in the same column followed by the same letter show results that are not significantly different ($p > 0.05$). Values shown are the mean ± standard error of mean (SEM). QE: quercetin equivalent; TFC: total flavonoid content.

The 3D response surface plot (Figure 1) shows the response values at the nodes and vertices of the triangle, representing the values of each independent variable. Although visually the highest response appears at approximately 75% methanol and 25% acetone, numerical optimization identified the optimal solvent mixture as a 50:50 acetone-methanol combination, based on the highest desirability value (0.81, Table 4). The resulting

linear model equation showed that all independent variables studied had a positive impact on the TFC. The highest positive coefficient was observed for acetone (1.84), while the lowest was for water (0.05). The variation in solvent extraction efficiency is attributed to differences in polarity, sample matrix, and the compounds being extracted (Jdaini *et al.*, 2023). This suggests that flavonoid present in the *P. oleracea* used are predominantly less polar flavonoid, such as isoflavones, flavanones, and flavones (Tzanova *et al.*, 2020).

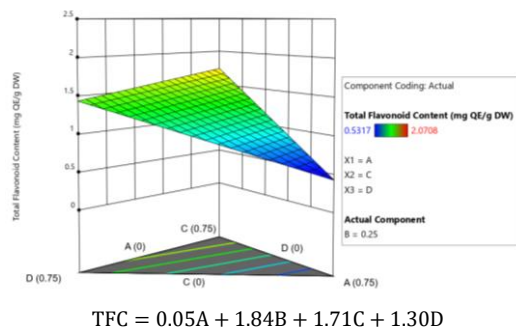


Figure 1. 3D response surface plot and model equation of TFC

Modelling and model evaluation

According to the ANOVA results (Table 3), the linear model achieved the desired outcomes for flavonoid response (p-value = 0.0028, PRESS = 3.41). The model adequacy was verified using the coefficients of determination (R^2) and adjusted R^2 . These coefficients show the proportion of the total variation in the response that can be predicted by the model. An R^2 value close to 1 is expected, showing a high level of model fit with the actual data. In this study, the R^2 value for the response was 0.71, with an adjusted R^2 of 0.63.

Table 3. The results of ANOVA analysis for the SCMD response

Parameter	TFC Linear
F	8.89
p-value	0.0028
R^2	0.71
Adjusted R^2	0.63
PRESS	3.41
Suggested	

PRESS: predicted residual error sum of squares; TFC: total flavonoid content

Optimization and validation solution

Desirability was the key parameter considered in selecting the optimal solution. A desirability value close to one shows that the independent variables can achieve the desired response. The acetone-methanol binary solvent was selected as the

optimal solution with a desirability value of 0.81 (Table 4). The presence of the ketone group ($C=O$) in acetone and the hydroxyl group ($-OH$) in methanol facilitates dipole-dipole interactions, which can form hydrogen bonds between the two molecules. Additionally, the nonpolar $-CH_3$ group in acetone and the $-CH$ group in methanol contribute to hydrophobic interactions (Idrissi *et al.*, 2013).

The SCMD model and the resulting formulation were validated through triplicate ($n=3$) trials. The validation results were then evaluated using the percentage residual standard error (%RSE). The obtained %RSE value of 3.65 shows that the SCMD-derived model and extraction solvent formulation for *P. oleracea* are reliable and applicable.

Table 4. The results of the optimization for total flavonoid extraction solvents in *P. oleracea* extract

	A	B	C	D	TFC
Prediction	0	0.5	0.5	0	1.78
Verification	0	0.5	0.5	0	1.84
Desirability					0.81
%RSE					3.65

A: water; B: acetone, C: methanol, D: ethanol; RSE: residual standard errors; TFC: total flavonoid content

Antioxidant capacity of optimal *P. oleracea* extract

The antioxidant capacity of the optimum formula obtained through SCMD was analyzed using FRAP, CUPRAC, and ABTS methods. These multiple methods were used to assess the extract ability to neutralize radicals through various reaction mechanisms. The reduction of Fe^{3+} to Fe^{2+} in the optimum extract was found to be 12.33 $\mu\text{mol TE/g DW}$, while the reduction of Cu^{2+} to Cu^+ was 38.57 $\mu\text{mol TE/g DW}$ (Table 5). The optimal *P. oleracea* extract also showed a hydrogen-donating capacity to neutralize ABTS radicals with a value of 21.16 $\mu\text{mol TE/g DW}$. Differences in radical neutralization capacities across methods are attributed to the distinct reaction mechanisms. The FRAP and CUPRAC methods measure metal ion reduction through electron transfer, while the ABTS method assesses radical neutralization through hydrogen donation (Aisyah *et al.*, 2023).

The results surpassed the antioxidant capacity of *P. oleracea* extracted with 70% ethanol, as reported by Aisyah *et al.* (2023), where ABTS neutralization was 15.98 $\mu\text{mol TE/g DW}$ and Cu^{2+} reduction was 33.22 $\mu\text{mol TE/g DW}$. Enhanced antioxidant capacity was achieved through the selection of an optimal extraction solvent formula using mixture design.

Table 5. The results of antioxidant capacity of optimal *P. oleracea* extract

	Antioxidant capacity ($\mu\text{mol TE/g DW}$)		
	ABTS	FRAP	CUPRAC
1	20.45 $\pm$ 0.64 ^a	10.22 $\pm$ 0.49 ^b	37.02 $\pm$ 1.86 ^a
2	21.51 $\pm$ 1.15 ^a	14.07 $\pm$ 0.22 ^a	41.54 $\pm$ 2.95 ^a
3	21.52 $\pm$ 2.18 ^a	12.69 $\pm$ 1.09 ^{ab}	37.14 $\pm$ 0.69 ^a
Mean	21.16	12.33	38.57

Numbers in the same column followed by the same letter show results that are not significantly different ($p > 0.05$). Values shown are the mean $\pm$ standard error of mean (SEM). ABTS: 2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic) acid; FRAP: ferric reducing antioxidant power; CUPRAC: cupric ion-reducing antioxidant capacity; DW: dried weight; TE: trolox equivalent.

Identification of secondary metabolites in the optimal *P. oleracea* extract

The secondary metabolites of the optimized *P. oleracea* extract were identified using the Vanquish UHPLC-Q Exactive Plus Orbitrap High-Resolution Mass Spectrometer. Identification was based on molecular formulas and retention times.

The identified metabolites had molecular weights ranging from 137.05 g/mol to 503.14 g/mol, with retention times between 1.10 and 18.87 minutes. The identification process for the secondary metabolites in the optimum SCMD formula yielded 30 identified compounds (Table 6). These compounds included 9 phenols, 8 flavonoids, 5 alkaloids, 5 indoles, and 3 quinolines.

Among the identified flavonoid, luteolin and apigenin were distinguished for the potential antioxidant properties. Luteolin contains a 1,4-pyrone structure with conjugated double bonds at C2 and C3, coupled with a 4-oxo group on the pyran ring C. This structure can stabilize phenoxyl radicals through electron delocalization across rings A, B, and C (Ahmadi *et al.*, 2020). Both Luteolin and Apigenin are known to induce the upregulation of nuclear factor erythroid 2-related factor 2 (Nrf2) and antioxidant response element (ARE) (Kashyap *et al.*, 2022; Zhang *et al.*, 2013). ARE is a promoter region responsible for the expression of antioxidant genes in response to oxidative stress (Ngo & Duennwald, 2022).

Table 6. Secondary metabolites of optimal *P. oleracea* extract

No	Compounds	Formula	Class
1	3-(3,4-Dihydroxyphenyl)-N-(3-oxopropyl)propanamide	C ₁₂ H ₁₅ NO ₄	Phenols
2	4-Hydroxybenzoic acid	C ₇ H ₆ O ₃	Phenols
3	Gentisic acid	C ₇ H ₆ O ₄	Phenols
4	Caffeic acid	C ₉ H ₈ O ₄	Phenols
5	Ferulic acid	C ₁₀ H ₁₀ O ₄	Phenols
6	2-Hydroxybenzoic acid	C ₇ H ₆ O ₃	Phenols
7	N-trans-Feruloyl 4-O-methyldopamine	C ₁₉ H ₂₁ NO ₅	Phenols
8	5-pentylresorcinol	C ₁₁ H ₁₆ O ₂	Phenols
9	4-Phenethylphenol	C ₁₄ H ₁₄ O	Phenols
10	Luteolin	C ₁₅ H ₁₀ O ₆	Flavonoid
11	3,4-Methylenedioxy-2',4'-dimethoxychalcone	C ₁₈ H ₁₆ O ₅	Flavonoid
12	Flavokawain A	C ₁₈ H ₁₈ O ₅	Flavonoid
13	Psoralidin	C ₂₀ H ₁₆ O ₅	Flavonoid
14	Okanin 3,4,3',4'-tetramethyl ether	C ₁₉ H ₂₀ O ₆	Flavonoid
15	2',4'-Dihydroxy-3,4-dimethoxychalcone	C ₁₇ H ₁₆ O ₅	Flavonoid
16	Lusianin	C ₁₇ H ₁₈ O ₅	Flavonoid
17	Apigenin	C ₁₅ H ₁₀ O ₅	Flavonoid
18	1-methylpyridinium-3-carboxylate	C ₇ H ₇ NO ₂	Alkaloid
19	Normorphine	C ₁₆ H ₁₇ NO ₃	Alkaloid
20	Oleracein E	C ₁₂ H ₁₃ NO ₃	Alkaloid
21	Oleracein A	C ₂₄ H ₂₅ NO ₁₁	Alkaloid
22	N-feruloyloctopamine	C ₁₈ H ₁₉ NO ₅	Alkaloid
23	Indoline-2-carboxylic acid	C ₉ H ₉ NO ₂	Indoles
24	trans-3-Indoleacrylic acid	C ₁₁ H ₉ NO ₂	Indoles
25	3-Indoleacetamide	C ₁₀ H ₁₀ N ₂ O	Indoles
26	Indole-3-acetic acid	C ₁₀ H ₉ NO ₂	Indoles
27	4-Indolecarbaldehyde	C ₉ H ₇ NO	Indoles
28	Salsolinol	C ₁₀ H ₁₃ NO ₂	Quinolines
29	8-hydroxyquinoline	C ₉ H ₇ NO	Quinolines
30	6-methylquinoline	C ₁₀ H ₉ N	Quinolines

Conclusions

In conclusion, SCMD was successfully applied to formulate extraction solvents, resulting in increased TFC in *P. oleracea* extracts. The linear model produced a p-value < 0.05, showing statistical significance. The optimal solvent formula was identified as an acetone-methanol mixture in a 50:50 ratio, with a desirability value of 0.809. Validation test confirmed that both the model and the solvent formula are suitable for studies on flavonoid extraction from *P. oleracea*. The extract prepared with the optimal solvent formula showed antioxidant capacity through both free radical reduction and metal chelation. Various secondary metabolites, including phenols, flavonoid, alkaloids, indoles, and quinolines, were identified in the extract. These results provide a foundation for further development of *P. oleracea* as a medicinal plant.

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References

- Ahmadi, S. M., Farhoosh, R., Sharif, A., & Rezaie, M. (2020). Structure-antioxidant activity relationships of luteolin and catechin. *Journal of Food Science*, 85(2), 298-305. <https://doi.org/10.1111/1750-3841.14994>
- Aisyah, S. I., Oktavia, A. W. P., Ayuningtyas, A. A., Putra, R. P., Prassiska, S., Jamilah, S., & Nurcholis, W. (2023). Short communication: Differences in phytochemical compounds and antioxidant activity of *Portulaca oleracea* and *Portulaca grandiflora*. *Biodiversitas*, 24(3), 1385-1390. <https://doi.org/10.13057/biodiv/d240307>
- Cavalcante, G. M., Cabral, A. E. da S., & Silva, C. C. (2018). Leishmanicidal activity of natural and synthetic flavonoids: A minireview. *Mintage Journal of Pharmaceutical and Medical Sciences*, 7, 25-34. <https://doi.org/10.37885/220207877>
- Chen, W. C., Wang, S. W., Li, C. W., Lin, H. R., Yang, C. S., Chu, Y. C., Lee, T. H., & Chen, J. J. (2022). Comparison of various solvent extracts and major bioactive components from *Portulaca oleracea* for antioxidant, anti-tyrosinase, and anti- α -glucosidase activities. *Antioxidants*, 11(2), 398. <https://doi.org/10.3390/antiox11020398>
- Dias, M. C., Pinto, D. C. G. A., & Silva, A. M. S. (2021). Plant flavonoids: Chemical characteristics and biological activity. *Molecules*, 26(17), 5377. <https://doi.org/10.3390/molecules26175377>
- Hwangdee, P., Charee, S., Kheowkrai, W., Junsiri, C., & Laloan, K. (2022). Application of the simplex-centroid mixture design to biomass charcoal powder formulation ratio for biomass charcoal briquettes. *Sustainability*, 14(7), 3940. <https://doi.org/10.3390/su14073940>
- Idrissi, A., Polok, K., Barj, M., Marekha, B., Kiselev, M., & Jedlovsky, P. (2013). Free energy of mixing of acetone and methanol: A computer simulation investigation. *The Journal of Physical Chemistry B*, 117(50), 16157-16164. <https://doi.org/10.1021/jp405090j>
- Jdaini, K., Alla, F., Mansouri, F., Parmar, A., & Elhoumaizi, M. A. (2023). Optimizing the extraction of phenolic antioxidants from date palm fruit by simplex-centroid solvent mixture design. *Heliyon*, 9(1), e12738. <https://doi.org/10.1016/j.heliyon.2022.e12738>
- Kashyap, P., Kumar, S., Riar, C. S., Jindal, N., Baniwal, P., Guiné, R. P. F., Correia, P. M. R., Mehra, R., & Kumar, H. (2022). Recent advances in drumstick (*Moringa oleifera*) leaves bioactive compounds: Composition, health benefits, bioaccessibility, and dietary applications. *Antioxidants*, 11(2), 402. <https://doi.org/10.3390/antiox11020402>
- Liga, S., Paul, C., & Péter, F. (2023). Flavonoids: Overview of biosynthesis, biological activity, and current extraction techniques. *Plants*, 12(14), 2732. <https://doi.org/10.3390/plants12142732>
- Makkiyah, F. A., Rahmi, E. P., Susantiningsih, T., Marliani, N., Arista, R. A., & Nurcholis, W. (2023). Optimization of *Graptophyllum pictum* leaves extraction using a simplex centroid design focused on extracting flavonoids with antioxidant activity. *Journal of Applied Pharmaceutical Science*, 13(5), 214-221. <https://doi.org/10.7324/japs.2023.117043>
- Maulana, F., Muhammad, A. A., Umar, A., Mahendra, F. R., Musthofa, M., & Nurcholis, W. (2022). Profiling metabolites through chemometric analysis in *Orthosiphon aristatus* extracts as α -glucosidase inhibitory activity and in silico molecular docking. *Indonesian Journal of Chemistry*, 22(2), 501-514. <https://doi.org/10.22146/ijc.71334>
- Ngo, V., & Duennwald, M. L. (2022). Nrf2 and oxidative stress: A general overview of mechanisms and implications in human disease. *Antioxidants*, 11(12), 2345. <https://doi.org/10.3390/antiox11122345>
- Nurcholis, W., Alfadzrin, R., Izzati, N., Arianti, R., Vinnai, B. A., Sabri, F., Kristóf, E., & Artika, I. M. (2022). Effects of methods and durations of extraction on total flavonoid and phenolic contents and antioxidant activity of java cardamom (*Amomum compactum* Soland. ex Maton) fruit. *Plants*, 11(17), 2221. <https://doi.org/10.3390/plants11172221>
- Panche, A. N., Diwan, A. D., & Chandra, S. R. (2016). Flavonoids: An overview. *Journal of Nutritional Science*, 5, e47. <https://doi.org/10.1017/jns.2016.41>
- Pandey, K. B., & Rizvi, S. I. (2009). Plant polyphenols as dietary antioxidants in human health and disease. *Oxidative Medicine and Cellular Longevity*, 2(5), 270-278. <https://doi.org/10.4161/oxim.2.5.9498>
- Patel, M. B., Shaikh, F., Patel, V., & Surti, N. I. (2017). Application of simplex centroid design in formulation and optimization of floating matrix tablets of metformin. *Journal of Applied Pharmaceutical Science*, 7(4), 23-30. <https://doi.org/10.7324/japs.2017.70403>
- Ponphaiboon, J., Krongrawa, W., Aung, W. W., Chinatangkul, N., Limmatvapirat, S., & Limmatvapirat, C. (2023). Advances in natural product extraction techniques, electrospun fiber fabrication, and the integration of experimental design: A comprehensive review. *Molecules*, 28(13), 5163. <https://doi.org/10.3390/molecules28135163>
- Putra, R. P., Aisyah, S. I., & Nurcholis, W. (2023). Benefits of total phenolic and flavonoid content of *Portulaca oleracea* as antioxidant and antidiabetic: A review. *Tropical Journal of Natural Product Research*, 7(2), 2293-2304. <https://doi.org/10.26538/tjnpr/v7i2.1>
- Putra, R. P., Aisyah, S. I., Kurniatin, P. A., & Nurcholis, W. (2024). Optimization of solvent sonication-maceration for

- enhancing total phenolic content and antioxidant activity of *Portulaca oleracea* L. extract using the simplex centroid design method. *Tropical Journal of Natural Product Research*, 8(3), 6552-6557. <https://doi.org/10.26538/tjnpr/v8i3.12>
- Rohman, S., & Aini, A. Q. (2023). Total phenolics and flavonoid content of maceration-based purslane (*Portulaca oleracea*) extract. *Nusantara Science and Technology Proceedings*, 2023(33), 421-424. <https://doi.org/10.11594/nstp.2023.3367>
- Sajid, M., Channakesavula, C. N., Stone, S. R., & Kaur, P. (2021). Synthetic biology towards improved flavonoid pharmacokinetics. *Biomolecules*, 11(5), 754. <https://doi.org/10.3390/biom11050754>
- Sicari, V., Loizzo, M. R., Tundis, R., Mincione, A., & Pellicanò, T. M. (2018). *Portulaca oleracea* L. extracts display antioxidant and hypoglycaemic effects. *Journal of Applied Botany and Food Quality*, 91, 39-46. <https://doi.org/10.5073/JABFQ.2018.091.006>
- Suhaimi, S. H., Hasham, R., Idris, M. K. H., Ismail, H. F., Ariffin, N. H. M., & Majid, F. A. A. (2019). Optimization of ultrasound-assisted extraction conditions followed by solid phase extraction fractionation from *Orthosiphon stamineus* Benth. leaves for antiproliferative effect on prostate cancer cells. *Molecules*, 24(22), 4183. <https://doi.org/10.3390/molecules24224183>
- Thao, T. T. P., Tu, P. T. C., & Men, T. T. (2023). A comparative study on polyphenol and flavonoid content, antioxidant and anti-inflammatory capacity of different solvent extracts from *Portulaca oleracea* in carrageenan-induced paw edema in mice. *Tropical Journal of Natural Product Research*, 7(10), 4152-4159. <https://doi.org/10.26538/tjnpr/v7i10.6>
- Tzanova, M., Atanasov, V., Yaneva, Z., Ivanova, D., & Dinev, T. (2020). Selectivity of current extraction techniques for flavonoids from plant materials. *Processes*, 8(10), 1222. <https://doi.org/10.3390/pr8101222>
- Wang, M., Li, C., Li, J., Hu, W., Yu, A., Tang, H., Li, J., Kuang, H., & Zhang, H. (2023). Extraction, purification, structural characteristics, biological activity and application of polysaccharides from *Portulaca oleracea* L. (purslane): A review. *Molecules*, 28(12), 4813. <https://doi.org/10.3390/molecules28124813>
- Zhang, Y. C., Gan, F. F., Shelar, S. B., Ng, K. Y., & Chew, E. H. (2013). Antioxidant and Nrf2-inducing activities of luteolin, a flavonoid constituent in *Ixeris sonchifolia* Hance, provide neuroprotective effects against ischemia-induced cellular injury. *Food and Chemical Toxicology*, 59, 272-280. <https://doi.org/10.1016/j.fct.2013.05.058>