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Optimal Extraction Solvent for Purslane (*Portulaca grandiflora* Hook.) Flowers: a Study on Phenolic and Flavonoid Contents and Antioxidant Activities Using Microwave-Assisted Extraction

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Purslane (*Portulaca grandiflora* Hook.) has the potential to act as an antioxidant due to the presence of secondary metabolites, such as phenolic compounds and flavonoids, which are abundant in its flowers. The efficiency of extracting these compounds can be influenced by the choice of extraction solvent. This study aimed to identify the optimal solvent for extracting purslane flowers using microwave-assisted extraction, evaluating total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activities (DPPH and FRAP methods). The optimal solvent, determined using the simplex-centroid design (SCD) method and Design Expert software®, is a mixture of 41% acetone and 59% methanol. The results obtained were consistent with predicted values from verification tests, yielding a TPC of 3.599 mg GAE/g fresh weight, TFC of 1.796 mg QE/g fresh weight, and antioxidant activities of 0.669 µmol TE/g fresh weight (DPPH) and 9.231 µmol TE/g fresh weight (FRAP), with a desirability value of 0.712. The SCD method effectively optimized the extraction solvent for purslane flowers concerning TPC, TFC, and antioxidant activity.

1. Introduction

Extensive research has been conducted on free radicals as intermediates in various diseases. Free radicals are molecules with unpaired electrons, making them highly unstable and reactive. They tend to attract electrons from their surroundings, damaging cells by attacking lipids, proteins, and DNA. Excessive free radicals can lead to oxidative stress in cells, resulting in degenerative diseases such as cancer, diabetes mellitus, cardiovascular diseases, and premature aging (Phaniendra et al., 2015).

Damage caused by free radicals can be mitigated using compounds known as antioxidants. Antioxidants donate electrons to neutralize free radicals, thereby inhibiting the chain reaction of free radical formation (Setiawan et al., 2018). While the human body can synthesize endogenous antioxidants, these may not suffice when there are excess free radicals, necessitating exogenous antioxidants from external sources (Kurusat, 2016).

Exogenous antioxidants can be derived from natural sources or chemically synthesized. However, toxicological studies on the long-term use of synthetic antioxidants have shown harmful effects, prompting researchers to seek natural alternatives (Anwar et al., 2018). Phenolic and flavonoid compounds are known to play a crucial role in the antioxidant activity of natural substances. Johari and Khong (2019) demonstrated that substances with higher total phenolic content exhibit greater antioxidant activity. One such plant known to contain significant phenolic and flavonoid compounds with antioxidant properties is purslane (*Portulaca grandiflora* Hook.).

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Purslane, also known as moss rose, Bombay silk, or eight o'clock flowers, belongs to the Portulacaeae family. Phytochemical analysis of purslane herbs by Anghel et al. (2013) revealed the presence of sterols, polyphenolic acids, flavonoids, polysaccharides, and reducing substances. Further research on the metabolite profile and its relationship with the biological activity of moss rose flower parts by Sporna-Kucab et al. (2022) indicated that the flower parts contain phenolic and flavonoid compounds that strongly correlate with antioxidant activity. These compounds include feruloylquinic acid, rosmarinic acid, quercetin-O-hexoside, luteolin, naringenin, apigenin, kaempferol, and sorbifolin.

Extraction is the primary process by which bioactive compounds are obtained from biomass materials. The aim of the extraction process is to maximize the yield of target compounds and achieve the highest biological activity from the extract. The results and biological activity of the extract are influenced by both the extraction technique and the solvent used (Winahyu et al., 2018). Various solvents, such as methanol, ethanol, acetone, and water, have been employed to extract bioactive compounds from plants. The diversity of bioactive compounds in plant materials and their different polarities towards various solvents necessitates carefully selecting an optimal solvent based on the plant and the target compounds to be isolated (Verdiana et al., 2018).

Most studies on purslane and its parts have focused on identifying bioactive compounds. However, no study has specifically examined the effect of solvents on the extraction of bioactive compounds from purslane flowers. This study aims to identify the optimal solvent for extracting purslane flowers using microwave-assisted extraction by evaluating total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activities determined by the DPPH and FRAP methods.

2. Materials and methods

2.1 Materials

Purslane with pink flowers was sourced from the Biopharmaca Conservation and Cultivation Station, Tropical Biopharmaca Research Center, IPB University, Bogor, Indonesia. The chemicals used in this study included acetone (pro-analysis), methanol (pro-analysis), ethanol (pro-analysis), aquadest, Folin–Ciocalteu phenol reagent, glacial acetic acid, HCl, sodium carbonate, sodium acetate, AlCl3, trolox, gallic acid, and quercetin, all procured from Merck-Millipore (Darmstadt, Germany). DPPH was obtained from HiMedia (Maharashtra, India), while FeCl3 and 2,4,6-tripydyl-s-triazine (TPTZ) were acquired from Sisco Research Laboratories Pvt. Ltd. (Maharashtra, India).

2.2 Experimental design and extract preparation

The extraction solvents were mixtures of water, acetone, methanol, and ethanol in various proportions, designed according to a simplex-centroid design (Design Expert version 13, State-Ease Inc., Minneapolis, MN, USA). Each mixture component was studied in the proportion range of 0 to 100%. The studied responses included total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activities (DPPH and FRAP). The final experimental design is presented in Table 1. Fifteen factorial experiments, each with three repetitions, were conducted.Purslane flower extract was obtained using microwave-assisted extraction (MAE) as described by Nurcholis et al. (2022), with modifications. Two grams of crushed fresh flowers were placed into an Erlenmeyer flask, and 20 mL of the solvent mixture was added. The flasks were exposed to a microwave oven (Sharp R-21D0(S)-IN) at 135 W for 3 minutes. The suspension was periodically cooled and filtered. A filtrate sample with a concentration of 10% (w/v) was subsequently used to analyze the responses.

2.3 Total phenolic content (TPC)

TPC was quantified based on the method of Yanuarti et al. (2017), with modifications, using Folin–Ciocalteu phenol reagent and gallic acid as a standard. Twenty microliters of the samples were mixed with 120 µL of 10% Folin–Ciocalteu phenol reagent in a microplate. The mixture was allowed to react for 5 minutes in a dark room, and then 80 µL of 10% sodium carbonate solution was added. The mixture was incubated for 30 minutes in a dark room until the color developed. The absorbance of the solution was read at 737 nm using a spectrophotometer (SPECTROstarNano, BMG LABTECH). TPC was expressed as milligrams of gallic acid equivalent per gram fresh weight (mg GAE/g FW).

2.4 Total flavonoid content (TFC)

TFC was quantified using the method of Yanuarti et al. (2017), with modifications, and quercetin as a flavonoid standard. The sample was added to 120 µL distilled water, 10 µL of 10% AlCl3, 60 µL of ethanol, and 10 µL of glacial acetic acid. The solution was incubated for 30 minutes, and the absorbance was measured at 415 nm using a nano-spectrophotometer (SPECTROstarNano, BMG LABTECH, Offenburg, Germany). TFC was expressed as milligrams of quercetin equivalent per gram of fresh weight (mg QE/g FW).

2.5 Antioxidant activity by DPPH method

The extracted sample (100 µL) was added to 100 µL of 125 µM DPPH solution. The mixture was incubated at 37°C for 30 minutes. The absorbance was measured at 515 nm using a spectrophotometer (SPECTROstarNano, BMG LABTECH). Antioxidant activity was indicated by a color change from dark purple to yellow, and free radical scavenging activity was expressed in µmol of trolox equivalent per gram of dry weight (µmol TE/g DW), with a Trolox standard of 0–50 µM.

2.6 Antioxidant activity by FRAP method

The FRAP solution was prepared by mixing acetate buffer (pH 3.6), 10 µM TPTZ dissolved in 40 mM HCl, and 20 mM FeCl3 solution in a ratio of 10:1:1, protected from light. Then, 300 µL of the FRAP solution was added to 10 µL of the sample. The mixture was vortexed and incubated at 37°C for 5 minutes. After incubation, the absorbance was recorded at 593 nm using a spectrophotometer (SPECTROstarNano, BMG LABTECH). The results of the FRAP assay were expressed as µmol of trolox equivalent per gram of dry weight (µmol TE/g DW), with a Trolox standard of 0–400 µM.

2.7 Data analysis

The obtained data were processed using Microsoft Excel version 2306 and Design Expert 13.0 up to the stage of formula verification. Tukey's test was conducted as a post hoc analysis using IBM SPSS Statistics 25.0.

3. Results and discussion

The variation in solvent composition produced diverse values for each response, as indicated in Table 1. Notably, the combinations of acetone-methanol, methanol, water, and methanol resulted in the highest TPC, TFC, DPPH, and FRAP values, respectively, with significant differences ($p<0.05$). Conversely, the water-methanol solvent yielded the lowest TPC, TFC, and FRAP values, while the ethanol solvent produced the lowest DPPH response.

Table 1: Experimental design and responses value of purslane flower extract

Run	Independent variable (%)				Experimental value			
	Water (A)	Acetone (B)	Methanol (C)	Ethanol (D)	TPC	TFC	DPPH	FRAP
1	0.00	0.00	50.00	50.00	1.220 ± 0.059^{bc}	1.078 ± 0.031^{e}	0.452 ± 0.005^{bc}	5.440 ± 0.423^{b}
2	50.00	0.00	50.00	0.00	0.938 ± 0.020^{a}	0.552 ± 0.004^{a}	0.438 ± 0.006^{b}	3.587 ± 0.050^{a}
3	0.00	0.00	0.00	100.00	1.399 ± 0.026^{cd}	1.397 ± 0.097^{fg}	0.405 ± 0.017^{a}	9.633 ± 0.398^{f}
4	0.00	50.00	0.00	50.00	2.037 ± 0.158^{ef}	1.564 ± 0.064^{h}	0.474 ± 0.012^{cd}	7.713 ± 0.330^{e}
5	50.00	50.00	0.00	0.00	2.190 ± 0.047^{f}	0.788 ± 0.015^{cd}	0.483 ± 0.009^{de}	5.627 ± 0.202^{bc}
6	0.00	0.00	100.00	0.00	1.450 ± 0.039^{d}	1.776 ± 0.087^{i}	0.556 ± 0.012^{g}	10.100 ± 0.172^{f}
7	50.00	0.00	0.00	50.00	1.199 ± 0.071^{bc}	0.604 ± 0.023^{ab}	0.496 ± 0.005^{def}	3.833 ± 0.122^{a}
8	0.00	33.33	33.33	33.33	1.262 ± 0.014^{bcd}	1.285 ± 0.007^{f}	0.452 ± 0.005^{bc}	7.567 ± 0.147^{e}
9	0.00	50.00	50.00	0.00	2.614 ± 0.170^{g}	1.523 ± 0.046^{gh}	0.601 ± 0.008^{h}	7.033 ± 0.100^{de}
10	25.00	25.00	25.00	25.00	1.087 ± 0.009^{ab}	0.890 ± 0.015^{d}	0.481 ± 0.019^{cde}	6.473 ± 0.302^{cd}
11	100.00	0.00	0.00	0.00	1.050 ± 0.050^{ab}	0.557 ± 0.038^{a}	0.716 ± 0.001^{j}	3.667 ± 0.040^{a}
12	33.33	33.33	33.33	0.00	1.830 ± 0.046^{e}	0.835 ± 0.019^{cd}	0.469 ± 0.002^{cd}	7.293 ± 0.491^{de}
13	33.33	33.33	0.00	33.33	1.388 ± 0.014^{cd}	0.730 ± 0.053^{bc}	0.508 ± 0.005^{ef}	5.58 ± 0.428^{b}
14	0.00	100.00	0.00	0.00	1.115 ± 0.046^{ab}	1.378 ± 0.093^{fg}	0.650 ± 0.016^{i}	5.040 ± 0.286^{b}
15	33.33	0.00	33.33	33.33	1.220 ± 0.020^{bc}	0.750 ± 0.043^{bcd}	0.519 ± 0.009^{f}	4.92 ± 0.341^{b}

The values are expressed as means ± SD. Different letters indicate significant differences ($p < 0.05$) by column.

3.1 Statistical analysis

Fifteen mixture formulations were conducted based on the experimental design, and the respective coefficients of determination (R^2) and ANOVA of the mathematical models were adjusted to the response functions, as shown in Table 2. A p-value less than 0.05 indicated a significant difference among runs at the 0.05 significance level. Based on the obtained p-values, the models for TFC, DPPH, and FRAP are statistically significant, while the model for TPC is not. The R^2 values indicate that the selected models explained 99.75%, 97.60%, 90.72%, and 51.18% of the variability in the TPC, TFC, DPPH, and FRAP data, respectively. Additionally, the adequate precision values for each response are greater than four, demonstrating that the selected models have a sufficiently strong signal to navigate the design space effectively during the optimization process.

Table 2: Analysis of Variance (ANOVA) results for solvent optimization

Response	Model	p-value	R^2	Adequate precision
TPC	special cubic	0.1403	0.9975	19.2264
TFC	quadratic	0.0016	0.9760	14.0111
DPPH	quadratic	0.0385	0.9072	8.7120
FRAP	linear	0.0418	0.5118	6.5453

The mathematical models obtained for TPC, TFC, DPPH, and FRAP are as follows:

$$TPC = 1{,}05A + 1{,}11B + 1{,}45C + 1{,}40D + 4{,}47AB - 1{,}21AC - 0{,}0642AD + 5{,}36BC + 3{,}16BD - 0{,}7802CD - 9{,}73ABC - 18{,}02ABD + 3{,}28ACD - 25{,}56BCD \quad (1)$$

$$TFC = 0{,}5581A + 1{,}40B + 1{,}76C + 1{,}40D - 1{,}02AB - 2{,}21AC - 1{,}52AD - 0{,}3018BC + 0{,}3524BD - 1{,}84CD \quad (2)$$

$$DPPH = 0{,}7052A + 0{,}6500B + 0{,}5536C + 0{,}3984D - 0{,}7157AB - 0{,}6646AC - 0{,}05540AD - 0{,}0752BC - 0{,}2061BD - 0{,}0630CD \quad (3)$$

$$FRAP = 2{,}74A + 6{,}41B + 8{,}07C + 7{,}72D \quad (4)$$

3.2 Effect of solvent system on the responses value

The interactions between water, acetone, methanol, and ethanol on TPC, TFC, DPPH, and FRAP responses are explained through mathematical equations (1-4). Positive coefficients indicate a direct proportional effect, whereas negative coefficients signify an inverse effect on the response values. These mathematical models were used to represent the simultaneous effect of the solvents on TPC, TFC, DPPH, and FRAP as contour plots, depicted in Figure 1. The color variations on the graph indicate different response values, with blue representing the lowest and red the highest.

Binary solvents with acetone mixtures are effective for extracting phenolic compounds. This effectiveness was demonstrated in a study by Nasr et al. (2019) using *Eucalyptus camaldulensis*, which showed that the water-acetone extract had the highest TPC value compared to single extracts and other organic solvent-water extracts. A 1:1 (v/v) solvent combination produces a unique polarity index. Additionally, the synergistic effect of the acetone-methanol solvent mixture allows for the extraction of more phenolic compounds from purslane flowers than other solvents. This synergistic effect was also observed in the phenolic extraction process from *Isatis tinctoria* leaves in a study by Wakeel et al. (2019).

Methanol provided the highest TFC and FRAP response values, as shown in Table 1. This result aligns with the study by Sari et al. (2021), which demonstrated that methanol extraction yields the highest flavonoid content in the extract of *Coccinia grandis* L. Flavonoid compounds are well-extracted in polar methanol solvents because plant flavonoids are generally bound to sugars as glycosides, increasing their polarity (Gazali et al., 2019). The flavonoids identified in purslane flowers by Sporca-Kucab et al. (2020) are conjugated with sugars, such as quercetin-O-hexoside I, quercetin-O-hexoside II, and luteolin-7-O-rutinoside. These conjugated compounds are more soluble in methanol than in other solvents.

Water produced the highest DPPH antioxidant activity response, while ethanol extraction resulted in the lowest antioxidant activity (Table 1). This finding is consistent with a study by Anggraini et al. (2019), which showed that the highest antioxidant activity in *Lepisanthes alata* fruit flesh is sequentially obtained from water, methanol, and ethanol extracts. Although water produced the highest DPPH antioxidant activity response, this value was inversely related to the TPC and TFC response values obtained in the water extract of purslane flowers. This discrepancy may be due to the extraction of other metabolites with antioxidant activity that are not phenolic compounds, such as malic acid, which is highly soluble in water and present in purslane flowers (Sporna-Kucab et al., 2022).

The FRAP method showed higher antioxidant activity values than the DPPH method. This difference is due to variations in the reaction mechanisms of the two tests. The preferred antioxidant reaction mechanism can be determined based on an antioxidant compound's bond dissociation energy (BDE) and ionization potential (IP) values. BDE is the energy required to break the O-H bond in the hydroxyl group of phenolic compounds, while IP is the energy required for a phenolic compound to release an electron and form a radical cation molecule (Marković et al., 2012).

A lower BDE value compared to IP indicates that the hydrogen atom transfer reaction is preferred over electron transfer, and vice versa. Zheng et al. (2017) demonstrated that the solvent influences the BDE and IP values of quercetin and its glycoside forms. Polar solvents stabilize charged species, providing lower IP values than those in gas or nonpolar solvents. However, polar solvents yield the highest BDE values, as shown in the study by Boli et al. (2019). Since the solvents used in purslane flower extraction (water, acetone, methanol, ethanol, and

their combinations) are polar or semi-polar, the IP value is lower than the BDE value. Consequently, the electron transfer mechanism is more likely to occur than hydrogen atom transfer, resulting in higher antioxidant activity in the FRAP test compared to the DPPH test.

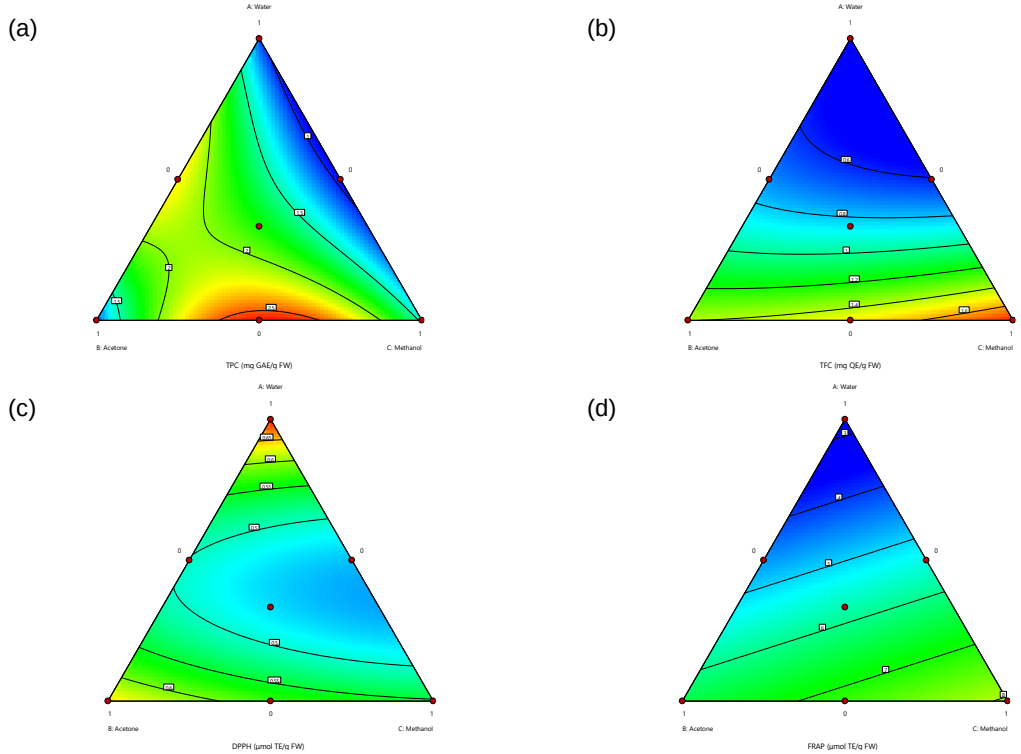

Figure 1: Effect of solvent on the (a) TPC, (b) TFC, (c) DPPH, and (d) FRAP of the purslane flower extract, which has been influenced by ethanol (D) at a level of 0.0

3.3 Optimization

The optimum extraction formulation for purslane flowers was identified using Design-Expert software with the Simplex-Centroid Design (SCD) method. Based on the highest desirability value, the selected combination comprised 41% acetone and 59% methanol, achieving a desirability value of 0.712, indicating a 71.2% alignment with the optimization target.

A validation experiment was conducted under optimum conditions to verify the model equations. The TPC, TFC, DPPH, and FRAP antioxidant activities of the purslane flower extract were 3.599 mg GAE/g FW, 1.796 mg QE/g FW, 0.669 µmol TE/g FW, and 9.231 µmol TE/g FW, respectively. These values exceeded the predicted results but remained within the 95% prediction interval (PI). The PI represents the expected range of subsequent response measurements under identical conditions at a specific significance level (Engelen et al., 2015). Therefore, the model equations are considered adequate for optimizing the extraction solvent for purslane flowers.

4. Conclusions

This study identified the optimal solvent for extracting purslane flowers using microwave-assisted extraction. The best formulation, determined by the Simplex-Centroid Design method, was 41% acetone and 59% methanol, achieving a 71.2% alignment with the optimization target. Validation confirmed the model's accuracy, with TPC, TFC, DPPH, and FRAP values within the 95% prediction interval. The results highlight the effectiveness of acetone-methanol mixtures in extracting phenolic compounds and enhancing antioxidant activities, providing a basis for optimizing extraction processes for bioactive compounds.

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