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Optimization of Solvent Type and Solvent-to-Solid Ratio for the Extraction of Phenolic Compounds and Antioxidant Capacity from *Portulaca oleracea* L.

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Abstract: *Portulaca oleracea* L. (Purslane) is a medicinal plant known for its high content of phenolic compounds with strong antioxidant properties. This study aims to investigate the influence of solvent type and solvent-to-solid ratio on the extraction efficiency of total phenolic content (TPC) and antioxidant capacity. We hypothesize that both solvent type and solvent-to-solid ratio significantly influence the yield of phenolic compounds and antioxidant activity. Five different solvents (water, ethanol, methanol, acetone, and ethyl acetate) and five solvent-to-solid ratios (3:1 to 15:1, v/w) were evaluated. The results showed that water extraction yielded the highest TPC (2.78 mg GAE/g DW), while methanol and ethyl acetate extracts demonstrated superior antioxidant capacity in CUPRAC and FRAP assays, respectively. Increasing the solvent-to-solid ratio enhanced TPC and antioxidant activity up to a saturation point. Pearson's correlation analysis revealed strong positive relationships between TPC and antioxidant activity. These findings provide insights into optimizing extraction conditions to enhance the bioactive properties of Purslane, facilitating its application in herbal medicine and nutraceuticals.

Keywords: antioxidant activity; phenolic compounds; *Portulaca oleracea* L.; extraction optimization; solvent extraction; solvent-to-solid ratio.

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1. Introduction

The use of active compounds from herbal plants has become a global trend in addressing various health issues. Among them, *Portulaca oleracea* L. (commonly known as Purslane) is widely recognized as a traditional medicinal plant [1]. This plant is rich in phenolic compounds, including caffeic acid, p-coumaric acid, scopoletin, ferulic acid, and chlorogenic acid, which have been reported to exhibit antioxidant properties [2]. The aerial parts of Purslane have traditionally been used as an antiseptic and for their analgesic properties to reduce pain and swelling [3]. Given its significant pharmacological potential, enhancing the accumulation of its bioactive metabolites, particularly phenolic compounds, is crucial for optimizing its

medicinal value [4]. However, the effects of extraction parameters on the yield and quality of these bioactive compounds remain insufficiently studied.

Phenolic compounds in plants are extracted to isolate active components from specific plant parts [5]. One of the main challenges in optimizing the medicinal potential of Purslane is determining the appropriate extraction parameters. The wide variety of available extraction solvents, combined with the lack of standardized guidelines for the solvent-to-solid ratio in natural product extraction, makes these factors particularly interesting for further research.

However, the diverse metabolic characteristics of natural products require a more selective approach in choosing the appropriate extraction solvent [6]. Additionally, optimizing the extraction process extends beyond solvent selection, as factors such as the solvent-to-solid ratio and particle size significantly influence extraction efficiency. Studies have shown that the optimal solvent-to-solid ratio depends on the mesh size of the plant material, highlighting the interaction between mass transfer phenomena and solvent penetration within the matrix [7]. Therefore, selecting the appropriate solvent type and solvent-to-solid ratio is crucial to maximizing the yield and bioactivity of medicinal compounds. We hypothesize that both solvent type and solvent-to-solid ratio significantly affect the phenolic yield and antioxidant activity of Purslane extracts.

Studies on extraction optimization, particularly those involving selecting an appropriate solvent type and solvent-to-solid ratio, are highly complex. Previous research has analyzed the synergistic effects of multiple extraction factors simultaneously, including their interactions [8–11]. However, simultaneous approaches using experimental design have certain limitations compared to single-factor studies, such as a more complex design and analysis, greater demands on time, materials, and data, and reduced suitability for exploratory studies due to the difficulty of isolating the specific effects of each factor.

To address these challenges, this study focuses on evaluating the effects of two single extraction factors: solvent type and solvent-to-solid ratio, in assessing their effectiveness in extracting phenolic compounds and their impact on the antioxidant capacity of Purslane. This approach aims to minimize the risk of misinterpretation due to factor interactions and to identify key parameters before advancing to multivariate optimization. The novelty of this study lies in its focus on analyzing the effects of each level of the two single extraction factors. Unlike previous studies that investigated the interactions between multiple factors, this research specifically examines the impact of selecting an appropriate single solvent type and solvent-to-solid ratio on the desired bioactive compounds, which contribute to the final quality of herbal medicine.

2. Materials and Methods

2.1. Materials.

Extraction solvents (ethanol pro-analysis, methanol pro-analysis, acetone pro-analysis, and ethyl acetate pro-analysis), Folin-Ciocalteu reagent, sodium carbonate (Na_2CO_3), copper(II) chloride dihydrate ($CuCl_2$), ammonium acetate ($C_2H_7NO_2$), Hydrochloric acid 37% (HCl), sodium acetate (CH_3COONa) were obtained from Merck KGaA (Darmstadt, Germany), gallic acid ($C_7H_6O_5$) (Merck KGaA, China), Neocuproine (Merck KGaA, Austria), 6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox), 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) were obtained from Sigma-Aldrich (St. Louis, USA), 2,4,6-Tripyridyl-s-triazine (TPTZ) and ferric chloride ($FeCl_3$)

were obtained from Sisco Research Laboratories Pvt. Ltd. (Maharashtra, India), and potassium persulfate ($K_2S_2O_8$) were obtained from FISHER Scientific.

2.2. Sample preparation.

The study was conducted from August to December 2023. Purslane was obtained from the Biopharmaca Conservation and Cultivation Unit at IPB University, Bogor, Indonesia. The aerial parts (above-ground biomass) were collected and dried in an oven at 45°C for 72 hours. The dried Purslane was then ground and sieved to obtain 80-mesh simplicia (equivalent to a particle size of approximately 180 µm), which was subsequently stored in a clean, sealed container at room temperature.

2.3. Experiment design and extraction.

The experiment was designed with two variables, each consisting of five levels: solvent type (water, ethanol, methanol, acetone, and ethyl acetate) and solvent-to-solid ratio (3:1, 5:1, 7:1, 10:1, and 15:1, v/w). Single-factor extraction for solvent type was conducted using a fixed solvent-to-solid ratio of 10:1 (v/w), while single-factor extraction for solvent-to-solid ratio was performed using methanol as the solvent. Purslane simplicia was extracted using a combined sonication-maceration method [12]. Sonication was carried out at room temperature for 30 minutes at 60 kHz using a Decon F5 Major sonicator (Decon Laboratories, USA), followed by maceration in a DAIHAN WiseBath water bath shaker (South Korea) at 25°C for 180 minutes. The maceration extract was then filtered and concentrated using a rotary evaporator (LabTech Ltd., USA) to obtain a final extract concentration of 0.2 g/mL. The physicochemical properties of the solvents are presented in Table 1.

Table 1. Physicochemical properties of solvents used in Purslane extraction.

Solvents	**Polarity index**	**Boiling point (°C)**	**Viscosity (cPoise)**	**Solubility in Water (% w/w)**
Water	9.0	100	1.00	100
Ethanol	5.2	78	1.20	100
Methanol	5.1	65	0.60	100
Acetone	5.1	56	0.32	100
Ethyl acetate	4.4	77	0.45	8.7

% w/w: weight/weight percent.

2.4. Total phenolic content (TPC) determination.

TPC was measured using the Folin-Ciocalteu method, as described by Nurcholis *et al.* [13]. A 10 µL sample extract was mixed with 160 µL of distilled water and 10 µL of 10% Folin-Ciocalteu reagent in a 96-well microplate (BiologiX), and then incubated for 5 minutes at room temperature. Next, 20 µL of 10% Na_2CO_3 was added, and the solution was incubated for 30 minutes. The absorbance was recorded at 750 nm using a nano spectrophotometer (SPECTROstarNano BMG LABTECH, Germany). Gallic acid standards with a concentration range of 0-300 ppm were used ($y = 0.001x + 0.013$, $R^2 = 0.998$). TPC values were expressed as mg gallic acid equivalent per gram dried weight (mg GAE/g DW). Each measurement was conducted in triplicate.

2.5. Antioxidant capacity assays.

The CUPRAC method was used to assess antioxidant capacity [14]. A 50 µL sample extract was mixed with 50 µL of 10 mM $CuCl_2$ x $6H_2O$, 7.5 mM neocuproine, and ammonium

acetate buffer (pH 7.0). The solution was incubated at room temperature for 30 minutes, and then the absorbance was measured at 450 nm. Trolox standards with a concentration range of 0-600 µmol were used ($y = 0.001x + 0.015$, $R^2 = 0.995$). Results were expressed as µmol Trolox equivalent per gram dried weight (µmol TE/g DW).

The FRAP method was used to measure antioxidant capacity [15]. The FRAP reagent was prepared by mixing acetate buffer (pH 3.6), 20 mM FeCl3, and 10 mM TPTZ. A 10 µL sample extract was then mixed with 300 µL of the FRAP reagent. The solution was incubated for 30 minutes, and the absorbance was measured at 593 nm. Trolox standards with a concentration range of 0-550 µmol ($y = 0.0008x + 0.003$, $R^2 = 0.995$) were used. Results were reported as µmol TE/g DW.

The antioxidant capacity was evaluated using the ABTS decolorization assay [16]. ABTS radical cations were generated by reacting 7.7 mM ABTS with 2.4 mM potassium persulfate. A 20 µL sample was mixed with 180 µL of the ABTS solution and incubated for 6 minutes in the dark. Absorbance was measured at 734 nm. Trolox standards with a concentration range of 0-500 µmol ($y = 0.1539x - 3.3843$, $R^2 = 0.995$) were used. Results were reported as µmol TE/g DW.

The DPPH decolorization assay was used to evaluate antioxidant capacity [17]. A 100 µL sample extract was mixed with 100 µL of 125 µM DPPH radical solution and incubated for 30 minutes in the dark. Absorbance was measured at 515 nm. Trolox standards with a concentration range of 0-60 µmol ($y = 1.2144x + 9.2224$, $R^2 = 0.997$) were used. Results were reported as µmol TE/g DW.

All antioxidant assays (CUPRAC, FRAP, ABTS, and DPPH) were performed in triplicate.

2.6. Data analysis.

Data were statistically analyzed using One-Way ANOVA followed by Tukey's post hoc test to determine significant differences between treatments ($p < 0.05$) with IBM® SPSS Statistics software version 25. Pearson's correlation analysis was then performed to assess the relationship between TPC and potential antioxidant capacity in Purslane extract, based on the correlation coefficient (r). All experiments were conducted in triplicate, and the data are presented as mean ± standard deviation (SD).

3. Results and Discussion

3.1. Determination of TPC and antioxidant in each solvent extract.

The comparative results for TPC and antioxidant capacity, depending on different solvent extracts of Purslane, are presented in Table 2. Both TPC and antioxidant capacity were significantly influenced by the type of solvent used, with values ranging from 0.60 to 2.78 mg GAE/g DW. The water extract exhibited the highest TPC (2.78 ± 0.01 mg GAE/g DW), followed by the ethanol extract (1.47 ± 0.05 mg GAE/g DW) and the methanol extract (1.48 ± 0.02 mg GAE/g DW), with no significant differences ($p > 0.05$). In contrast, the acetone and ethyl acetate extracts had the lowest TPC values (0.96 ± 0.04 and 0.60 ± 0.02 mg GAE/g DW, respectively).

The TPC values obtained in this study were lower compared to similar studies, such as those reporting values ranging from 56.2 to 142.2 mg GAE/g in Iran [18], where the highest TPC was obtained from methanol extract. Some authors reported TPC values for Purslane from

Taiwan ranging from 19.67 to 219.27 mg/g GAE [19], with the highest TPC found in the ethanol extract. On the other hand, the highest TPC value in the water extract in this study (2.78 ± 0.01 mg GAE/g DW) was higher than that reported by Karoune *et al*. [20], who found a TPC of 1.970 ± 0.081 mg GAE/g using water as the extraction solvent.

Table 2. TPC and antioxidant capacity of Purslane extract obtained with different solvents.

Extraction solvents	TPC	CUPRAC	FRAP	ABTS	DPPH
	mg GAE/g DW	µmol TE/g DW			
Water	2.78 ± 0.01[a]	33.19 ± 1.40[c]	26.92 ± 0.41[b]	35.97 ± 1.40[a]	1.16 ± 0.01[c]
Ethanol	1.47 ± 0.05[b]	55.99 ± 1.08[b]	24.48 ± 1.70[b]	21.25 ± 0.39[b]	2.84 ± 0.04[a]
Methanol	1.48 ± 0.02[b]	69.70 ± 1.32[a]	10.30 ± 0.50[c]	22.73 ± 0.64[b]	2.77 ± 0.01[b]
Acetone	0.96 ± 0.04[c]	36.02 ± 1.26[c]	2.47 ± 0.07[d]	18.69 ± 0.40[c]	0.94 ± 0.01[d]
Ethyl acetate	0.60 ± 0.02[d]	34.67 ± 1.20[c]	38.79 ± 1.20[a]	0.48 ± 0.01[d]	0.28 ± 0.01[e]

Numbers in the same column followed by the same letter indicate results that are not significantly different ($p > 0.05$). TPC: total phenolic content; GAE: gallic acid equivalent; TE: Trolox equivalent; DW: dried weight. Each value represents the mean ± standard deviation of triplicate measurements.

Polar solvents in previous studies successfully extracted more phenolics from Purslane compared to non-polar solvents. However, these previous findings suggest that, in addition to the solvent type, the accumulation of phenolic compounds extracted from Purslane may also be influenced by genetic variation between species or varieties of Purslane used [20,21] and environmental conditions such as light, soil type, water availability, and temperature, as described by Petropoulos *et al*. [22].

The antioxidant capacity of Purslane from different solvent extracts was measured using various assays (CUPRAC, FRAP, ABTS, and DPPH), as their characteristics depend on the mechanism of antioxidant metabolites in the extract. The reducing power assay showed the highest antioxidant capacity in the methanol extract (69.70 ± 1.32 µmol TE/g DW) using the CUPRAC method and in the ethyl acetate extract (38.79 ± 1.20 µmol TE/g DW) using the FRAP method. On the other hand, differences were observed in the ABTS and DPPH radical scavenging assays, where the water extract (35.97 ± 1.40 µmol TE/g DW) exhibited the highest ABTS scavenging activity, while the ethanol extract (2.84 ± 0.04 µmol TE/g DW) exhibited the highest DPPH scavenging.

These variations across assays can be attributed to differences in the antioxidant mechanisms each assay detects. CUPRAC and FRAP operate on the Single Electron Transfer (SET) mechanism, involving redox reactions where antioxidants donate electrons to oxidants, generating measurable colorimetric changes [23]. ABTS and DPPH, in contrast, primarily rely on the Hydrogen Atom Transfer (HAT) mechanism, which assesses the hydrogen-donating ability of antioxidants to neutralize free radicals [24]. This mechanistic distinction may explain the differential responses of extracts across assays, highlighting that solvent choice affects the types of antioxidant compounds extracted and their modes of action.

3.2. Determination of TPC and antioxidant in each solvent-to-solid ratio.

Table 3 shows the TPC and antioxidant capacity in different solvent-to-solid ratio extracts of Purslane, with values ranging from 0.72 to 5.60 mg GAE/g DW. TPC increased significantly as the solvent-to-solid ratio increased. The highest TPC was found at the 15:1 (v/w) ratio (5.60 ± 0.06 mg GAE/g DW), followed closely by the 10:1 and 7:1 ratios. The lowest value was observed at the 3:1 ratio.

As with TPC, antioxidant activity assessed using CUPRAC and FRAP increased with higher ratios, peaking at 15:1 (29.29 ± 0.29 and 35.81 ± 0.14 µmol TE/g DW, respectively).

These results are consistent with prior findings on Centella asiatica and Phyllanthus niruri, which also demonstrated higher extraction efficiency at elevated solvent ratios [25, 26].

Interestingly, radical scavenging activity did not follow this trend. The highest ABTS and DPPH activity was found at lower ratios (3:1 and 5:1), likely due to early saturation of the matrix-solvent system. This phenomenon can be explained by mass-transfer equilibrium, where increasing the solvent volume beyond a certain point yields diminishing returns in radical-scavenging capacity.

Table 3. TPC and antioxidant capacity of Purslane extract obtained with different solvent-to-solid ratios.

Solvent-to-solid ratio (v/w)	TPC	CUPRAC	FRAP	ABTS	DPPH
	mg GAE/g DW	µmol TE/g DW			
3:1	0.72 ± 0.02[d]	10.48 ± 0.14[d]	13.55 ± 0.16[e]	5.09 ± 0.06[a]	0.54 ± 0.01[d]
5:1	3.13 ± 0.06[c]	22.56 ± 0.73[c]	24.09 ± 0.27[d]	1.28 ± 0.00[b]	1.12 ± 0.01[a]
7:1	5.39 ± 0.05[b]	21.68 ± 0.23[c]	26.67 ± 0.05[c]	1.23 ± 0.00[bc]	1.09 ± 0.01[a]
10:1	5.46 ± 0.03[b]	26.87 ± 0.13[b]	27.42 ± 0.35[b]	1.19 ± 0.00[cd]	0.83 ± 0.01[b]
15:1	5.60 ± 0.06[a]	29.29 ± 0.29[a]	35.81 ± 0.14[a]	1.13 ± 0.00[d]	0.78 ± 0.03[c]

[3] Numbers in the same column followed by the same letter indicate results that are not significantly different ($p > 0.05$). TPC: total phenolic content; GAE: gallic acid equivalent; TE: Trolox equivalent; DW: dried weight; v/w: volume/weight. All values are means of triplicate measurements.

3.3. Effect of solvent type on TPC and antioxidant of Purslane extracts.

This study investigated five solvents with varying polarity and physicochemical properties (Table 1): water, ethanol, methanol, acetone, and ethyl acetate. The findings suggest that the extraction solvent with the highest polarity, water, significantly influenced the highest TPC values in Purslane extracts. In contrast, the TPC values of the ethanol and methanol extracts did not show significant differences ($p > 0.05$).

On the other hand, semi-polar solvents such as acetone and ethyl acetate resulted in lower TPC values. This finding aligns with the "like dissolves like" solubility principle [27], where phenolic compounds are polar due to the presence of hydroxyl groups bound to sugars as glycosides [28]. Therefore, their solubility is higher in polar solvents like water, ethanol, and methanol, compared to solvents with lower polarity, such as acetone and ethyl acetate [29–31].

The measurement of antioxidant capacity using the reducing power approach revealed that the methanol extract exhibited the highest value in the CUPRAC method, while the ethyl acetate extract showed the highest value in the FRAP method. These findings align with previous studies by Cai *et al*. [32], which highlight methanol's effectiveness in extracting compounds with high reducing power, likely due to its ability to solubilize a broad spectrum of phenolic antioxidants.

In previous studies, Uddin *et al*. [33] and Sallam and Anwar [34] reported that methanol extracts of Purslane yielded the highest total phenolic content compared to other solvents, which correlated with enhanced antioxidant activity. However, the FRAP assay's peak in the ethyl acetate extract suggests the presence of specific antioxidant compounds within this fraction, which exhibit a higher ferric-reducing capacity. This could be due to the extraction of moderately polar antioxidants that are less readily soluble in methanol [35].

Differences were also observed in the ABTS and DPPH radical scavenging assays, where the water extract exhibited the highest ABTS scavenging activity, while the ethanol extract showed comparatively lower activity in the highest DPPH scavenging. The superior ABTS scavenging ability of the water extract may be attributed to its efficiency in extracting

hydrophilic antioxidants, such as certain phenolic acids and glycosides, which are particularly effective at neutralizing ABTS radicals [36].

The relatively low DPPH scavenging activity observed in the ethanol extract, despite ethanol's general efficacy in extracting phenolics, warrants further investigation. This discrepancy could stem from variations in the specific phenolic profile extracted with ethanol in this instance, or from potential interferences from co-extracted compounds affecting the DPPH assay [37]. These findings suggest that solvents with high polarity, ranging from 9.0 to 5.1 (Table 1), such as water, ethanol, and methanol, are suitable for extracting phenolic compounds with antioxidant potential from Purslane.

3.4. Effect of solvent-to-solid on TPC and antioxidant of Purslane extracts.

The use of a high solvent-to-solid ratio was found to be beneficial for the extraction of phenolic compounds and to support an increase in antioxidant capacity, as measured by reducing power methods (CUPRAC and FRAP). This finding is supported by Tan *et al*. [25], who stated that a higher solid-to-solvent ratio can increase concentration gradients, resulting in enhanced diffusion rates and allowing the solvent to extract the solids more effectively.

The solubility of phenolic compounds depends on several factors, including the polarity of the solvent used, the degree of phenolic polymerization, interactions with other plant constituents, and the formation of insoluble complexes [38]. According to Frank *et al*. [39], strong interactions between the compounds and the solvent can modify the activity coefficient, thereby increasing the compounds' solubility. Furthermore, the solvent-to-solid ratio plays a crucial role in influencing solubility and equilibrium constants, ultimately maximizing the total phenolic yield at the highest solvent-to-solid ratio [40].

However, the increased time required to reach equilibrium must be considered, as the yield of active components will not continue to increase once equilibrium is reached [41]. Hamdam *et al*. [42] noted that the solid-to-solvent ratio can significantly influence the equilibrium constant and characterized the relationship between yield and solvent use as an exponential increase followed by a steady state, resulting in maximum yields. This may explain why lower solvent-to-solid ratios provided more optimal ABTS and DPPH antioxidant capacity values than higher ratios.

Although the amount of phenolic compounds generally increases with a higher solid-to-solvent ratio, the increase in phenolic yield does not necessarily correlate with antioxidant capacity due to the equilibrium factors. Therefore, it is essential to evaluate the influence of the solid-to-solvent ratio during the optimization of phytochemical extraction from different plant materials. This approach will help efficiently use solvents and solvent mixtures for extracting phytochemicals, avoid saturation effects, and reduce the cost of solvent disposal.

3.5. Correlation between TPC and antioxidant capacity of Purslane extracts.

Phenolic compounds are characterized by the presence of an aromatic ring containing hydroxyl substituents [43]. This structure often accounts for their antioxidant properties, as they donate a hydrogen atom via an electron-transfer process, converting the phenolic compound into a phenoxyl radical that stabilizes [44]. This relationship presents an interesting trend to investigate through a linear correlation approach. In this study, Pearson's correlation analysis between TPC and antioxidant capacities measured by various methods was conducted to evaluate the relationship between TPC and the antioxidant capacity of Purslane extract. The

results in Figure 1 revealed distinct patterns depending on the solvent type (Figure 1a) and solvent-to-solid ratio (Figure 1b).

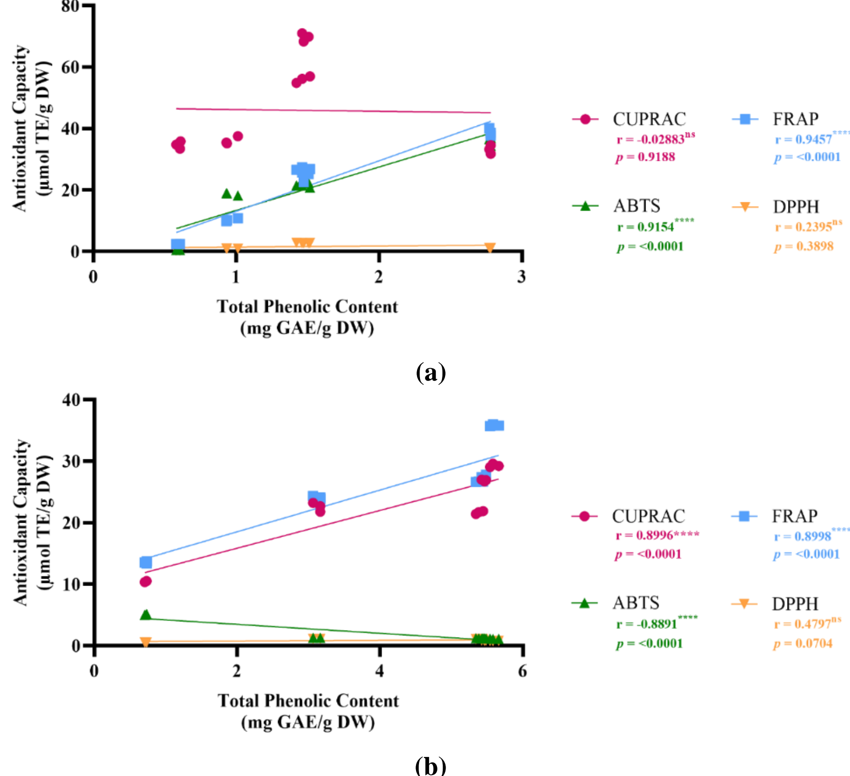

Figure 1. Scatter plot displaying Pearson's correlations between TPC and antioxidant capacity of Purslane obtained by: (**a**) Different solvents; (**b**) Solvent-to-solid ratio. "r" indicates correlation coefficient and the sign * ($p < 0.0001$, significant); ^{ns} ($p > 0.05$, not significant) indicates the significance level.

Based on solvent type, TPC showed a very strong positive correlation with FRAP (r = 0.9457) and ABTS (r = 0.9154), indicating significant associations (p < 0.0001). However, no significant correlations were observed with CUPRAC (r = -0.02883, p = 0.9188) or DPPH (r = 0.2395, p = 0.3898). In contrast, when examining the solvent-to-solid ratio, TPC exhibited significant positive correlations with CUPRAC (r = 0.8996, p < 0.0001) and FRAP (r = 0.8998, p < 0.0001). A significant but negative correlation was observed with ABTS (r = -0.8891, p < 0.0001), while no significant correlation was found with DPPH (r = 0.4797, p = 0.0704).

The strong correlations between TPC and antioxidant capacities measured by FRAP and ABTS can be explained by the sensitivity of these methods to phenolic compounds. In the FRAP method, antioxidant capacity is measured through the SET mechanism, where antioxidant compounds like polyphenols with hydroxyl (-OH) groups reduce ferric ions (Fe^{3+} to Fe^{2+}) [45]. The chemical structure of phenolic compounds, rich in hydroxyl groups, indicates that higher TPC content will show significant antioxidant activity in this method. A similar result was reported in an earlier study by Uddin *et al.* [33], who explained that the FRAP method is particularly suitable for measuring the antioxidant activity of compounds that operate through electron transfer mechanisms.

Meanwhile, the ABTS method involves the inhibition of the $ABTS^{\bullet+}$ radical formed through a combination of SET and HAT mechanisms. The ABTS method is known to be more sensitive to various types of antioxidants [46], including both polar compounds and water-soluble phenolic compounds, which are dominant in Purslane extract. Re *et al.* [47] stated that the ABTS method exhibits high sensitivity to polyphenolic compounds due to the enhanced

reactivity of the ABTS radical cation. This explains the strong correlation between TPC and antioxidant capacity measured using this method.

The weak correlation between TPC and antioxidant capacity measured by the DPPH method can be explained by limitations of the method for certain types of phenolic compounds and reaction conditions. The DPPH method works by measuring the antioxidant's ability to donate a hydrogen atom (HAT) to neutralize DPPH free radicals [48]. However, DPPH is more selective for compounds that act via the HAT mechanism, whereas some phenolic compounds in Purslane extract are more effective via the SET mechanism, which this method does not fully detect.

Furthermore, the DPPH reaction occurs in an organic medium such as methanol, which may affect the solubility of certain phenolic compounds in Purslane extract, thereby inhibiting optimal reaction. As a result, not all polyphenolic compounds in the extract may react with DPPH, leading to a weak correlation. According to Rumpf *et al*. [49], the DPPH method has lower sensitivity to hydrophilic antioxidants compared to methods like FRAP and ABTS, which are more responsive to phenolic compounds.

The weak negative correlation between TPC and antioxidant capacity measured by the CUPRAC method is attributed to differences in the method's sensitivity to specific antioxidants and measurement conditions. As explained by Apak *et al*. [50], the CUPRAC method operates based on the ability of antioxidants to reduce copper ions (Cu^{2+} to Cu^{+}) via the SET mechanism. However, not all phenolic compounds primarily function via SET; some exhibit a stronger tendency toward HAT or a combination of both. Additionally, the chemical structure of polyphenols significantly influences their reduction potential towards Cu^{2+}, particularly when steric effects or functional groups hinder electron transfer.

Moreover, the presence of non-phenolic compounds in plant extracts can contribute more substantially to antioxidant capacity in the CUPRAC assay than polyphenols [51], leading to a weak or even negative correlation between TPC and the measured antioxidant activity. Furthermore, certain phenolic compounds may preferentially form complexes with Cu^{2+} rather than reduce it to Cu^{+} [52], thereby lowering the detected antioxidant capacity. The stability of reaction products also plays a crucial role, as the Cu^{+} formed in the process may undergo disproportionation or reoxidation back to Cu^{2+} [53], ultimately reducing the measured antioxidant activity. Therefore, the weak negative correlation between TPC and CUPRAC antioxidant capacity can be attributed to a combination of factors, including differences in reduction mechanisms, the influence of non-phenolic compounds, copper complexation, and the stability of reaction products. Based on the results, the phenolic compounds in Purslane extract are likely responsible for the antioxidant properties, either through the SET or HAT mechanism.

These findings are supported by recent studies from Indonesian medicinal plant extracts, including *Averrhoa bilimbi* (starfruit), which demonstrated maximum FRAP and ABTS activity using acetone-methanol combinations [54] and *Orthosiphon aristatus* (Java tea), where phenolic content correlated more strongly with antioxidant capacity than flavonoid content [55]. Similarly, solvent optimization studies on *Psidium guajava* reported that a ternary solvent system (water, ethanol, and acetone) achieved optimal phenolic yield and antioxidant activity [56]. Research on *Morinda citrifolia* (noni) leaves also corroborated that higher solvent-to-solid ratios (up to 1:20) enhance both phenolic and flavonoid content and DPPH scavenging activity [57]. Additionally, comparative studies of Curcuma species confirm the strong predictive value of total phenolics for DPPH-based antioxidant capacity [58].

4. Conclusions

This study demonstrated that both solvent type and solvent-to-solid ratio significantly affect the extraction efficiency of phenolic compounds and antioxidant capacity from Purslane (*Portulaca oleracea* L.). Water was identified as the most effective solvent for extracting total phenolic content (TPC), whereas methanol and ethyl acetate extracts exhibited superior antioxidant activities in CUPRAC and FRAP assays, respectively. The results also showed that increasing the solvent-to-solid ratio improved TPC and antioxidant activities up to an optimal point, beyond which no further benefit was observed.

Pearson's correlation analysis indicated strong positive relationships between TPC and antioxidant capacities, particularly in FRAP and ABTS assays, suggesting that phenolic compounds are key contributors to antioxidant activity. These insights are valuable for optimizing extraction parameters in phytochemical research and may facilitate the development of more effective herbal formulations for use in pharmaceutical and nutraceutical industries. Future studies should consider multivariate optimization and compound-specific profiling to enhance extract standardization and therapeutic efficacy.

Author Contributions

Conceptualization, W.N.; methodology, A.Z. and W.N.; software, A.Z.; validation, S.I.A. and W.N.; formal analysis, A.Z.; investigation, A.Z.; resources, S.I.A. and W.N.; data curation, A.Z.; writing—original draft preparation, A.Z.; writing—review and editing, A.Z., S.I.A., and W.N.; visualization, A.Z.; supervision, S.I.A. and W.N.; project administration, W.N.; funding acquisition, W.N. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest

The authors declare no conflict of interest.

Abbreviations

The following abbreviations are used in this manuscript:

Abbreviation	Definition
WS-Ceria-NPs	*Withania somnifera*-derived Cerium Oxide Nanoparticles
ROS	Reactive Oxygen Species
RONS	Reactive Oxygen and Nitrogen Species
SOD	Superoxide Dismutase
DPPH	2,2-diphenyl-1-picrylhydrazyl
LPS	Lipopolysaccharide
SAED	Selected Area Electron Diffraction
FFT	Fast Fourier Transform
HRTEM	High-Resolution Transmission Electron Microscopy
PBS	Phosphate-buffered Saline
BSA	Bovine Serum Albumin
NOX	Nitric Oxide Assay
XRD	X-ray Diffraction
SEM	Scanning Electron Microscopy
EDS/EDX	Energy Dispersive X-ray Spectroscopy
FTIR	Fourier Transform Infrared Spectroscopy
UV-Vis	Ultraviolet-visible Spectroscopy
SD	Standard Deviation
ANOVA	Analysis of Variance

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