

Perbandingan Metode *Continuous Percolation* dan *Ultrasound-Assisted Extraction* (UAE) terhadap Kadar Total Fenolik Ekstrak Etanol 70% Gulma Krokot (*Portulaca oleracea* L.)

Comparison of Continuous Percolation and Ultrasound-Assisted Extraction (UAE) Methods on Total Phenolic Content of 70% Ethanol Extract of Purslane (Portulaca oleracea L.)

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Abstrak

Latar Belakang: *Portulaca oleracea* L. (krokot) mengandung senyawa fenolik dengan potensi farmakologis tinggi. Optimalisasi metode ekstraksi penting untuk memaksimalkan potensi bioaktif tanaman ini. **Tujuan:** Penelitian ini bertujuan membandingkan efisiensi metode *Continuous Percolation* (CP) dan *Ultrasound-Assisted Extraction* (UAE) dalam mengekstraksi kadar total fenolik (TPC) dari ekstrak etanol 70% krokot. **Metodologi:** Serbuk kering krokot diekstraksi dengan CP dan UAE menggunakan rasio bahan:pelarut 1:10 (b/v). Uji kualitatif FeCl_3 mengkonfirmasi keberadaan senyawa fenolik. TPC ditentukan menggunakan metode Folin-Ciocalteu dan dinyatakan dalam mg ekuivalen asam galat (GAE)/g ekstrak. Analisis statistik mencakup uji normalitas, uji Levene, dan uji Mann-Whitney U. **Hasil dan Pembahasan:** UAE menghasilkan TPC lebih tinggi secara signifikan ($420,04 \pm 0,40$ mg GAE/g) dibanding CP ($354,30 \pm 0,00$ mg GAE/g; $p = 0,034$), meskipun CP menghasilkan massa ekstrak lebih besar. Analisis statistik menunjukkan keunggulan UAE dalam mengekstraksi senyawa fenolik secara selektif. **Kesimpulan:** UAE lebih efektif dibandingkan CP dalam mengekstraksi senyawa fenolik dari krokot, kemungkinan karena efek kavitasi yang meningkatkan transfer massa. Metode ini direkomendasikan untuk ekstraksi senyawa fenolik, khususnya dari bahan tumbuhan yang bersifat termolabil.

Kata kunci: *Portulaca oleracea*; kadar total fenolik; *ultrasound-assisted extraction*; *continuous percolation*; ekstrak etanol; analisis fitokimia.

Abstract

Background: *Portulaca oleracea* L., commonly known as Krokot, is rich in phenolic compounds with notable pharmacological benefits. Optimizing extraction methods is critical to maximize its bioactive potential. **Objective:** This study aimed to compare the efficiency of *Continuous Percolation* (CP) and *Ultrasound-Assisted Extraction* (UAE) in obtaining total phenolic content (TPC) from 70% ethanol extract of *P. oleracea*. **Methods:** Dried powdered *P. oleracea* was subjected to CP and UAE using a 1:10 (w/v) plant-to-solvent ratio. Qualitative screening with FeCl_3 confirmed the presence of phenolics. TPC was quantified using the Folin-Ciocalteu method, expressed as mg gallic acid equivalent (GAE)/g extract. Statistical analysis included normality testing, Levene's test, and the Mann-Whitney U test. Results: UAE yielded significantly higher TPC (420.04 ± 0.40 mg GAE/g) compared to CP (354.30 ± 0.00 mg GAE/g; $p = 0.034$), although CP produced a higher extract mass. Statistical results confirmed the superiority of UAE in selectively extracting phenolic constituents. **Conclusion:** UAE is more effective than CP in extracting phenolic compounds from *P. oleracea*, likely due to enhanced cavitation-induced mass transfer. This method is recommended for maximizing phenolic recovery in plant-based extractions, especially for thermolabile compounds.

Keywords: *Portulaca oleracea*; total phenolic content; *ultrasound-assisted extraction*; *continuous percolation*; ethanol extract; phytochemical analysis.

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1. INTRODUCTION

Medicinal plants have long played a crucial role in traditional healthcare systems, providing bioactive compounds that contribute to the treatment and prevention of various diseases (1). These therapeutic properties are primarily attributed to the presence of secondary metabolites, including phenolic compounds, flavonoids, tannins, alkaloids, and terpenoids (2,3), which exhibit a broad spectrum of pharmacological activities such as antioxidant, anti-inflammatory, antibacterial, and wound healing effects (4).

Portulaca oleracea L., commonly known as Krokot, is a wild plant species widely distributed in tropical and subtropical regions (5). Often regarded as a weed, this plant has nonetheless been traditionally utilized for its medicinal benefits and as a food source in several cultures (6,7). Phytochemical investigations have revealed that *P. oleracea* contains various bioactive constituents, including flavonoids and tannins, which contribute to its reported antibacterial and antioxidant properties (8–10).

Among the phenolic compounds present in Krokot, flavonoids are known to disrupt bacterial cell membranes and interfere with microbial protein synthesis (11,12), while tannins can exert antimicrobial effects through protein precipitation and membrane damage (13). Quantifying the total phenolic content (TPC) of such plants is a vital step in validating their pharmacological potential. Ethanol is frequently employed as an extraction solvent due to its safety profile, availability, and efficiency in extracting a wide range of phytochemicals (14–17). The Folin–Ciocalteu method remains a standard analytical approach for determining TPC, favored for its simplicity, sensitivity, and reliability under alkaline conditions (18,19).

Several extraction techniques apply to medicinal plants, including maceration, percolation, reflux, and more advanced methods such as Continuous Percolation and Ultrasound-Assisted Extraction (UAE) (20,21). Continuous Percolation, an enhancement of traditional percolation, involves a constant flow of solvent through the plant material using a mechanical pumping system, which facilitates solvent penetration and may increase extraction efficiency (22). In contrast, UAE employs ultrasonic waves to disrupt plant cell walls, thereby accelerating the release of intracellular compounds (23); this approach is particularly beneficial for thermolabile phenolics, enabling higher yields within shorter extraction times (24). In purslane (*Portulaca oleracea* L.), aqueous infusion and decoction have been directly compared: decoction significantly outperforms infusion for recovering total phenolics (377.60 ± 2.44 vs 121.33 ± 1.16 mg GAE/g dry extract) (25). Separately, ultrasound-assisted extraction combined with maceration has been applied to *P. oleracea*, where pulsed sonication improved extraction yield and 48-h maceration produced the highest total phenolic content among tested conditions (19.24 ± 1.01 mg GAE/g) (26).

To date, comparative studies examining the efficiency of Continuous Percolation and UAE in extracting total phenolic compounds from Krokot remain limited. Therefore, this study aims to evaluate and compare the total phenolic content obtained from Krokot, using both extraction methods. The findings are expected to provide insights into the optimal extraction technique for maximizing the recovery of phenolic compounds from this widely available medicinal plant.

2. METHODS

Plant Material and Chemicals

The plant material used in this study, *Portulaca oleracea* L. (Krokot), was collected from Cengkir Village, Kepohbaru District, Bojonegoro, East Java, Indonesia (coordinates: $-7.217539, 112.074914$). The plant specimen was taxonomically identified at the UPT Herbal Materia Medica Laboratory, Batu, and authenticated under the reference number: 074/015/102.20-A/2023. The following reagents and materials were used in this study: distilled water (aquadest), Folin–Ciocalteu reagent (Supelco, Sigma-Aldrich, Germany), gallic acid standard (Sigma-Aldrich, Germany), sodium bicarbonate (Na_2CO_3) (Sigma-Aldrich, Germany), iron (III) chloride hexahydrate ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$) (Emsure, Merck, Germany), and Whatman filter paper grade 4 (125 mm diameter).

Instruments

The main instruments used in this study included an analytical balance (Durascale DAB-E223, Indonesia), drying oven (Mettler 30-1060, Germany), UV-Vis spectrophotometer (DLab SP UV 1000), beaker glasses (Iwaki, Indonesia), condenser (Iwaki, Indonesia), ultrasonic cleaner (BK-2400, Indonesia), mesh 40 sieve (JRP,

Indonesia), volumetric flasks (Herma, Indonesia), and an aquarium pump (Kiyosaki, China) used as part of the continuous percolation setup.

Procedure

Research Design

This study employed an experimental design to quantify the total phenolic content (TPC) of Krokot (*Portulaca oleracea* L.) extracted with 70% ethanol and to evaluate the effect of two extraction techniques Continuous Percolation and Ultrasound-Assisted Extraction (UAE). The experimental workflow (UAE parameters and the Folin–Ciocalteu TPC assay) was adapted with minor modifications from a validated purslane protocol (25), in which UAE uses direct pulsed sonication followed by room-temperature maceration, and TPC is determined by the Folin–Ciocalteu method. Accordingly, the extraction method served as the independent variable and the TPC of the ethanolic extracts as the dependent outcome (25).

Extraction Procedure

The first extract was obtained using the Continuous Percolation method with a solid-to-solvent ratio of 1:10 (w/v). A total of 100 grams of dried powdered Krokot. was weighed and placed on a layer of filter paper inside the extraction chamber. A 70% ethanol solution was continuously circulated through the plant material using an aquarium pump for 1 hour (27). This system allowed for constant solvent flow, promoting efficient diffusion of phenolic compounds from the plant matrix into the solvent phase.

The second extract was obtained using the Ultrasound-Assisted Extraction (UAE) method with a solid-to-solvent ratio of 1:10 (w/v), adapted with slight modifications from a previous study. A total of 50 grams of powdered sample was dissolved in 500 mL of 70% ethanol in a closed Erlenmeyer flask sealed with aluminum foil. The solution was subjected to ultrasonic extraction in an ultrasonic bath for 60 minutes (28). Both extracts were subsequently concentrated by drying in an oven at 60°C for 48 hours or until a dry extract was obtained. Extraction yield was calculated using the following formula:

$$\text{Extract yield (\%)} = \left(\frac{\text{Weight of dry extract (gram)}}{\text{Initial weight of plant material (gram)}} \right) \times 100 \quad (1)$$

Where:

Weight of dried extract = mass of the dry extract obtained after evaporation or drying

Initial weight of plant material = mass of the plant powder used for extraction

Equation (1). Calculation of Extract Yield

Phytochemical Screening for Phenolic Compounds

A preliminary qualitative test was conducted to identify the presence of phenolic compounds. One gram of each extract was dissolved in 2 mL of 70% ethanol. One milliliter of this solution was then reacted with two drops of 1% ferric chloride (FeCl₃) solution. The appearance of a blue to bluish-black coloration was interpreted as a positive result, indicating the presence of phenolic compounds (29).

Determination of Total Phenolic Content (TPC)

The total phenolic content (TPC) was determined using the Folin–Ciocalteu method. A 0.5 mL aliquot of the extract solution was mixed with 2 mL of Folin–Ciocalteu reagent in a test tube and allowed to stand at room temperature for 4 minutes. Subsequently, 1.5 mL of 20% sodium carbonate (Na₂CO₃) solution was added, and the mixture was shaken for 1 minute. The solution was then diluted with distilled water to a final volume of 10 mL and incubated at room temperature for 120 minutes. Absorbance was measured at a wavelength of 750 nm using a UV-Vis spectrophotometer. Each sample was analyzed in triplicate (30).

The concentration of phenolic compounds in the extract, expressed as gallic acid equivalents (GAE), was calculated using the linear regression equation obtained from the standard calibration curve of gallic acid. The calibration curve was prepared using standard gallic acid solutions at concentrations of 10, 20, 30, 40, and 50 µg/mL, with the resulting regression equation:

$$y = 0.0202x - 0.0836, R^2 = 0.9987 \quad (2)$$

Where:

y = absorbance of the sample measured at 750 nm (AU)

x = concentration of gallic acid ($\mu\text{g/mL}$)

R^2 = coefficient of determination, reflecting the linearity and goodness of fit of the standard curve

Equation (2). Calculation of Total Phenolic Content (TPC)

where y represents absorbance and x represents the concentration of gallic acid ($\mu\text{g/mL}$). The TPC of each sample was then calculated and expressed as mg GAE per gram of dry extract using Equation (3).

$$\text{TPC (mg GAE/gram)} = \frac{C \times V \times Fp}{m} \quad (3)$$

Where:

C = concentration of gallic acid from calibration (mg/mL)

V = volume of extract (mL)

Fp = dilution factor

m = weight of extract (g)

Equation (3). Calculation of Total Phenolic Content (TPC)

Data Analysis

Data were analyzed in IBM SPSS Statistics 25 (IBM Corp., Armonk, NY, USA). Normality (Shapiro–Wilk) and variance homogeneity (Levene) were assessed; the Continuous Percolation group showed no within-group variance, precluding formal normality testing. Owing to non-normality and heteroscedasticity, differences in TPC between Continuous Percolation and UAE were evaluated using the Mann–Whitney U test. Statistical significance was set at $p < 0.05$. Results are reported as mean $\pm$ SEM.

3. RESULTS

Extraction Outcomes and Yield

The dried ethanolic extracts of *Portulaca oleracea* L. (Krokot) were successfully obtained using two different methods: Continuous Percolation (CP) and Ultrasound-Assisted Extraction (UAE). As shown in Table 1, CP produced 14.448 g of dried extract from 100 g of plant material, while UAE yielded 5.291 g from 50 g of material under identical drying conditions (60°C for 14 days). The corresponding extract yields were 14.45% for CP and 10.58% for UAE (Table 2).

Table 1. Extraction results of Krokot using different methods

Method	Sample Weight (gram)	Solvent Volume (mL)	Drying Temperature (°C)	Drying Time (days)	Dried Extract (gram)
Continuous Percolation	100	1000	60	14	14.448
Ultrasound-Assisted Extraction	50	500	60	14	5.291

Table 2. Extract yield of Krokot

Method	Plant Material (gram)	Dried Extract (gram)	Yield (%)
Continuous Percolation	100	14.448	14.45
Ultrasound-Assisted Extraction	50	5.291	10.58

Qualitative Screening for Phenolic Compounds

Qualitative phytochemical screening using the ferric chloride (FeCl_3) test indicated the presence of phenolic compounds in both extracts. A bluish-black color developed in each test reaction (Table 3), confirming positive results for phenolics in both CP and UAE extracts. This visual change is consistent with known reactions between phenolics and Fe^{3+} ions. The visual documentation of the reaction is presented in Figure 1.

Table 3. Qualitative test for phenolic compounds in Krokot extracts

Test Type	Sample	Reagent	Color Change	Result (+/-)	Photographic Documentation
Polyphenol	Continuous Percolation dry extract	1% FeCl_3	Bluish-black	(+)	Figure 1(c)
	Ultrasound-Assisted Extraction dry extract	1% FeCl_3	Bluish-black	(+)	Figure 1(d)

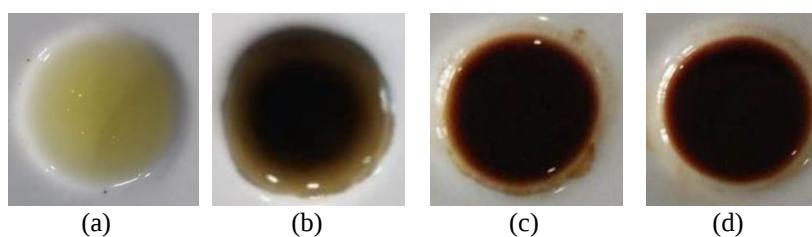


Figure 1. Ferric chloride (FeCl_3) test for the identification of phenolic compounds, (a) Negative control (70% ethanol); (b) Positive control (*Areca catechu* L. seed extract); (c) Dry extract of Krokot obtained using Continuous Percolation method; (d) Dry extract of Krokot obtained using Ultrasound-Assisted Extraction (UAE) method.

Total Phenolic Content (TPC)

The TPC of each extract was determined spectrophotometrically using the Folin–Ciocalteu method. The calibration curve generated from gallic acid standards in the range of 10–50 $\mu\text{g/mL}$ yielded a linear regression equation of $y = 0.0202x - 0.0836$ with $R^2 = 0.9987$ (Figure 2), indicating excellent linearity.

Quantitative analysis revealed that the UAE extract contained a higher mean TPC (420.040 ± 0.396 mg GAE/g) compared to the CP extract (354.297 ± 0.000 mg GAE/g) as shown in Table 4. Notably, the CP group displayed no variance across triplicates, indicating highly consistent extraction but limiting statistical modeling assumptions.

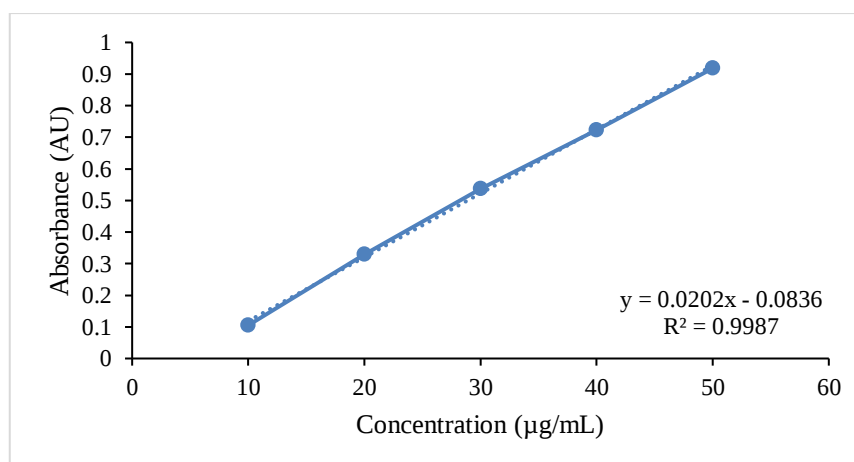


Figure 2. Linear regression calibration curve of gallic acid standard.

Table 4. Total phenolic content of Krokot extracts (mean $\pm$ SEM, n = 3)

Method	Replicate	Absorbance (AU)	Concentration		TPC (mg GAE/gram)	Mean TPC (mg GAE/g $\pm$ SEM)
			(μ g/mL)	(mg/mL)		
Continuous Percolation	1	0.811	44.287	0.044	354.297	354.297 $\pm$ 0.000
	2	0.811	44.287	0.044	354.297	
	3	0.811	44.287	0.044	354.297	
Ultrasound-Assisted Extraction	1	0.975	52.406	0.052	419.248	420.040 $\pm$ 0.396
	2	0.978	52.554	0.053	420.436	
	3	0.978	52.554	0.053	420.436	

Shapiro–Wilk analysis indicated that the UAE group was not normally distributed ($p = 0.000$), while the CP group lacked variance, rendering the normality test invalid for that group. Levene’s test further confirmed heterogeneity of variances between groups based on both mean ($p = 0.016$) and trimmed mean ($p = 0.024$), as detailed in Table 5.

Due to the violation of parametric assumptions, the Mann–Whitney U test was used to assess statistical differences. The test showed a statistically significant difference in TPC between CP and UAE groups ($U = 0.000$, $Z = -2.121$, $p = 0.034$), suggesting that the UAE method is more effective in extracting phenolic compounds from *P. oleracea* compared to Continuous Percolation.

Table 5. Summary of Statistical Analyses for Total Phenolic Content (TPC) by Extraction Method

Test	Group/Comparison	N	Test Statistic	p-value	Interpretation
Shapiro–Wilk Normality	Ultrasound-Assisted Extraction	3	W = 0.750	0.000	Not normally distributed
	Continuous Percolation	3	–	–	Not valid: no variance among triplicates
Levene’s Test (Homogeneity)	Based on Mean	–	F = 16.000	0.016	Variances not homogeneous
	Based on Trimmed Mean	–	F = 12.602	0.024	Variances not homogeneous
Mann–Whitney U Test	Continuous Percolation vs. UAE	3 vs 3	U = 0.000; Z = -2.121	0.034	Significant difference in TPC between groups

4. DISCUSSION

Compared with Continuous Percolation (CP), Ultrasound-Assisted Extraction (UAE) yielded a significantly higher TPC in purslane (420.04 $\pm$ 0.40 vs 354.30 $\pm$ 0.00 mg GAE/g; 70% ethanol). This trend is directionally consistent with prior purslane work showing that sonication can enhance extraction performance UAE combined with maceration increased extraction yield relative to maceration alone. Nonetheless, that study also reported the highest TPC under 48-h maceration rather than short-pulse UAE conditions, indicating that phenolic recovery is sensitive to sonication power/time and protocol (31). Using aqueous systems, another purslane study found decoction produced markedly greater TPC than infusion (377.60 $\pm$ 2.44 vs 121.33 $\pm$ 1.16 mg GAE/g dry extract), further underscoring the method-dependence of phenolic extraction (25). Taken together, literature suggests that more disruptive or thermally assisted techniques can outperform milder methods under appropriate conditions consistent with UAE > CP here, while absolute TPC values remain contingent on solvent composition and process parameters.

The enhanced performance of the UAE is consistent with the findings of Liu et al. (2023) who demonstrated that ultrasonic cavitation disrupts plant cell walls and improves the mass transfer of intracellular compounds, significantly increasing flavonoid yields from *P. oleracea* under optimized ultrasound conditions (32). Similar results were obtained by González-Silva et al. (2022), who reported that UAE provided 1.7-fold higher phenolic

yields from *Psidium cattleianum* compared to conventional extraction (33). The cavitation effect generated by ultrasound promotes faster cell wall rupture and solvent penetration, contributing to the observed increase in TPC (34).

Despite CP producing a larger mass of extract, the lower concentration of phenolics suggests that it may be less efficient in targeting specific phytoconstituents. This phenomenon is comparable to findings in other studies, such as those on *Myrtus communis* and *Corchorus olitorius*, where UAE yielded 20–35% higher TPC than percolation or maceration (35,36).

Statistical analysis further supported these results. Due to the lack of variance in CP replicates and the non-normal distribution in the UAE group, the Mann–Whitney U test was employed, showing a statistically significant difference between the methods ($p = 0.034$). The result highlights the reliability of the UAE as a high-efficiency method, even under conditions of variable data structure.

Moreover, the TPC value obtained from UAE (~420 mg GAE/g) is within the previously reported range for *P. oleracea* extracts (300–480 mg GAE/g), suggesting methodological consistency with existing literature (37). Various parameters such as ultrasonic intensity, solvent composition, and extraction time are known to influence phenolic yield and should be considered for further optimization (38,39).

5. CONCLUSION

This study demonstrated that both Continuous Percolation (CP) and Ultrasound-Assisted Extraction (UAE) methods successfully extracted phenolic compounds from *Portulaca oleracea* L. using 70% ethanol. However, UAE yielded a significantly higher total phenolic content (420.04 ± 0.40 mg GAE/g) compared to CP (354.30 ± 0.00 mg GAE/g), as confirmed by statistical analysis ($p = 0.034$). These findings highlight the superior efficiency of the UAE, likely attributable to enhanced mass transfer and cell disruption induced by ultrasonic cavitation. While CP produced a higher extract mass, its phenolic concentration was lower, indicating reduced selectivity toward bioactive constituents. The results support the application of UAE as a more effective method for maximizing phenolic recovery in plant-based extractions, especially for thermolabile compounds. Future research should explore optimization of ultrasound parameters to further enhance extraction yields and evaluate the bioactivity of the resulting extracts for pharmaceutical applications.

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