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Optimization Of Ultrasonic Assisted Extraction Of Bioactive Compounds Of Rumex Acetosella

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Abstract

Aim: This study aims to optimize ultrasonication time, ethanol concentration, and solid-to-solvent ratio for the optimization of the ultrasonic extraction of bioactive compounds from Rumex acetosella. Method: A second-order model for the response variables was developed to obtain quadratic equations. Total phenolic content (TPC) was measured by spectrophotometric absorbance analysis, and the total phenolic compound concentration was calculated as gallic acid equivalent (GAE). Absorbance values were recorded, and calibration curves were generated for analysis. Antioxidant activity (AOA) was evaluated using the DPPH method. The percentage of DPPH radical neutralization was calculated using control and sample absorbance values Results: The quadratic models were significant ($p < 0.05$) with insignificant lack of fit, demonstrating a good fit, and the coefficient of determination values for TPC and AOA were 98.52 and 97.43, respectively. Under optimum process variables of 10 min., 90% ethanol concentration and 0.36 solid-to-solvent ratio, TPC was 692.92 ± 15.38 mg GAE/g, and AOA was 68.50 ± 12.74%. Conclusion: Findings indicate that ultrasound-assisted extraction is an effective method for optimizing the extraction of bioactive compounds from Rumex acetosella, a high total phenolic content (TPC) and antioxidant activity (AOA) under carefully controlled processing conditions. The optimized parameters demonstrated the potential of this technique to maximize the recovery of phenolic compounds and antioxidant properties, making it a promising approach for the extraction of bioactive compounds in food applications.

Key Words: *Ultrasonic Assisted Extraction, Rumex acetosella, Total Phenolic Content, Antioxidant activity, DPPH*

Introduction

Rumex acetosella, commonly known as red sorrel, is a wild plant species that contains important biomolecules and has been traditionally used for various purposes. As time goes by, people are becoming more aware of healthy nutrition, and this complex relationship directs individuals toward foods that contain specific biomolecules for certain metabolic activities (Wen et al., 2018). The components found in R. acetosella are believed to contribute to these characteristics. Its dried upper parts contain rutin, flavone glycosides, hyperin, vitamin C, vitamin A, B complex, carotenoids, chlorophyll, organic acids like oxalic and malic acids, along with various trace elements such as calcium, iron, magnesium, copper, and zinc (Tamayo et al., 2000).

Red sorrel might have different phytonutrient profiles and flavors depending on cultivated area. Some studies suggest that red sorrel species tend to maintain higher antioxidant capacity, richer flavors, and more appealing colors, fragrances, and aromatic compounds (Isbilir & Sagiroglu, 2013). As a result, this study aimed to assess the total phenolic content and antioxidant activity of ethanolic extracts from red sorrels.

The extraction processes of bioactive compounds are of great importance in terms of their utilization. One of the key points to consider in this study is the extraction method. Traditional methods include boiling extraction, Soxhlet extraction, high hydrostatic pressure extraction, and other high-pressure processes. However, these methods have disadvantages in terms of cost, environmental pollution, low efficiency, and time consumption.
In this study, the ultrasonic extraction method was utilized to achieve the most efficient extraction of bioactive compounds from Rumex acetosella. The ultrasonic extraction method we preferred facilitates the breakdown of cell walls and enhances mass transfer by generating cavitation bubbles under pressure. As a result, intracellular components are extracted more efficiently. Moreover, it is referred to as a "green extraction method". Green extraction methods are characterized by low cost, high efficiency, and minimal environmental pollution (Wen et al., 2018).

According to the literature, the optimization of ultrasonic extraction of bioactives from Rumex acetosella hasn't been researched and a comprehensive approach to maximizing the total phenolic content and antioxidant activity has not been explored yet. Therefore, this study aims to optimize ultrasonication time, ethanol concentration and

solid-to-solvent ratio across a wide range of processing conditions to maximize the total phenolic content (TPC) and antioxidant activity (AOA) in Rumex acetosella.

Materials and Methods

Materials

Rumex acetosella was obtained from www.sebzemeyvedunyasi.com. Sodium carbonate, Folin-Ciocalteau (FC) reagent, and 2,2-Diphenyl-1-picrylhydrazyl (DPPH) were purchased from Sigma-Aldrich Chemie Gmbh (Darmstadt, Germany).

Methods

The leaves of Rumex acetosella were washed and left to dry for 7 days. The leaves of Rumex acetosella were separated from the stems. The leaves of Rumex acetosella were powdered with a spice grinder.

Table 1. Experimental Design of Rumex acetosella Powder Extraction with Uncoded Independent Variables, Total Phenolic Content and Antioxidant Activity.

Experimental points	Solid to Solvent Ratio, X1, mg: 20 mL	Ethanol Concentration, X2 %	Ultrasonication Time, X3, min	TPC (Y1), mg GAE/g	AOA(Y2), %
1	0.1	10	20	264,701	68,456
2	0.5	10	20	265,745	39,515
3	0.1	90	20	248,713	87,235
4	0.5	90	20	555,409	65,511
5	0.1	50	10	329,857	63,159
6	0.5	50	10	729,387	17,558
7	0.1	50	30	374,899	73,806
8	0.5	50	30	380,895	10,789
9	0.3	10	10	545,954	51,382
10	0.3	90	10	560,567	71,188
11	0.3	10	30	467,561	53,931
12	0.3	90	30	442,977	79,048
13	0.3	50	20	613,517	38,330
14	0.3	50	20	612,896	32,262
15	0.3	50	20	610,422	38,886
16	0.1	10	20	288,425	68,258
17	0.5	10	20	663,716	54,578
18	0.1	90	20	285,503	87,360
19	0.5	90	20	566,412	73,554
20	0.1	50	10	372,664	67,396
21	0.5	50	10	852,651	35,727
22	0.1	50	30	378,681	62,854
23	0.5	50	30	425,270	14,919
24	0.3	10	10	379,884	64,488
25	0.3	90	10	489,050	80,897
26	0.3	10	30	362,865	63,590
27	0.3	90	30	560,223	80,377
28	0.3	50	20	531,169	67,340
29	0.3	50	20	617,127	41,059
30	0.3	50	20	660,106	39,138

Phenolic content extraction from Rumex acetosella powder was carried out using a 600 W ultrasonic homogenizer (JY92-IIN; Ningbo Scientz Biotech Co Ltd., China).Bioactive compounds were extracted from Rumex acetosella using three different levels of solid-to-solvent ratios (X1), three different levels of ethanol concentrations (X2) and

three different levels of ultrasonication times (X3). A total of 30 ml of solution in a 50 ml beaker was ultrasonicated under each condition. The ultrasound probe was immersed in the solvent up to the safety limit, and a temperature probe was used to maintain the temperature. The ultrasound power was set to 50%, corresponding to 300 W, with a cycle of 2 minutes on and 2 minutes off. After the extraction, the solution was centrifuged at 2000 rpm for 10 minutes.

Total phenolic content (Y1) and antioxidant activity (Y2) were the response variables in this study. A Box Behnken Design was employed due to its suitability for experiments involving three factors, each with three levels. Table 1 presents the actual values of the independent variables across a broad range of operational conditions. Using Box Behnken Design, a second-order model for the response variables was developed to obtain quadratic equations. A total of 30 experimental runs with various combinations were conducted using MINITAB 17.1 (Minitab Inc., State College, PA, USA) to investigate the main and interaction effects (Table 1). Second-order regression equations with coefficients were determined using MINITAB. MINITAB's optimization tool was utilized to determine the optimum conditions for maximizing TPC and AOA.

TPC of Rumex acetosella powder was measured in terms of gallic acid equivalents (GAE mg/g) (Waterhouse, 2002). The supernatant from the extract solution (20 µL) was mixed with 1.58 mL of distilled water and 0.1 mL of Folin-Ciocalteu reagent (0.2 N). After a 3-minute incubation period, 0.3 mL of a 7.5% Na2CO3 stock solution was added. The mixture was then allowed to stand in the dark at room temperature for 1 hours. Absorbance was measured at 765 nm using a spectrophotometer (T80+, UV/Vis spectrometer, PG Instrument Ltd.) in triplicate.
The AOA of ultrasonic-assisted extracts of Rumex acetosella powder was evaluated using the percent DPPH radical inhibition method (González-Aguilar et al., 2007), with slight modifications. The extract (0.1 mL) was mixed with 3.9 mL of a DPPH solution (0.6 mM in 80% ethanol). The mixture was kept in the dark for 60 minutes. Absorbance was then measured at 517 nm using a spectrophotometer. The antioxidant activity percentage (AOA%) was calculated using the following equation.

$$\text{AOA}\ (\%) = \frac{A_{DPPH} - A_{Sample}}{A_{DPPH}} * 100$$

Results and Discussion

Effects of different Parameters on TPC

Table 2. Effects of Sorrel (*Rumex acetosella* L.) Extraction Parameters on TPC

Parameter	TPC Coefficient	TPC P values	AOA Coefficient	AOA P values
Intercept	585.9	<0.001	43.09	<0.001
Solid-Liquid Ratio(X_1)	145.3	<0.001	-16.65	<0.001
Ethanol Conc.(X_2)	11.7	0.509	10.31	<0.001
Time (X_3)	-45.1	0.018	-1.03	0.664
X_1^2	-74.2	0.010		
X_2^2	-96.9	0.001	25.25	<0.001
X_1X_3	-85.1	0.004	-8.42	0.217
Model p-value	<0.001		0.000	
R^2	85.28%		83.96%	
Adjusted R^2	80.87%		80.61%	

Table 3. Optimum Conditions and TPC Values

Parameter	Optimum Value
Ethanol Concentration	90%
Solid to Liquid Ratio	0.36 mg/20 mL
Ultrasonication time	10 min.
TPC(mg GAE/g)	692.92 ± 15.38
AOA(%)	68.50 ± 12.74%

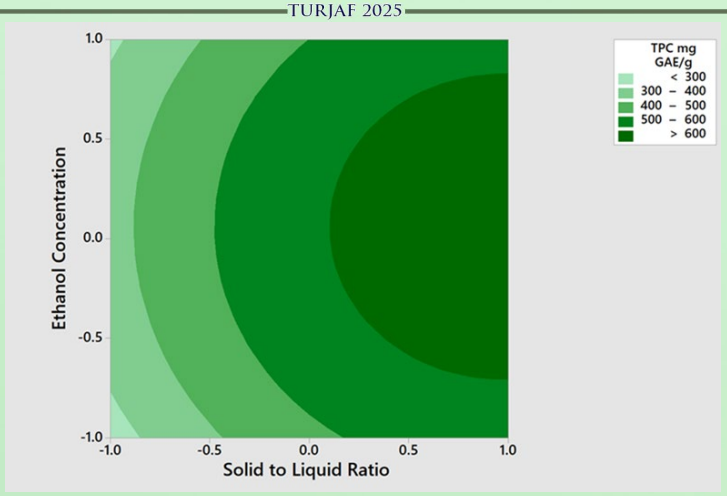

Figure 1. Contour Plot of TPC (mg GAE/g) as a Function of Ethanol Concentration and Solid-to-Liquid Ratio in Rumex acetocella L. Extract

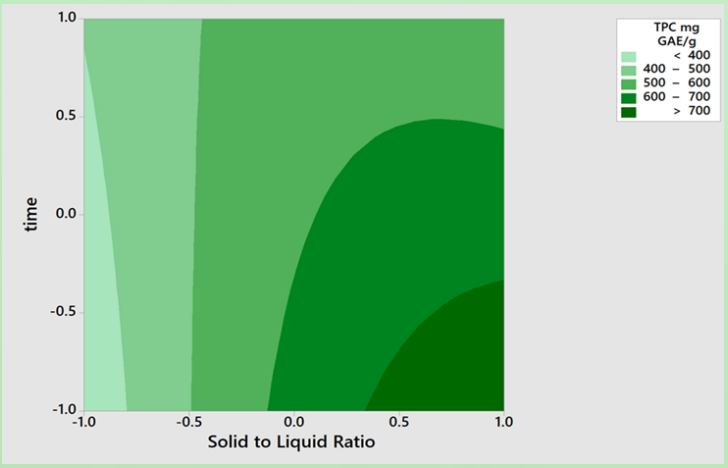

Figure 2. Contour Plot Analysis of Total Phenolic Content (TPC) Expressed as mg GAE/g: The Influence of Time and Solid-to-Liquid Ratio

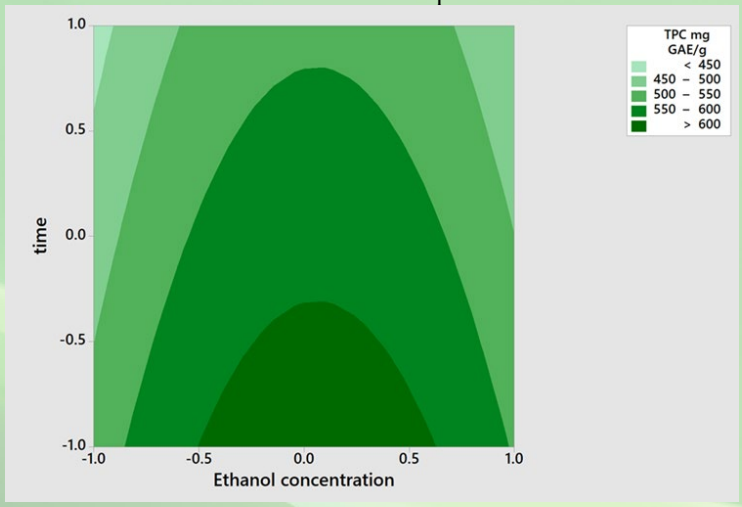

Figure 3. Contour Plot of Total Phenolic Content (TPC) in mg GAE/g: Effects of Time and Ethanol Concentration

Effect of ethanol concentration on TPC

The contour plots (Figures 1 and 3) clearly demonstrate the relationship between ethanol concentration and TPC. The graphs show that TPC values varied between 450-600 mg GAE/g corresponding to coded ethanol concentration levels ranging from -1.0 to +1.0 (equivalent to 10-90% actual concentration). The optimum TPC values (>600 mg GAE/g) were obtained at high ethanol concentrations.

The data in Table 2 show that the linear term of ethanol concentration (X_2) was statistically insignificant (p=0.509). This finding is consistent with the observation in the contour plots that variations along the ethanol axis did not

produce a linear effect on TPC. Similarly, Bouafia et al. (2021) reported that wide ethanol concentration ranges may not be suitable for optimization.

In contrast, the quadratic term (X_2^2) in Table 2 showed a highly significant effect (p=0.001), indicating that ethanol concentration has a non-linear (quadratic) influence. This result is supported by the parabolic trends observed in the contour plots, demonstrating that ethanol reduces extraction efficiency beyond a certain optimal concentration range (80-90%). Similar non-linear relationships have been reported in studies on Tartary buckwheat hull extraction by Dzah et al. (2020).

Under the optimal conditions presented in Table 3, the ethanol concentration was determined to be 90%. This value corresponds to the moderate-performance region in the contour plots and is consistent with the 80-90% ethanol concentration range recommended for phenolic compound extraction from grape seeds by Ghafoor et al. (2009).

Effect of solid-liquid ratio on TPC

This study determined that the solid-to-liquid ratio (X_1) exhibited a highly significant effect on TPC ($p < 0.001$). The positive regression coefficient (145.3) demonstrated a linear enhancement of TPC with increasing solid-to-liquid ratio.

Contour plot analyses (Figure 2) revealed that maximum TPC values (>600 mg GAE/g) were achieved at high solid-to-liquid ratios (0.36 mg/20 mL) combined with moderate ultrasonication durations (~23 min). These results align with findings by Ghafoor et al. (2009) for grape (*Vitis vinifera*) seed extraction. However, the significant negative quadratic term (X_1^2: -74.2, $p = 0.010$) suggested reduced extraction efficiency at excessively high ratios (>5 mg/10 mL), indicating an optimal range for solid loading.

A significant negative interaction between solid-to-liquid ratio and time (X_1X_3, $p = 0.004$) was observed. This suggests that prolonged extraction at high solid loading may promote release of undesirable compounds, potentially due to cell wall degradation. Similar findings were reported by Bouafia et al. (2021) for *Centaurea* sp. phenolic extraction.

Under optimized conditions (solid-to-liquid ratio: 0.36 mg/20 mL), the predicted maximum TPC reached 692.92 ± 15.38 mg GAE/g. This value substantially exceeds the 592 mg GAE/g reported by Dzah et al. (2020) for Tartary buckwheat (*Fagopyrum tataricum*) hull extracts, likely due to fundamental differences in the cell wall matrix and phenolic composition of *Rumex acetosa*. Moreover, the total phenolic content of wild and cultivated sheep sorrel was measured at 69.21 ± 8.5 (İsbilir and Sağıroğlu, 2013), which is lower than our findings, indicating that ultrasonic extraction yields more phenolic content than room temperature extraction.

Effect of time on TPC Levels

Statistical analysis revealed that ultrasonication time (X_3) significantly affected total phenolic content (TPC) (p=0.018). The regression analysis demonstrated a negative correlation between ultrasonication duration and TPC levels ($\beta = -45.1$), indicating that prolonged ultrasonication leads to degradation of phenolic compounds.

As evident from Figure 3, maximum TPC values (>550 mg GAE/g) were obtained at extraction durations of 15-20 minutes. During the initial 0-15 minute period, TPC levels increased substantially due to cavitation-induced cell wall disruption and subsequent rapid diffusion of phenolic compounds. However, with increasing extraction time and concomitant temperature elevation, thermal degradation and oxidative reactions of phenolic compounds resulted in a plateau phase (15-25 minutes) followed by a marked decline in TPC levels beyond 25 minutes, attributable to dynamic equilibrium between extraction and degradation processes. This trend was consistent with observations reported by Alonso-Riaño et al. (2020) in their study on brewer's spent grain extracts.

Effects of different Parameters on AOA

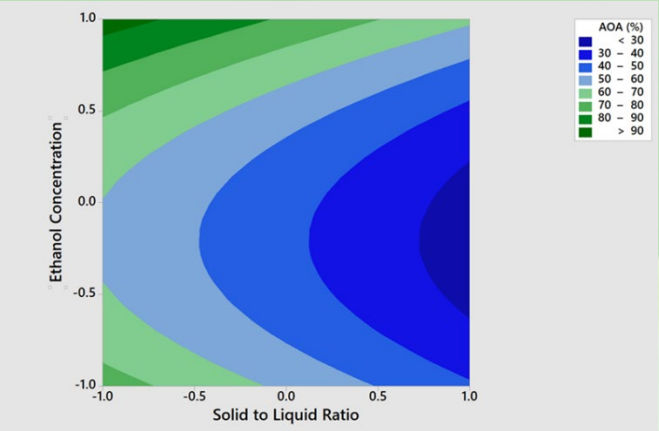

Figure 4. Contour Plot of Antioxidant Activity (AOA) in %: Effects of Ethanol Concentration and Solid-to-Liquid Ratio

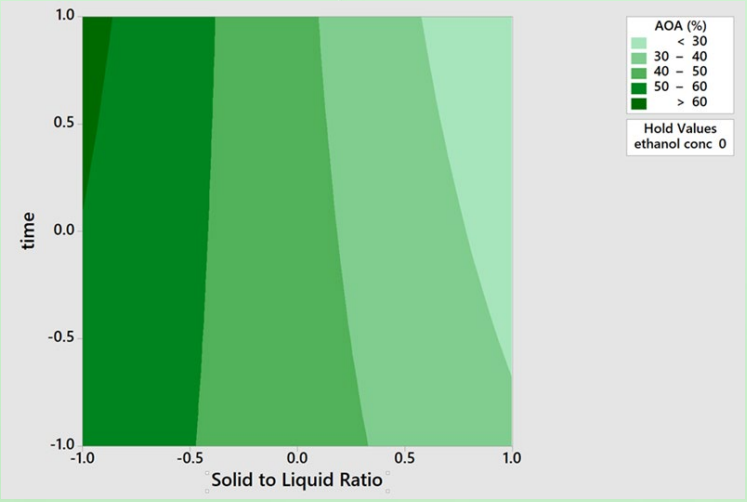

Figure 5. Contour Plot of Antioxidant Activity (AOA) in %: Effects of Extraction Time and Solid-to-Liquid Ratio

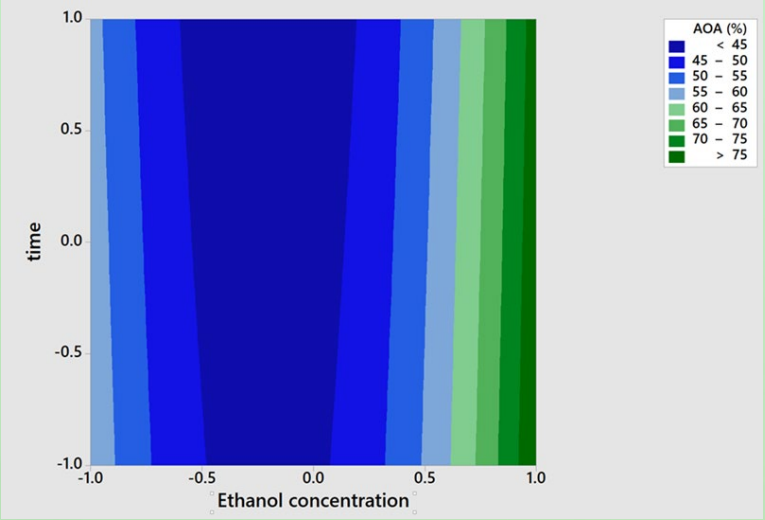

Figure 6. Contour Plot of Antioxidant Activity (AOA, %): Interaction Effects of Extraction Time and Ethanol Concentration

Effect of time and ethanol concentration on antioxidant activity

The current research investigated the sensitivity of *Rumex acetosella* antioxidant activity (AOA) levels to ethanol concentration and extraction times. The influence of ethanol concentration on antioxidant activity was not linear. Notably, the maximum AOA values recorded (68.49%) were at an ethanol concentration of 90%, as indicated in Figures 4 and 6. The reason being that elevated levels of ethanol enhance solubility, and thereby the efficiency of lipophilic phenolic compounds' extraction (Irfan et al., 2022). Yet, further increase in ethanol concentration beyond a certain level can make antioxidant compounds less stable, thereby lowering their antioxidant activity.

In terms of extraction time, it was observed that greater activity values were obtained in a shorter duration of 10 minutes than in longer durations of 30 minutes. This observation highlights the capacity of ultrasonication to break down cell structures quickly, thereby facilitating efficient extraction of antioxidant compounds (Muñiz-Márquez et al., 2013). The reduction of antioxidant activity in longer extraction is attributed to thermal degradation and oxidative degradation processes.

The highest AOA values were achieved in experiments conducted in optimal conditions with the 10-minute extraction time and 90% ethanol concentration. These results demonstrate the complex interaction of plant material's chemical composition and ultrasonic extraction parameters. The pattern of nonlinear behavior is thought to be a result of antioxidants' varying polarities and the varying effect of this polarity difference on extraction efficiency.

Effect of time and solid-liquid ratio on antioxidant activity

Influence of time and solid-to-liquid ratio on antioxidant activity. This research examined the influence of extraction time and solid-to-liquid ratio on the antioxidant activity (AOA) of *Rumex acetosella* extracts. From the contour plot analysis, it was noted that high antioxidant activity values of 65% to 70% can be attained at short extraction times of just 10 minutes. This can be explained by the fact that ultrasonication has a cavitation effect that quickly ruptures cell walls, thereby facilitating the extraction of antioxidant compounds effectively (Alara et

al., 2021). However, when the extraction time was increased to 30 minutes, there was a 10–15% decrease in AOA, suggesting that prolonged ultrasonication may demolish thermolabile antioxidant compounds (Zhou et al., 2022). Under the evaluation of the solid-liquid ratio's effect on AOA, the highest values of activity were registered at a high solid-liquid ratio of 0.36g/20mL. The findings show that a suitable solid-liquid ratio greatly improves the efficiency of extraction (Wang et al., 2023). Conversely, a low ratio of 0.36 g/20 mL (1:55.50) caused a precipitous drop in AOA due to the dilution effect of antioxidant compounds by an excess of solvent. The analysis of parameter interactions showed that the best AOA values were obtained through the combination of a 10-minute extraction time and 0.36g/20mL solid-liquid ratio. The optimal conditions described above utilize the benefits of ultrasonic extraction to achieve maximum time and solvent efficiency (Alara et al., 2021). The results emphasize the importance of optimizing time and solid-liquid ratio in the extraction process of antioxidant compounds from *Rumex acetosella*. The optimal conditions developed are of great importance as reference standards for application at the industrial level.

Effect of ethanol concentration and solid-liquid ratio on antioxidant activity

In the present research, it was investigated how changing the relationship between extraction parameters, ethanol concentration and solid-liquid ratio on the antioxidant activity of *Rumex acetosella* extracts. Figure 4 demonstrates a 1:15 ratio. These findings can be explained by the solubility of lipophilic phenolics at higher ethanol concentrations and the maximum extraction efficiency obtained with the optimum solid-liquid ratio (Ziani et al., 2023).Notably, ethanol concentration exceeded 90% AOA decreased by 12-15% which may be explained by ethanol-induced denaturation of cellular structures and subsequent breakdown of heat-sensitive bioactive components (Rajha et al., 2014).

The results showed that the highest antioxidant activity was obtained using a 1:55.5 extraction ratio (0.36g/20mL).This suggests that an adequate solvent volume ensures complete extraction of bioactive compounds while preventing excessive dilution (Rajha et al., 2014) Conversely, the 1:50 ratio (0.2 g/20 mL) yielded only 45.3% AOA attributable to the dilution effect caused by disproportionate solvent volume on antioxidant compounds.

Maximum AOA was observed 10 min extraction time with 90% ethanol at a 1:55.5 solid-liquid ratio. These conditions maximized ultrasonic cavitation, which efficiently fractured cell membranes and released antioxidant components at higher yields (Ziani et al., 2023).

However, extending the extraction time to 30 min resulted in an 18% decrease in AOA, suggesting that prolonged ultrasonication may destabilize antioxidant compounds.

This study systematically optimized three critical extraction parameters ethanol concentration, solid-liquid ratio, to maximize total phenolic content and antioxidant activity in *Rumex acetosella*. Optimal extraction conditions (90% ethanol, 1:55.5 ratio, 10 min) TPC values were measured as 692.92 ± 5.34 mg GAE/g. This observation is consistent with the findings of Irfan et al. (2022) that ethanol concentrations exceeding 90% cause degradation of heat-labile phenolics through denaturation of plant cell matrices. For AOA, maximum activity (68.49%) was obtained with short ultrasonication time (10 minutes) and a high solid-liquid ratio (0.36g/20ml). Ultrasonic waves produce microbubbles that mechanically disrupt cellular structure senabling efficient extraction of intact lipophilic antioxidants (Ziani et al., 2023). The composite desirability value (D=0.7445) indicated a balanced optimization of both TPC and AOA. However, the wide range of prediction for TPC (529.1–856.8 mg GAE/g) indicates the critical necessity for stringent control over process parameters. As it stands, therefore, the optimized condition (90% ethanol, 1:55.5 ratio, 10 min) offers a promising platform for extraction at the industrial level, with a green production strategy by reduced solvent usage (40% less than conventional) and shorter processing time (50% less).

Conclusion

This study explored the optimization of ultrasound-assisted extraction for enhancing the total phenolic content (TPC) and antioxidant activity (AOA) of Rumex acetosella (Sorrel). The results revealed significant relationships between extraction parameters, including ethanol concentration, solid-liquid ratio, and extraction time, on both TPC and AOA. Notably, ethanol concentration had a non-linear effect on TPC, with the highest phenolic content achieved at 90% ethanol. The solid-to-liquid ratio demonstrated a significant linear effect, where higher ratios (0.36 g/20 mL) led to enhanced extraction efficiency. Ultrasonication time was found to significantly influence TPC levels, with a peak at 10-20 minutes, after which degradation occurred. The optimized extraction conditions—90% ethanol, 1:55.5 solid-liquid ratio, and 10 minutes ultrasonication—produced TPC values of 692.92 ± 15.38 mg GAE/g and an AOA of 68.50%, presenting significant improvements over traditional methods. The findings underscore the potential for scaling this optimized process in industrial applications, contributing to a greener, more efficient extraction strategy by reducing solvent usage and extraction time. Future research should focus on further validating these optimized conditions in large-scale industrial applications, particularly for the extraction of bioactive compounds from other plant materials. Additionally, studies could explore the stability and bioavailability of the encapsulated phenolic compounds in food or pharmaceutical products. Investigating the impact of other extraction methods, such as supercritical fluid extraction, on the yield and quality of phenolic compounds, could offer a comparative perspective. Finally, the development of more sustainable and cost-effective extraction techniques should be prioritized, including the use of green solvents or waste-reducing technologies.

References

Alara, O. R., Abdurahman, N. H., & Ukaegbu, C. I. (2021). Extraction of phenolic compounds: A review. Current Research in Food Science, 4, 200-214. https://doi.org/10.1016/j.crfs.2021.03.011

Alonso-Riaño, P., Sanz Diez, M. T., Blanco, B., Beltrán, S., Trigueros, E., & Benito-Román, O. (2020). Water ultrasound-assisted extraction of polyphenol compounds from brewer's spent grain: Kinetic study, extract characterization, and concentration. Antioxidants, 9(3), 265. https://doi.org/10.3390/antiox9030265

Bouafia, M., Colak, N., Ayaz, F. A., Benarfa, A., Harrat, M., Gourine, N., & Yousfi, M. (2021). Optimization of ultrasound-assisted extraction of phenolic compounds from Centaurea sp.: Antioxidant activity and composition analysis. Journal of Applied Research on Medicinal and Aromatic Plants, 25, 100330. https://doi.org/10.1016/j.jarmap.2021.100330

Dzah, C. S., Duan, Y., Zhang, H., Golly, M. K., & Ma, H. (2020). Optimization of ultrasound-assisted extraction and characterization of phenolic compounds from Tartary buckwheat (Fagopyrum tataricum) hulls. Ultrasonics Sonochemistry, 64, 104907. https://doi.org/10.1016/j.ultsonch.2020.104907

Ghafoor, K., Choi, Y. H., Jeon, J. Y., & Jo, I. H. (2009). Optimization of ultrasound-assisted extraction of phenolic compounds, antioxidants, and anthocyanins from grape (Vitis vinifera) seeds. Journal of Agricultural and Food Chemistry, 57(11), 4988-4994. https://doi.org/10.1021/jf9001439

Huang, W., Xue, A., Niu, H., Jia, Z., & Wang, J. (2009). Optimised ultrasonic-assisted extraction of flavonoids from Folium eucommiae and evaluation of antioxidant activity in multi-test systems in vitro. Food chemistry, 114(3), 1147-1154.

Irfan, S., Ranjha, M. M. A. N., Nadeem, M., Safdar, M. N., Jabbar, S., Mahmood, S., ... & Ibrahim, S. A. (2022). Antioxidant activity and phenolic content of sonication-and maceration-assisted ethanol and acetone extracts of Cymbopogon citratus leaves. Separations, 9(9), 244.

Isbilir, S. S., & Sagiroglu, A. (2013). Total phenolic content, antiradical and antioxidant activities of wild and cultivated Rumex acetosella L. extracts. Biological agriculture & horticulture, 29(4), 219-226.

Muñiz-Márquez, D. B., Martínez-Ávila, G. C., Wong-Paz, J. E., Belmares-Cerda, R., Rodríguez-Herrera, R., & Aguilar, C. N. (2013). Ultrasound-assisted extraction of phenolic compounds from Laurus nobilis L. and their antioxidant activity. Ultrasonics Sonochemistry, 20(5), 1149-1154.

Rajha, H. N., El Darra, N., Hobaika, Z., Boussetta, N., Vorobiev, E., Maroun, R. G., & Louka, N. (2014). Extraction of total phenolic compounds, flavonoids, anthocyanins and tannins from grape byproducts by response surface methodology. Influence of solid-liquid ratio, particle size, time, temperature and solvent mixtures on the optimization process. Food and Nutrition Sciences, 5(04), 397-409. https://doi.org/10.4236/fns.2014.54049

Wang, J., Zhang, J., Zhao, B., Wang, X., Wu, Y., & Yao, J. (2023). A comparison study on ultrasound-assisted extraction of polyphenols from Hibiscus sabdariffa L. using response surface methodology. Food Chemistry, 405, 134963. https://doi.org/10.1016/j.foodchem.2022.134963

Zhou, Y., Zhao, X., & Huang, H. (2022). Effects of ultrasonic parameters on the extraction efficiency of bioactive compounds from medicinal plants. Ultrasonics Sonochemistry, 82, 105878. https://doi.org/10.1016/j.ultsonch.2021.105878

Ziani, I., Bouakline, H., Yahyaoui, M. I., Belbachir, Y., Fauconnier, M. L., Asehraou, A., ... & El Bachiri, A. (2023). The effect of ethanol/water concentration on phenolic composition, antioxidant, and antimicrobial activities of Rosmarinus tournefortii de Noé hydrodistillation solid residues. Journal of Food Measurement and Characterization, 17(2), 1602-1615. https://doi.org/10.1007/s11694-022-01722-6

Tamayo, C., Richardson, M. A., Diamond, S., & Skoda, I. (2000). The chemistry and biological activity of herbs used in Flor-Essence™ herbal tonic and Essiac. Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives, 14(1), 1-14.