Chemical and Process Engineering: New Frontiers, 2025, 46(3), e98
DOI: 10.24425/cpe.2025.153683

Micellar-mediated extraction of Paeonia lactiflora – design of experiment

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Article info:
Received: 18 April 2025
Revised: 03 July 2025
Accepted: 10 July 2025

Abstract
This study aimed to develop a micellar-mediated extraction (MME) method with elements of experimental planning. The scope of work included preparing a series of micellar extracts from the root of *Peoniae lactiflora*. Extractions were performed under ultrasonic conditions in a solution with ethoxylated fatty alcohol. The variable parameters were time, concentration of plant material, and citric acid. The properties of extracts, such as antioxidant activity, total phenolic and flavonoid content, were measured using a spectrophotometric method. The findings demonstrated clear relationships between extraction time and raw material amount with the measured outputs. Longer extraction times led to higher values across all output variables. Similarly, increasing the amount of raw material resulted in elevated concentrations of polyphenols and flavonoids. Interestingly, higher citric acid content enhanced the concentration of flavonoids. This study successfully demonstrated that MME, when combined with a well-designed experimental plan, offers a powerful and adaptable strategy for obtaining antioxidant-rich plant extracts with optimized bioactive content. The results provided an indication enabling to obtain extracts with high concentrations of antioxidants.

Keywords
micelle-mediated extraction (MME), ethoxylated fatty alcohol, flavonoids, polyphenols, antioxidant activity

1. INTRODUCTION

Scientific studies show that the root, seeds, and petals of *Peonia lactiflora* are rich in various nutrients such as vitamins, proteins, sugars, and mineral components. It is worth mentioning that the bioactive compounds contained in these raw materials have potent antioxidant activity. The root and bark of the Chinese peony are the most commonly used for medicinal purposes. Phytochemical studies conducted to date have isolated 451 compounds from *Peoniae lactiflora* P. The plant contains monoterpenoid glycosides, flavonoids, tannins, triterpenoids, stilbenes, steroids, and phenols. Studies of the pharmacological activity of isolated compounds from Chinese peony have shownlabel that they have antioxidant, anti-inflammatory, antimicrobial, antiviral, anticancer, as well as cardiovascular and neuroprotective activities (Kumar et al., 2023). The characteristic monoterpenoid glycosides of plants of the genus *Peoniae* are albiflorin, benzoylalbiflorin, 4-O-galloylalbiflorin, 6-O-galloylalbiflorin, galloylpeoniflorin, and oxypeoniflorin (Feng et al., 2022). The main compound found in Chinese peony root extract is peoniflorin, which has 15 times higher concentration than other compounds in this raw material. It is worth noting that peoniflorin is found in all plants of the peony family, but its highest concentration is found in *Peoniae lactiflora* P. Differences in the concentrations of phytochemical compounds of plants are due to the variability of parameters such as temperature, soil type, and rainfall intensity, as well as the time of harvesting (Kim et al., 2021). Peonol (2'-hydroxy-4'-methoxyacetophenone) is a characteristic phenolic component in Chinese peony root extract. There are many scientific studies describing the analgesic and anti-inflammatory effects of peonol and the previously mentioned peoniflorin (Zhang and Dai, 2012). The significant pharmacological potential of peonol remains untapped due to its poor solubility, bioavailability, low stability, and high volatility at room temperature. Research is currently underway to increase the stability of this compound and use it in pharmaceutical products (Li et al., 2021). Quercetin, epicatechin, kemferol, and their glycosides are the predominant flavonoids found in white peony root extract (Chen et al., 2015; Wang et al., 2022). Quercetin, like kemferol, is a natural flavonol with antioxidant, anti-inflammatory, anti-fibrotic, immunomodulatory, anticancer, neuroprotective, and vascular-protective activities. The wide range of activities, availability, and bioactivity of this compound have led to increased interest in quercetin among researchers. Studies have shown that the compound has applications in the treatment of diabetes, immune system diseases, bacterial and viral infections, and supports cancer therapies (Zhao et al., 2021). Epicatechin is a flavonoid compound from the catechin group. Its main properties are antioxidant, anti-inflammatory, neuroprotective, and supportive of the circulatory system. In addition, epicatechin exhibits antimicrobial

activity against drug-resistant pathogens. Preclinical studies have proven that epicatechin also has chemopreventive and anticancer activities (Prakash et al., 2019).

The present study used MME to prepare peony extracts, an attractive alternative to classical extraction methods. The process itself is relatively simple, inexpensive, and environmentally friendly. Unlike traditional methods, it does not require toxic organic solvents. In this method, the extraction solvent is an aqueous surfactant solution, which allows the desired components to be dissolved in hydrophobic micelles. The method allows to increase bioavailability and reduce the side effects of extracted active substances, protecting them from degradation. The MME method takes advantage of the solubilizing properties of micelles, thanks to which it is possible to extract a variety of substances, including insoluble or those with limited solubility. Micelles are ordered aggregates formed from surfactant molecules after reaching a critical micellar concentration (CMC) (Śliwa et al., 2013; Śliwa et al., 2016b; Śliwa and Śliwa, 2020; Śliwa et al., 2019b; Śliwa and Śliwa, 2021). An essential issue in MME is the selection of an appropriate surfactant, whose structure depends primarily on the type of substance we are studying. Non-ionic, anionic, cationic, and amphoteric surfactants or their mixtures are used in this method. The degree of binding of active substances into micelles is also influenced by the concentration of the surfactant used, which should not take lower values than its critical micellization concentration (CMC). Turbidity point and CMC depend on the surfactant's structure. As the number of non-polar groups in the hydrophilic part and carbon atoms in the hydrophobic part of the surfactant increases, CMC and turbidity temperature values decrease. Studies have shown that non-ionic surfactants are better solubilizers than other types of surfactants. They generally have lower critical micellar concentrations, lower toxicity, and higher efficiency even at low concentrations. Studies also show that the extraction efficiency of polyphenolic compounds depends on the number of ethoxyl groups and the hydrophobic chain length of surfactant molecules. The most promising non-ionic surfactants for micellar extraction of polyphenols from plant raw materials are C9-11 Pareth-5 (Rokanol NL5), C9-11 Pareth-5 (C9/11E5), PPG-6 Steareth-7 (C18E7P6), Oleth-10, Oleth-5, Poloxamer 407 NF (302637), as well as Steareth-20 (Brij S20) (Sazdanić et al., 2023; Śliwa et al., 2019a; Śliwa and Śliwa, 2020). The time and temperature of conducting MME are selected individually for the raw material being extracted. The test substance's physicochemical form, mass, and fineness affect the extraction efficiency. The finer the raw material, the better the active substances are extracted (Śliwa et al., 2016b; Śliwa and Śliwa, 2021). An undeniable advantage of MME is using a surfactant solution as a solvent, which fits the principle of "green chemistry." Extracts prepared with this method can potentially be used in the cosmetic, food or pharmaceutical industries. The technique was initially used for the extraction and pre-concentration step of inorganic analytes (Pocurull et al., 2020). Studies conducted by Zarei et al. (2018) showed that the MME method can be successfully applied in the pharmaceutical industry to monitor paminophenol contamination in pharmaceutical preparations. This method was also used to prepare organic extracts of calendula flowers, elderberry flowers, tri-colored violet, or tri-colored marsh marigold (Śliwa et al., 2016a; Śliwa et al., 2019a; Śliwa and Śliwa, 2020). The obtained extracts were components of cosmetic emulsions (Śliwa et al., 2018c, 2018b, 2018a, 2018d). Micellar-mediated extracts with alkylpolyglucosides from basil were also obtained, with potential use in mild cleansing cosmetics (Śliwa et al., 2025).

Design of experiments (DoE) refers to the process of planning, designing, and analyzing a given experiment to obtain valid and objective conclusions (Jiju, 2014; Konkol, 2008). Typical applications of DoE can include various types of chemical synthesis, crystallization processes, polymerization and hydrogenation reactions, biocatalysis and enzymatic catalysis, or monitoring of processes (Bochentyn and Kusz, 2015; Jiju, 2014). In the MME method, many process parameters can be changed, such as the concentration of surfactant, plant raw material, preservative, pH adjuster, process temperature or ultrasonic power. Therefore, it is worthwhile to perform experimental planning to reduce the number of unnecessarily tested samples and plan the process properly.

The aim of our study was to develop an MME method with elements of experimental planning to prepare antioxidant-rich plant extracts from the root of *Peonia lactiflora*. The study investigates the effects of extraction time, concentration of plant material, and citric acid on the properties of the extracts, such as antioxidant activity, total phenolic, and flavonoid contents. The novelty of this study lies in the combination of MME with a well-designed experimental plan to optimize the bioactive content of plant extracts. This approach offers a robust and adaptable strategy for obtaining high-quality antioxidant-rich extracts, which can be used in various industries such as food, cosmetics, and pharmaceuticals. The study also highlights the importance of using a surfactant solution as a solvent, which aligns with the principles of "green chemistry" and reduces the need for toxic organic solvents.

2. EXPERIMENTAL DETAILS AND RESULTS

2.1. Materials and chemicals

Solvents and chemical reagents of analytical purity, i.e., aluminum chloride, methanol, sodium nitrite, sodium carbonate, and citric acid, were purchased from POCh (Gliwice, Poland). Sodium benzoate and sodium hydroxide were purchased from Chempur (Piekary Śląskie, Poland). Folin–Ciocalteau solution, 2,2-diphenyl-1-picrylhydrazyl (DPPH) and quercetin ($\geq$ 98% HPLC) were purchased from Sigma Aldrich (Poznań, Poland). Rokanol NL5 (C9-11 Pareth-5, Table 1) was purchased from PCC Exol SA (Brzeg Dolny, Poland). *Peonia lactiflora* root by Nanga (Błękwit, Poland) was used for the extraction.

Table 1. Properties of the surfactant used in the extraction process (Śliwa et al., 2019b).

Rokanol NL5	
Producer	PCC Exol SA
INCI	C9-11 Pareth-5
Carbon chain	$C_9 - C_{11}$
OE[a]	5
Viscosity [mPa·s]	6880 (@25°C)
Surface tension [mN/m]	27
HLB	8.6
CMC [mg/dm^3]/[mmol/dm^3]	159.8/0.420
N^b_{agg}	76

[a]Oxyethylene units, [b]Aggregation number

2.2. Methods

2.2.1. The extraction procedure

The ultrasonic assistance micelle-mediated extraction (UAMME) was used to isolate the flavonoids from the herb. A 1% aqueous solution of C9-11 Pareth-5 was used as the extraction medium. This surfactant is effective in micellar extraction from plant material, based on our previous work (Śliwa et al., 2019a; Śliwa and Śliwa, 2020). Extraction was performed according to the following scheme: 0.5, 1.5, 2.5 g of dry herb and 0.25, 0.75, 1.25 g of citric acid were poured into 100 cm^3 of surfactant solution and placed for 20, 30, 40 minutes in a thermostatic ultrasonic bath (50 Hz, 300 W, 25 °C). After the allotted time, the mixtures were filtered (Büchner funnel with a medium-grade qualitative filter paper of 80 g/m^2), and the filtrates were analyzed. Each experiment was repeated three times, and the results are the mean values.

2.2.2. Total flavonoids, polyphenol content, and antioxidant activity measurements

The total flavonoid content in the extracts and concentrates was determined colorimetrically, as in (Shraim et al., 2021). First, 1 cm^3 of the sample was diluted in 5 cm^3 of distilled water and 0.3 cm^3 of 5% $NaNO_2$ solution and left for 5 min. Next, 0.6 cm^3 of 10% $AlCl_3$ solution was added, and after 6 min, 2 cm^3 of 1 M NaOH was admixed. The obtained mixture was made up to 10 cm^3 with distilled water, and the absorbance of the sample was measured directly at $\lambda = 510$ nm using a Macherey–Nagel Nanocolor spectrophotometer. Based on a standard curve (Fig. 1), the results were expressed as quercetin content (mg/cm^3). The procedure for standards was analogous, but instead of 1 cm^3 of sample, 1 cm^3 of quercetin standard solution with appropriate concentrations was added.

The total polyphenol concentration in the extracts and concentrates was determined using the Folin–Ciocalteau (FC) methodology, as in Cybul and Nowak (2008). First, 1 cm^3 of the sample was mixed with 5 cm^3 of the FC reagent for 4 min. Next, 4 cm^3 of 7.5% sodium carbonate was added,

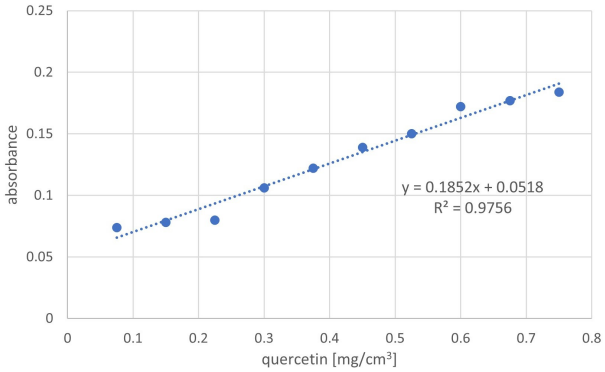

Figure 1. The calibration curve showing the dependence of absorbance at a wavelength of $\lambda = 510$ nm on the concentration of the quercetin standard. The trend line equation was used to determine the total flavonoid content in the extracts. The correlation coefficient R^2 is included below the equation.

and the solutions prepared in this way were kept in the dark for two hours. After that, the absorbance of the samples was recorded at a wavelength $\lambda = 765$ nm using a Macherey–Nagel Nanocolor spectrophotometer. The results, expressed as quercetin content (mg/cm^3), were calculated based on a standard curve (Fig. 2). The procedure for standards was analogous, but instead of 1 cm^3 of sample, 1 cm^3 of quercetin standard solution with appropriate concentrations was added.

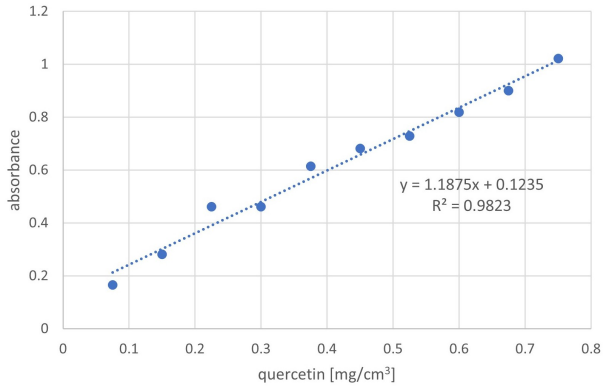

Figure 2. The calibration curve showing the dependence of absorbance at a wavelength of $\lambda = 765$ nm on the concentration of the quercetin standard. The trend line equation was used to determine the total polyphenol content in the extracts. The correlation coefficient R^2 is included below the equation.

The antioxidant activity was studied as DPPH (1,1-diphenyl-2-picrylhydrazyl) scavenging potential, according to the procedure described by Shabir et al. (2011): 2 cm^3 of DPPH solution (0.05% w/w) in methanol was added to 4 cm^3 of each analysed sample and 4 cm^3 of distilled water and left to react at room temperature for 30 min without light; next, the absorbance of the obtained mixtures was measured at $\lambda = 517$ nm using Macherey–Nagel Nanocolor spectrophotometer. Each measurement was taken three times.

Statistical and multivariate regression analyses were performed using GraphPad Prism 10.

2.3. Results and discussion

The raw material used in the study contains monoterpenoid glycosides, flavonoids, tannins, triterpenoids, stilbenes, steroids, and phenolics and exhibits a range of pharmacological activities. The MME method under ultrasonic conditions was used to make the extracts. The nonionic surfactant C9-11 Pareth-5, which belongs to the group of ethoxylated fatty alcohols, was used as the solvent. Elements of the experimental planning method were used to develop and analyze the obtained results.

2.3.1. Antioxidant properties of the micellar-mediated extracts.

Figure 3 illustrates the relationship between extraction time and the concentrations of total polyphenols, flavonoids, and antioxidant activity. All measured parameters show a steady increase with prolonged extraction time. Notably, the free radical inhibition percentages remain relatively consistent across the extracts. Interestingly, the curves for polyphenol and flavonoid concentrations rise linearly and intersect almost perpendicularly. Generally, the highest antioxidant activity (69.5%), along with peak concentrations of polyphenols (1.864 mg/cm^3) and flavonoids (0.8074 mg/cm^3), was observed in samples extracted for 45 minutes.

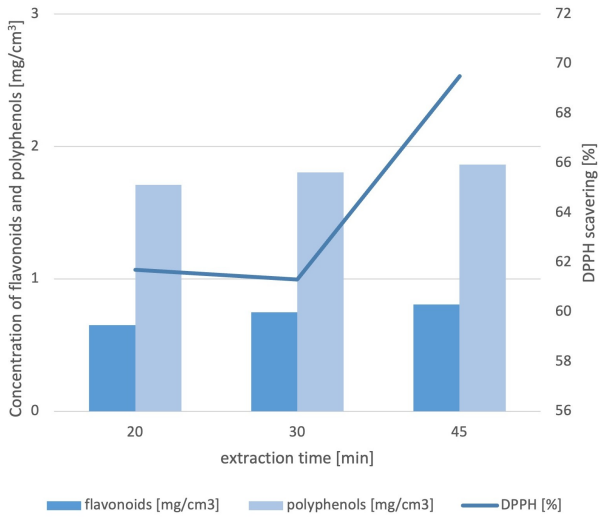

Figure 3. Relationship between polyphenol, flavonoid concentration, antioxidant activity, and the extraction time.

Figure 4 illustrates the relationship between the tested output variables and the amount of raw material used. The total concentrations of polyphenols and flavonoids increase linearly with the weight of dried peony root added to the samples. The highest levels were observed in samples containing 2.5 g of the root, reaching 2.362 mg/cm^3 for polyphenols and 1.445 mg/cm^3 for flavonoids. In contrast, the DPPH free radical inhibition initially rises but then declines. Typically, antioxidant activity is expected to increase or stabilize alongside rising levels of polyphenolic and flavonoid compounds, as these molecules exhibit antioxidant properties. The observed decrease in % inhibition at 2.5 g is likely due to increased sample turbidity caused by the high concentration of plant material.

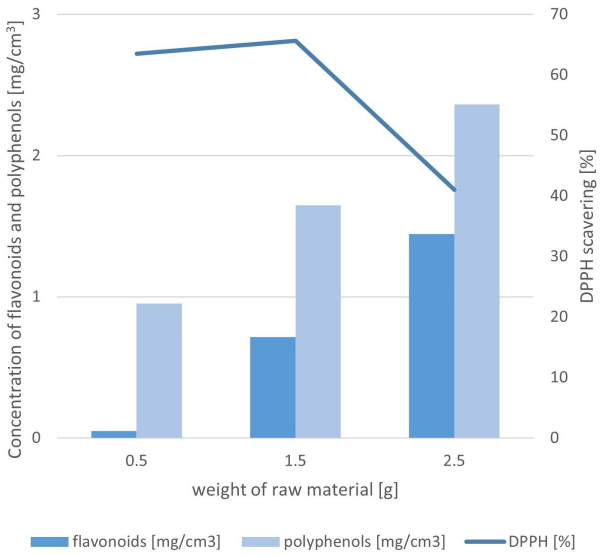

Figure 4. Relationship between polyphenol, flavonoid concentration, antioxidant activity, and raw material weight.

Figure 5 shows how the quality of extracts responds to increasing amounts of citric acid. The concentration of flavonoids exhibits a slight decrease, remaining relatively stable around 1.0 mg/cm^3. In contrast, a clear decline in antioxidant activity is observed, dropping from 62.3% to 36.7% as the citric acid content increases. Interestingly, only the total polyphynol concentration increases with decreasing pH, reaching a maximum of 4.088 mg/cm^3 in samples containing 1.25 g of citric acid.

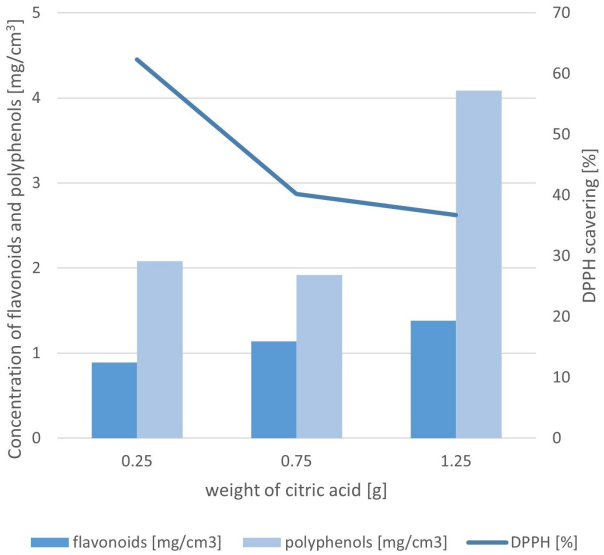

Figure 5. Relationship between polyphenol, flavonoid concentration, antioxidant activity, and the amount of citric acid.

2.3.2. Multivariate regression analysis

The study used the simplest experiment planning approach, involving three independent variables. Two response models were applied: linear $y = ax_1 + bx_2 + cx_3$ and quadratic $y = ax_1 + bx_2 + cx_3 + dx_1^2 + ex_2^2 + fx_3^2$. The multiple regression model is used to quantify the relationship between multiple independent variables and the dependent variable. Pearson's correlation coefficient is used to calculate the relationship between quantitative variables. The sign of the correlation coefficient indicates the direction of the correlation, while the absolute value of this coefficient indicates the strength of the correlation between the variables. When the absolute value of Pearson's coefficient is close to 0, it means that the variables are not correlated, while its value is close to 1 then the correlation between the variables is strong (Uy and Telford, 2009). Models are evaluated by their correlation coefficient R^2.

The results of the multiple regression analysis of the linear and the quadratic models for the total concentration of polyphenols in peony micellar extracts are summarized in Table 2 and Table 3, respectively. Below are equations describing the models:

$$y = 0.016x_1 + 0.707x_2 - 0.3911x_3 \quad (1)$$

$$y = 0.0461x_1 + 1.159x_2 - 1.435x_3 - 0.0007x_1^2 - 0.1742x_2^2 + 0.8392x_3^2 \quad (2)$$

The correlation coefficient describes a measure of the model's usefulness. In the case of the linear model used to analyze the relationship of variables for the total concentration of polyphenols, it appears that it describes very well the correlation of extraction time and the amount of added raw material. The relationship between the amount of citric acid and the dependent variable shows an average correlation ($R^2 = 0.7073$). As for the quadratic model, its correlation coefficient is 0.998, which means that it is highly useful for predicting the value of the independent variable.

Multivariate linear and quadratic regression analysis for the total concentration of flavonoids in the extract is shown in Tables 4 and 5. Below the linear and quadratic model

Table 2. Summary results of multivariate regression analysis of the linear model for the total concentration of polyphenols in peony micellar extracts, where x_1: extraction time, x_2: weight of peony root and x_3: citric acid weight.

	χ_1	χ_2	χ_3
Regression coefficient	0.016	0.707	–0.3911
Standard error	0.0068	0.1615	0.3404
Confidence level –95%	–0.0007	0.3119	–1.224
Confidence level 95%	0.0326	1.102	0.4417
T-statistic of the correlation coefficient significance	1.657	7.9	2.187
p-value test	0.1487	0.0002	0.0714
VIF collinearity test	8.274	10.28	3.416
Correlation coefficient R^2	0.8791	0.9027	0.7073

Table 3. Summary of the results of multivariate regression analysis of the quadratic model for the total concentration of polyphenols in peony micellar extracts, where x_1: extraction time, x_2: weight of peony root and x_3: citric acid weight.

	χ_1	χ_2	χ_3	χ_1^2	χ_2^2	χ_3^2
Regression coefficient	0.0461	1.159	–1.435	–0.0007	–0.1742	0.8392
Standard error	0.0298	0.4251	0.9872	0.0005	0.1329	0.6692
Confidence level –95%	–0.0487	–0.1935	–4.577	–0.0023	–0.5971	–1.291
Confidence level 95%	0.1409	2.512	1.707	0.0009	0.2487	2.969
T-statistic of the correlation coefficient significance	1.548	2.727	1.454	1.319	1.311	1.254
p-value test	0.2195	0.0721	0.242	0.2789	0.2811	0.2987
VIF collinearity test	3					
Total R^2	0.998					

Table 4. Summary of the multiple linear model regression analysis for the total concentration of flavonoids in peony micellar extracts, where x_1: extraction time, x_2: weight of peony root and x_3: citric acid weight.

	χ_1	χ_2	χ_3
Regression coefficient	–0.005	0.5626	0.3283
Standard error	0.003	0.0712	0.1501
Confidence level –95%	–0.0123	0.3884	–0.039
Confidence level 95%	0.0024	0.7369	0.6956
T-statistic of the correlation coefficient significance	1.657	7.9	2.187
p-value test	0.1487	0.0002	0.0714
VIF collinearity test	8.274	10.28	3.416
Correlation coefficient R^2	0.8791	0.9027	0.7073

Table 5. Summary of the results of the multiple quadratic model regression analysis for the total concentration of flavonoids in peony micellar extracts, where x_1: extraction time, x_2: weight of peony root and x_3: citric acid weight.

	χ_1	χ_2	χ_3	χ_1^2	χ_2^2	χ_3^2
Regression coefficient	–0.0247	0.8274	-0.2218	0.0004	–0.0707	0.3741
Standard error	0.0292	0.4164	0.9671	0.0005	0.1302	0.6556
Confidence level –95%	–0.1176	–0.4977	–3.299	–0.0012	–0.4849	–1.712
Confidence level 95%	0.0682	2.153	2.856	0.002	0.3436	2.46
T-statistic of the correlation coefficient significance	0.8453	1.987	0.2293	0.81	0.5428	0.5707
p-value test	0.46	0.1411	0.8334	0.4772	0.625	0.6082
VIF collinearity test	3					
Total R^2	0.994					

equations for the total concentration of flavonoids in peony micellar extracts:

$$y = -0.005x_1 + 0.5626x_2 + 0.3283x_3 \quad (3)$$

$$y = -0.0247x_1 + 0.8274x_2 - 0.2218x_3 + 0.0004x_1^2 - 0.0707x_2^2 + 0.3741x_3^2 \quad (4)$$

The multiple linear regression analysis revealed that the concentration of flavonoids in the extract is most strongly influenced by the amount of raw material, indicating a high degree of correlation. Extraction time also demonstrated a strong predictive relationship, reinforcing its importance as a key process parameter. In contrast, the amount of citric acid showed the weakest correlation, as reflected by the lowest R^2 value among the evaluated variables. When a quadratic regression model was applied, the coefficient of determination (R^2) significantly improved, reaching a value of 0.9941. This result suggests that the quadratic model offers an excellent fit and accurately captures the nonlinear relationships between the independent variables and the response variable.

Finally, the results of the multiple linear and quadratic regression analyses for the prediction of total antioxidant activity of the obtained extracts are summarized in Tables 6 and 7.

Below are equations describing the models:

$$y = 1.517x_1 + 1.4x_2 + -27.16x_3 \quad (5)$$

$$y = 3.062x_1 + 29.14x_2 - 28.47x_3 - 0.04012x_1^2 - 11.49x_2^2 + 9.171x_3^2 \quad (6)$$

Similar to the trends observed for polyphenol and flavonoid concentrations, the total antioxidant activity of the extract shows a strong correlation with both the amount of raw material used and the duration of the extraction process. This is supported by the high coefficients of determination (R^2), which are 0.8791 and 0.9027, respectively. In contrast, the amount of citric acid added exhibits the weakest correlation with antioxidant activity. The results of the multivariate regression analysis using the quadratic model confirm the appropriateness of this model for describing antioxidant potential. The model yielded an R^2 value of 0.9897, indicating that it accounts for 98.97% of the variability in the observed data.

The Figure 6 presents the results of Pearson's correlation analysis, highlighting the relationships between the studied variables and the concentrations of polyphenols, flavonoids, as well as the total antioxidant potential. The analysis reveals

Table 6. Summary of the results of the multiple linear model regression analysis for the total antioxidant activity of peony micellar extracts, where x_1: extraction time, x_2: weight of peony root and x_3: citric acid weight.

	x_1	x_2	x_3
Regression coefficient	1.517	1.4	–27.16
Standard error	0.469	11.11	23.42
Confidence level –95%	0.3693	–25.79	–84.46
Confidence level 95%	2.665	28.59	30.15
T-statistic of the correlation coefficient significance	3.234	0.126	1.16
p-value test	0.0178	0.9039	0.2903
VIF collinearity test	8.274	10.28	3.416
Correlation coefficient R^2	0.8791	0.9027	0.7073

Table 7. Summary of results of multiple quadratic model regression analysis for total antioxidant activity of Chinese peony extracts, where x_1: extraction time, x_2: weight of peony root and x_3: citric acid weight.

	x_1	x_2	x_3	x_1^2	x_2^2	x_3^2
Regression coefficient	3.062	29.14	–28.47	–0.04012	–11.49	9.171
Standard error	2.057	29.34	68.15	0.03512	9.173	46.2
Confidence level –95%	–3.484	–64.24	–245.3	–0.1519	–40.68	–137.9
Confidence level 95%	9.607	122.5	188.4	0.07166	17.70	156.2
T-statistic of the correlation coefficient significance	1.489	0.9932	0.4177	1.142	1.253	0.1985
p-value test	0.2333	0.3938	0.7043	0.3363	0.2991	0.8553
VIF collinearity test	3					
Total R^2	0.9897					

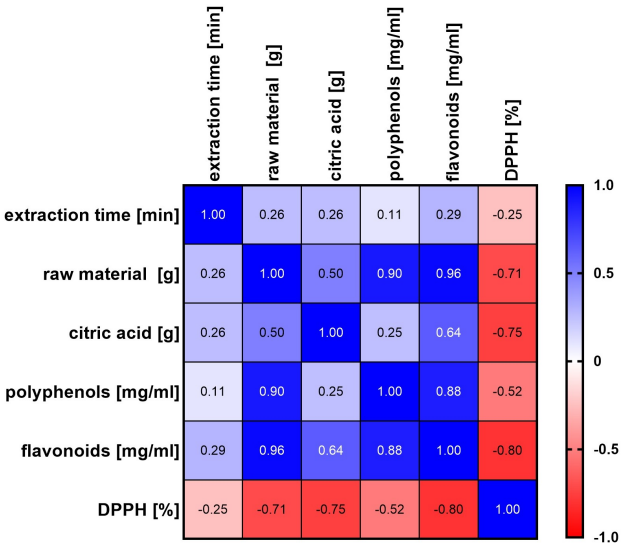

Figure 6. Map of Pearson's correlation coefficients. The correlation coefficient values range from –1 to +1, where +1 (dark blue) indicates a perfect positive linear correlation, 0 (white) indicates no linear correlation, and –1 (dark red) indicates a perfect negative linear correlation.

that all parameters are strongly correlated with the amount of raw material used in the extracts. Additionally, a strong positive correlation is observed between flavonoid and polyphenol concentrations. In contrast, antioxidant activity is most closely associated with flavonoid content and, to a lesser extent, with the amount of citric acid added.

3. CONCLUSIONS

The MME applied in this study proved to be an effective approach for isolating active compounds, such as polyphenols, from the dried root of *Paeonia lactiflora* P. This technique is highly efficient, cost-effective, straightforward, and environmentally friendly. One of its key advantages lies in its flexibility — the method allows for the adjustment of various process parameters, including surfactant concentration, plant material load, type and amount of preservative, pH regulator, extraction temperature, and ultrasonic power. This flexibility makes it well-suited for optimization through experimental design, enabling researchers to reduce the number of unnecessary test samples and improve process planning.

The input variables investigated in this study included extraction time, the amount of raw material, and the quantity of

citric acid added to the extracts. A simple experimental design was employed, where each parameter was tested at three levels. The results were then analyzed using both multiple linear and quadratic regression models. The output variables were the total concentrations of polyphenols and flavonoids, as well as the antioxidant potential of the extracts.

Generally, based on the regression coefficients and other statistical parameters, the quadratic regression model provides a better prediction of the total flavonoid and polyphenol content and the antioxidant activity of micellar extracts from *Peonia lactiflora* root. The findings demonstrated clear relationships between extraction time and raw material amount with the measured outputs. Longer extraction times led to higher values across all output variables. Similarly, increasing the amount of raw material resulted in elevated concentrations of polyphenols and flavonoids. Interestingly, higher citric acid content enhanced the concentration of flavonoids but corresponded with a reduction in antioxidant activity. In the case of polyphenols, no distinct trend was observed in response to citric acid variation.

This study successfully demonstrated that MME, when combined with a well-designed experimental plan, offers a powerful and adaptable strategy for obtaining antioxidant-rich plant extracts with optimized bioactive content.

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