Influence of Solvent Polarity on the Kinetics of Microwave-assisted Extraction, Composition, and Antiradical Activity of Black Locust Flowers (*Robinia pseudoacacia flos*) Extracts

doi: https://doi.org/10.15255/CABEQ.2025.2455

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Original scientific paper
Received: October 30, 2025
Accepted: February 11, 2026

The present study investigated the influence of solvent polarity on the kinetics of microwave-assisted extraction of phenolic compounds from black locust flowers. Extractions were conducted in an open-vessel microwave system operating at 462 W, with a constant liquid-to-solid ratio of 10 mL g^{-1}. Extraction kinetics were simulated using Ponomarev's model and an unsteady-state diffusion model. Furthermore, the effect of solvent polarity was evaluated with respect to the mineral composition and antiradical activity of the extracts.

Among the tested solvents, the 50 % (v/v) ethanolic extract showed the highest total phenolic content (1.94 g gallic acid equivalents/100 g d.m.) and flavonoid content (0.97 g rutin equivalent/100 g d.m.), while simultaneously exhibiting the strongest antiradical activity (half-maximal inhibitory concentration of 0.424 mg mL^{-1}). Correlation analysis indicated that the antiradical activity is not solely attributable to the presence of phenolic compounds, but also to other bioactive compounds present in the extracts. Solvent polarity significantly affected macro- and micro-element content, with potassium identified as the predominant macroelement.

The absence of detectable heavy metals, specifically lead and cadmium, confirms the safety profile and suitability of the extracts for further application. The extracts, particularly those obtained using 50 % (v/v) ethanol, demonstrate significant potential as bioactive ingredients for functional product development. These findings support the replacement of synthetic additives with natural alternatives, aligning with the principles of sustainable and green chemistry.

Keywords
Robinia pseudoacacia flos, microwave-assisted extraction, kinetics, phenolic compounds, mineral composition

Introduction

Natural antioxidants are playing an increasingly important role across various industrial sectors, driven by growing consumer interest in safe and healthy-promoting products.[1] The global market for natural antioxidants is projected to reach USD 1.50 billion by 2030, with a compound annual growth rate of 3.9 % during the period from 2025 to 2030.[2] These compounds serve as promising therapeutic agents for the prevention and management of oxidative stress-related pathologies.[3] Moreover, they function as natural preservatives by inhibiting oxidation, extending shelf life, and preserving nutritional quality.[4] Such extracts also serve as effective skin-protective agents against harmful environmental factors, decelerating the aging process and preserving epidermal integrity.[5] Within the spectrum of natural antioxidants, phenolic compounds play a pivotal role due to their abundance and pronounced bioactivity.[6]

In addition to traditional extraction techniques,[7] advanced extraction methods are increasingly being employed to achieve efficient and selective extraction of these compounds.[8,9] Traditional techniques often require longer extraction times, higher solvent consumption, and may lead to the degradation of thermosensitive compounds.[10] Conversely, advanced techniques, such as ultrasound-assisted extraction (UAE), supercritical fluid extraction, and microwave-assisted extraction (MAE), accelerate the process by reducing solvent use and lowering extraction temperatures.[8,11] In a scientific and industrial context, MAE represents an effective and sustainable method with the potential to enhance extract quality and reduce environmental impact.[12] The advantages of MAE primarily relate to process

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intensification, including reductions in extraction time, solvent use, and energy consumption.[13] During MAE, microwave radiation causes *in situ* heating of the aqueous phase inside plant cells, leading to an increase in internal pressure. This pressure increase, often associated with osmosis, induces structural changes in the cell membrane, resulting in rupture or increased permeability of the cell wall.[14] Consequently, bioactive ingredients are released more rapidly and efficiently.

A thorough understanding of extraction kinetics is fundamental to the rational design, optimization, and successful scale up of processes aimed at isolating bioactive compounds from plant materials.[15] This knowledge enables accurate prediction of extraction duration, enhances efficiency, reduces costs, and helps identify the key factors influencing process performance in industrial applications.[16]

Black locust (*Robinia pseudoacacia*) is recognized as a plant with multiple values across various industries and daily life.[17] Lu *et al.*[11] reported that the flower contains a variety of bioactive compounds, including flavonoids, phenolic acids, alkaloids, triterpenes, and steroids. The qualitative and quantitative composition of these compounds depend on species, geographical origin, growing conditions, flowering stage, and harvest time.[18,19] These compounds are responsible for its biological activities,[20] making it a potential source of natural antioxidants for various applications.[21,22] In our previous research, the optimal conditions for UAE of phenolic compounds from this plant material were proposed using central composite design.[23] However, the potential of MAE technique remains unexplored, presenting both a challenge and an opportunity for developing an innovative extraction procedure.

To date, no systematic investigation has addressed the influence of solvent polarity on the efficiency of MAE of phenolic compounds from black locust flowers, nor have the extraction kinetics of this process been reported in the literature. Therefore, this study aimed to evaluate the kinetics of MAE of phenolic compounds from this plant material, systematically analyzing different solvent systems. The safety of the obtained extracts was assessed through mineral composition analysis, while correlation analysis was employed to elucidate the origin of their antiradical activity.

Materials and methods

Chemicals and reagents

Ethanol (96 %, v/v), sodium carbonate, methanol (Zorka Pharma, Šabac, Serbia), rutin trihydrate standard (purity 97 %; Alfa Aesar–Johnson Matthey, Heysham, UK), Folin-Ciocalteu's reagent, gallic acid (purity of 97 %), aluminium(III)-cloride, potassium acetate, 2,2-diphenyl-1-picrylhydrazyl (DPPH), butylhydroxytoluene (BHT), and multielement standard solutions IV (containing Al, Ag, B, Ba, Be, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, Se, Ti, V, and Zn) at a concentration of 1,000 mg L^{-1} (Sigma-Aldrich, St. Louis, Missouri, USA) were used.

Plant material

The dried black locust flowers (*Robiniae pseudoacaciae flos*) were purchased from Dr. Josif Pančić Institute (Belgrade, Serbia). The moisture content of the plant material was determined to be 10.6 % (w/w) by drying the sample in a laboratory oven at 105 °C until a constant mass was achieved. The plant material was subsequently ground in an electric mill (Bosch, model TSM6A013B, 180 W). The particle size distribution was determined using a laboratory vibrating sieve shaker (ANALYSETTE 3 PRO, Fritsch GmbH, Idar-Oberstein, Germany). A fraction with a particle size of 0.5 mm was used for MAE of phenolic compounds.

Microwave-assisted extraction of phenolic compounds from black locust flowers

Distilled water, ethanol, 50 % (v/v) ethanol, methanol, and 50 % (v/v) methanol were used to prepare black locust flower extracts. Ground dried flowers (5.0 g) were subjected to the MAE with 50 mL of solvent, using extraction times ranging from 15 to 240 s. All extractions were performed individually for each specified time point. An open-vessel microwave system (Vivax MWO-2070 BL) was employed at a constant microwave power of 462 W due to technical limitations, in the absence of a temperature control system. After the extraction, the extracts were cooled and then vacuum filtered. The resulting liquid extracts were centrifuged using a TH16B centrifuge (Hong Kong, China) at 6,000 rpm for 15 min. Finally, the prepared extracts were stored at 4 °C in a refrigerator prior to further analysis.

The extraction kinetics of phenolic compounds were simulated based on Ponomarev's model (Eq. 1) and unsteady-state diffusion model in its linear form (Eq. 2). The reference value of total phenolic content (TPC) in the dry flowers was determined for each solvent system. Plant material (10.0 g) was placed in a 250 mL flask and subjected to MAE with 100 mL of solvent for 5 min. After vacuum filtration and centrifugation, the remaining plant material was extracted again with fresh solvent (100 mL) using MAE for 5 min.

$$\frac{q_0 - q_i}{q_0} = b' + k't \tag{1}$$

$$\ln\frac{q_i}{q_0} = \ln(1 - b'') - k''t \tag{2}$$

where q_0 is the initial TPC in the plant raw material, q_i is the TPC after time t, b' and b'' are the leaching coefficients according to Ponomarev's model and the unsteady-state diffusion model, respectively, and k' and k'' are the slow extraction coefficients of Ponomarev's model and unsteady-state diffusion model, respectively.

Determination of total phenolic and total flavonoid content

The TPC was determined spectrophotometrically using the Folin-Ciocalteu reagent[23] and reported as grams of gallic acid equivalents per 100 grams of dry matter (g GAE/100 g d.m.). A ten-fold dilluted Folin-Ciocalteu reagent (1 mL) was added to 0.1 mL of extract sample. The prepared sample was incubated with 7 % (w/v) sodium carbonate solution for 90 min. Subsequently, the absorbance of the sample was measured at 765 nm on a Varian Cary-100 spectrophotometer (Mulgrave, VIC, Australia). Instead of the sodium carbonate solution, the negative control contained an equivalent amount of distilled water.

The TFC was also determined spectrophotometrically, based on the colorimetric reaction with aluminium chloride.[24] The content was expressed as gram of rutin equivalents per 100 grams of dry matter (g RE/100 g d.m.). The extract samples (0.5 mL) were mixed with 1 M potassium acetate solution (0.1 mL) and 10 % (w/v) aluminium chloride solution (0.1 mL), and incubated for 90 min. The negative control contained an equivalent volume of distilled water instead of aluminium chloride. The absorbance of the samples was subsequently measured at 415 nm.

ICP-OES analysis

Black locust flower extracts obtained under optimal extraction times were used to determine the content of macro- and microelements using inductively coupled plasma-optical emission spectrometry (ICP-OES). Prior to analysis, alcoholic samples were tenfold-diluted with distilled water, while the aqueous extract was injected directly into the ICP-OES system (Optima 8300, PerkinElmer Inc., Waltham, MA, USA) without dilution. The calibration curves were prepared by diluting the Multistandard IV multielemental standard solution. The concentrations of the standards were adjusted to fall within the expected concentration ranges for the tested elements. Quantitative analysis of all samples was performed using a device equipped with a segmented-array charge-coupled device detector. The plasma power was set to 1,400 W, with the plasma operating in the axial mode. Argon was used as the plasma gas, with a cooling gas flow of 10 L min^{-1} and an auxiliary gas flow of 0.5 L min^{-1}. A standard cyclonic spray chamber was employed, with a nebulizer flow rate of 1.5 L min^{-1}. The sample injection speed was maintained at 1 mL min^{-1}. Data acquisition and processing were carried out using WinLab32 software (version 5.5.0.0714, PerkinElmer Inc., Waltham, MA, USA). The wavelength data used for detecting each element, together with the correlation coefficients (R^2), detection limits, and linearity ranges, are provided in Table S1.

DPPH assay

The antiradical activity of black locust flower extracts was determined using the DPPH assay according to Daulay *et al.*[25] with slight modification. A series of extract solutions at different concentrations was prepared by diluting the stock solution (10 mg mL^{-1}). An ethanolic solution of DPPH radicals at a concentration of $3\cdot10^{-4}$ mol L^{-1} (1 mL) was added to 2.5 mL of each sample. Sample absorbance at 517 nm was recorded using ethanol as the reference, following 30 min of incubation in the dark at room temperature. The negative control sample was prepared by mixing 1 mL of the DPPH ethanolic solution and 2.5 mL of ethanol. The commercially available synthetic antioxidant BHT was used as a positive control. The percentage inhibition of DPPH radicals (I_{DPPH}) was calculated using Eq. 3:

$$I_{\mathrm{DPPH}}(\%) = \frac{A_c - A_s}{A_c} \cdot 100 \tag{3}$$

where, A_s is the absorbance of the sample treated with DPPH radical solution, and A_c is the absorbance of the negative control solution. The antiradical activity of the samples was evaluated based on the half-maximal inhibitory concentration (IC_{50}). It was determined by interpolating the relationship between DPPH inhibition and sample concentration.

Electrical energy consumption

The electrical energy consumption (E) of MAE and other extraction methods, as reported in the literature, used for phenolic compounds from black locust flowers was calculated using Eq. 4:

$$E(\mathrm{kWh}) = P \cdot t \tag{4}$$

where P denotes the power (in kW), and t represents the time of operation (in h).

Statistical analysis

The data are presented as the mean ± standard deviation of three measurements. Statistical differences between analyzed groups were estimated using IBM SPSS Statistics 27 software (International Business Machines Corporation, Armonk, NY,

USA) according to Tukey's post hoc test. Additionally, Dunnett's post hoc test was used to compare the obtained IC_{50} values of the extract samples with the positive control sample (BHT).

The kinetic extraction models were systematically compared by evaluating the coefficient of determination (R^2), root mean square error (RMSE), and average absolute relative error (AARE), as defined in Eqs. (5–7).

$$R^2 = 1 - \frac{\sum_{i=1}^{n}\left(q_{exp} - q_{pred}\right)^2}{\sum_{i=1}^{n}\left(q_{exp} - \overline{q}_{exp}\right)^2} \quad (5)$$

$$\mathrm{RMSE} = \sqrt{\frac{1}{n}\sum_{i=1}^{n}\left(\frac{q_{exp} - q_{pred}}{q_{exp}}\right)^2} \quad (6)$$

$$\mathrm{AARE} = \frac{1}{n}\sum_{i=1}^{n}\left(\left|\frac{q_{exp} - q_{pred}}{q_{exp}}\right|\right) \quad (7)$$

where q_{exp} is the experimental data, $\overline{q}_{exp}$ is the mean value of the experimental data, q_{pred} is the predicted data, and n is the number of samples.

Results and discussion

Kinetic modeling of phenolic compounds extraction from black locust flowers

The MAE of phenolic compounds from black locust flowers was performed in an open vessel system without a temperature control system. The advantage of this system lies in its ability to be easily scaled up to an industrial level, enabling the processing of larger quantities of plant material.[26] A critical parameter for optimal MAE operation is microwave power. Selecting an appropriate power level is crucial to preventing excessive temperature elevation. Excessive heating can accelerate the degradation of thermolabile bioactive compounds and generate overpressure within the microwave chamber.[27] During the extraction of phenolic compounds from the flowers, microwave power was maintained at a constant level of 462 W to ensure process stability. Application of higher power was not feasible due to technical limitations, as it caused localized overheating of the solvent system and increased the risk of bioactive compound degradation.[28] Conversely, operation at lower power resulted in reduced TPC.

The choice of solvent is also essential in MAE and depends on the solubility of the compound to be extracted.[29] Research has shown that the efficiency and selectivity of MAE significantly depend on the dielectric constant of the solvent.[30] Ethanol and methanol are known to be relatively efficient absorbers of microwave energy and are therefore frequently used as solvents for extracting polar compounds, such as phenolic compounds.[29] A small amount of water in the extracting solvent facilitates easier penetration into the cells of the plant matrix. It promotes more efficient heating, which results in increased mass transfer of bioactive components in the extract.[31] Based on these findings, water, ethanol, 50 % (v/v) ethanol, methanol, and 50 % (v/v) methanol were selected for extracting phenolic compounds from flowers.

The extraction time significantly influences the process efficiency and should be carefully considered during the extraction process. Generally, extending the extraction time increases the yield of extracted compounds, but there is a risk of their possible degradation.[27] Most often, the maximum MAE time is 15–20 min, although some data indicate that a satisfactory extraction yield can be achieved within just a few seconds.[27] Available studies on extraction kinetics reported that the yield of phenolic compounds increases during the process until approximately 4 min, after which it decreases due to degradation.[31] Therefore, the kinetic studies of MAE of TPC from black locust flowers were conducted for up to 4 min.

Two extraction kinetic models, the Ponomarev's model and the unsteady-state diffusion model, were chosen to simulate the MAE process. While Ponomarev's model represents an empirical fitting approach, the unsteady-state diffusion model is grounded in the physical principles of mass transfer and diffusion.[32] The selection of Ponomarev's model and the unsteady-state diffusion model was motivated by their complementary strengths in describing the MAE process. Ponomarev's model provides a simple and accurate empirical fitting of the extraction kinetics. In contrast, the unsteady-state diffusion model is grounded in the physical principles of mass transfer and diffusion, enabling a mechanistic interpretation of the extraction process. The combined use of these models ensures both reliable empirical description and theoretical insight, thus providing a more comprehensive understanding of MAE compared to conventional mathematical models. The plots of extraction kinetics for the TPC from black locust flowers using different solvents are presented in Fig. 1.

Both extraction phases, comprising the initial rapid phase and the subsequent slower phase, were observed in this process. The rapid phase corresponds to the steeper slope of the kinetic curve,[33] reflecting the accelerated release of phenolic compounds from disrupted cell structures and surface layers. The slower extraction phase results from the

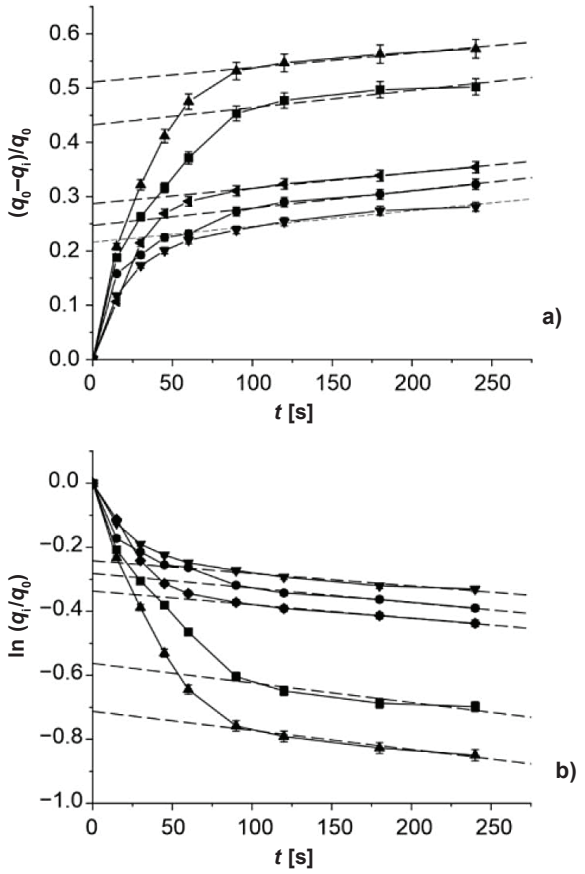

Fig. 1 – *Extraction kinetics of phenolic compounds from black locust flowers according to (a) Ponomarev's model, and b) the unsteady-state diffusion model. Solvents are indicated by symbols: water (■), ethanol (●), 50 % ethanol (▲), methanol (▼), 50 % methanol (◀).*

diffusion of phenolic compounds from intact plant cells, that is, from within the internal plant tissues. This phase is marked by a significantly slower increase in yield, indicating the limited rate of mass transfer at this stage. The values of the rapid extraction period (REP) and the extraction degree (ED) are shown in Table 1. The REP was observed up to 90 s, during which between 23.85 % and 53.15 % of phenolic compounds were released from the plant matrix, depending on the solvent used. The highest ED (53.15 %) was achieved using 50 % (v/v) ethanol. It provided an optimal polarity balance, facilitated enhanced penetration, and release of phenolic compounds from the plant matrix. Pure alcoholic solution is known to denature proteins due to broken intramolecular hydrogen bonds, which may cause plant cell pores to close and restrict mass transfer.[34] Water is a highly polar solvent, which limits its penetration through lipophilic membranes and consequently reduces its extraction efficiency.[35]

The kinetic parameters obtained from the Ponomarev's model (b' and k') and unsteady-state diffusion model (b' and k'') for solvents of different polarity and extraction time range of 90–240 s are presented in Table 1. Unlike the washing coefficients, which showed only slight differences between the two kinetic models, the slow extraction coefficients were consistently higher in the unsteady-state diffusion model compared to Ponomarev's model. For instance, in 50 % (v/v) ethanol, k'' reached $0.592 \cdot 10^{-3}$ s^{-1} compared to $0.264 \cdot 10^{-3}$ s^{-1}

Table 1 – *Extraction kinetic and statistical parameters obtained from Ponomarev's and unsteady-state diffusion models*

Model	Parameters	Solvent				
		Water	Ethanol	50 % (v/v) Ethanol	Methanol	50 % (v/v) Methanol
	REP [s]	90	90	90	90	90
	ED [%]	45.33	27.27	53.15	23.85	31.08
Ponomarev's model (90 – 240 s)	b'	0.434	0.248	0.512	0.217	0.288
	$k'\cdot10^3$ [s^{-1}]	0.311	0.317	0.264	0.283	0.282
	R^2	0.866	0.976	0.953	0.935	0.990
	RMSE	0.00705	0.00285	0.00337	0.00429	0.00166
	AARE	1.46	0.78	0.61	1.58	0.45
Unsteady-state diffusion model (90 – 240 s)	b''	0.432	0.246	0.510	0.216	0.286
	$k''\cdot10^3$ [s^{-1}]	0.599	0.452	0.592	0.383	0.422
	R^2	0.873	0.979	0.958	0.938	0.991
	RMSE	0.01314	0.00385	0.0071	0.00566	0.00229
	AARE	1.99	0.91	0.88	1.79	0.51

for k', nearly a twofold difference. This suggests that the diffusion model emphasizes the contribution of solvent polarity and cell wall permeability to the release of phenolic compounds. In contrast, Ponomarev's model provides a more conservative empirical fit of data. Ponomarev's model provided lower RMSE and AARE values across all solvents, indicating a slightly better statistical fit to the experimental data. In contrast, the unsteady-state diffusion model, although yielding comparable R^2 values (0.873–0.991), exhibited higher RMSE and AARE values, particularly for water and 50 % (v/v) ethanol.

Since TPC did not change significantly during the extraction period from 90 s to 240 s, 90 s was selected as the optimal extraction time for phenolic compounds. In our previous study, the impact of these same solvents on the UAE kinetics of phenolic compounds from flowers was reported.[36] In that study, the ED ranged between 79.97 % and 88.68 % of phenolic compounds at 60 °C for the REP of 30 min. A comparison of the results indicated that UAE gives a higher amount of phenolic compounds than MAE. The effectiveness of UAE can be explained by its mechanism involving the generation of sonic waves. They induce vibration and rupture of the cell walls in plant materials, releasing intracellular compounds.[37] In addition to the superior results in terms of TPC, other factors important for selecting the extraction technique should also be considered.

Total phenolic content in black locust flower extracts

The TPC was determined spectrophotometrically using the non-selective Folin–Ciocalteu reagent. It can react not only with phenolic compounds but also with other reducing compounds, such as vitamins, amino acids, peptides, carbohydrates, organic acids, etc.[38] Consistent with the literature, the term TPC was retained in this study, although it does not represent a strictly accurate measure of total phenolics. Importantly, this assay provides only an indirect estimation of antioxidant potential, as it reflects the overall reducing capacity of the analyzed sample. After modeling the extraction kinetics, black locust flower extracts obtained after 90 s of extraction were selected for further analysis. The TPC, TFC, and antiradical activity are presented in Table 2. TPC and TFC values ranged from 0.75 g GAE/100 g d.m. to 1.94 g GAE/100 g d.m. and from 0.38 g RE/100 g d.m. to 0.97 g RE/100 g d.m., respectively.

The results obtained indicate that solvent polarity, as defined by dielectric constant values (Table 2), exerts a significant influence on the extraction efficiency of phenolic and flavonoid compounds. Specifically, binary mixtures of alcohol and water yielded higher TPC and TFC compared to pure alcohols (ethanol and methanol). This behavior is expected, as water facilitates the formation of hydrogen bonds with the hydroxyl groups of phenolic compounds, thereby increasing their solubility.[39] However, despite water having the highest dielectric constant among the tested solvents (ε_r = 78.4), the TPC and TFC in the aqueous extract was approximately 30 % and 20 % lower than that obtained with 50 % (v/v) ethanol (ε_r = 51). Based on the obtained data, 50 % (v/v) ethanol is the most suitable solvent for extracting phenolic and flavonoid compounds from black locust flowers using MAE. Otherwise, the results align with our previous research, which also indicated that TPC from black locust flowers is higher in water-alcoholic solutions.[36] However, in the case of UAE, the highest TPC of 3.78 g GAE/100 g d.m. was observed in the 50 % (v/v) methanolic extract. It was nearly four times higher than the value obtained for the same solvent using MAE. Comparing these two different studies suggests that, in addition to solvent polarity, the extraction technique itself significantly

Table 2 – *Influence of solvent dielectric properties on total phenolic content (TPC), total flavonoid content (TFC), and antiradical activity of black locust flower extracts produced by microwave-assisted extraction for 90 s*

Solvent	Dielectric constant (ε_r)	TPC (g GAE/100 g d.m.)	TFC (g RE/100 g d.m.)	Antiradical activity	
				IC_{50} [mg mL^{-1}]	Concentration range [mg mL^{-1}]
Water	78.4[46]	1.36 ± 0.04^{c}	0.79 ± 0.02^{c}	0.503 ± 0.015^{a,b*}	0.0027 – 3.0198
Ethanol	25.3[46]	1.39 ± 0.04^{c}	0.81 ± 0.01^{c}	0.575 ± 0.017^{b*}	0.0069 – 1.6900
50 % (v/v) Ethanol	51.0[47]	1.94 ± 0.06^{d}	0.97 ± 0.03^{d}	0.424 ± 0.013^{a*}	0.0063 – 3.1370
Methanol	33.0[46]	0.75 ± 0.02^{a}	0.38 ± 0.01^{a}	1.129 ± 0.034^{c*}	0.0115 – 5.9500
50 % (v/v) Methanol	60.9[48]	1.04 ± 0.03^{b}	0.62 ± 0.02^{b}	1.685 ± 0.051^{d*}	0.0096 – 4.9980
BHT				0.037 ± 0.001	0.0066 – 0.2497

Values sharing the same superscript letter within a column are not significantly different according to Tukey's post hoc test ($p > 0.05$).

*Statistically significant differences in IC_{50} values compared to the positive control (BHT), as determined by Dunnett's post hoc test ($p < 0.05$).

influences the TPC. Uzelac *et al.*[19] reported the TPC of approximately 25 mg GAE/g d.m. and 33 mg GAE/g d.m. for 70 % (v/v) ethanolic and 80 % (v/v) methanolic extracts of black locust flowers, respectively. The extracts were prepared by shaking at room temperature for 75 min. Our study showed that the TPC in the extract prepared with 50 % (v/v) ethanol was roughly 20 % lower. This difference may be attributed to variations in solvent concentration and extraction time. Efficient extraction of phenolic compounds can be achieved even under conditions of lower solvent concentration, which holds practical significance in both laboratory and industrial settings. Tian *et al.*[18] found the TPC of 0.69 g GAE/100 g d.m. for a 70 % (v/v) ethanol extract of black locust flowers, prepared by mixing at 25 °C for 40 min. This value was significantly lower than the 1.94 g GAE/100 g d.m. obtained in our study.

Antiradical activity of black locust flower extracts

The antiradical activity of the optimal black locust flower extracts and the synthetic antioxidant BHT was evaluated using the DPPH assay and IC_{50} value (Table 2). Among the analyzed samples, the 50 % (v/v) ethanolic extract showed the highest antiradical activity (IC_{50} = 0.424 mg mL^{-1}). This activity was expected, as the extract with the highest TPC and TFC was prepared using this solvent. Although these extracts exhibited activity, their effectiveness was approximately ten times weaker than that of the synthetic antioxidant BHT. Although natural extracts cannot completely replace synthetic antioxidants in all applications, their use remains valuable in the food, pharmaceutical, and cosmetics industries due to their additional health benefits and natural origin. Interestingly, the extracts obtained using water and pure ethanol showed similar antiradical activity, whereas the methanolic extracts exhibited lower activity. A similar effect of solvent polarity on antiradical activity was observed in our previous study, which involved the application of UAE.[36] These findings confirm that the choice of solvent and extraction technique significantly influence the quality and bioactivity of extracts, playing a crucial role in the development of effective natural antioxidants.

A correlation analysis between IC_{50} values and TPC or TFC was conducted to identify the classes of compounds responsible for the antiradical activity of the extracts (Fig. 2). The higher coefficient of determination (R^2 = 0.613) for TFC compared to that for TPC (R^2 = 0.491) indicates that the antiradical activity is predominantly associated with flavonoids present in the extracts. These results are in agreement with previous reports highlighting flavonoids as key contributors to antioxidant activity in

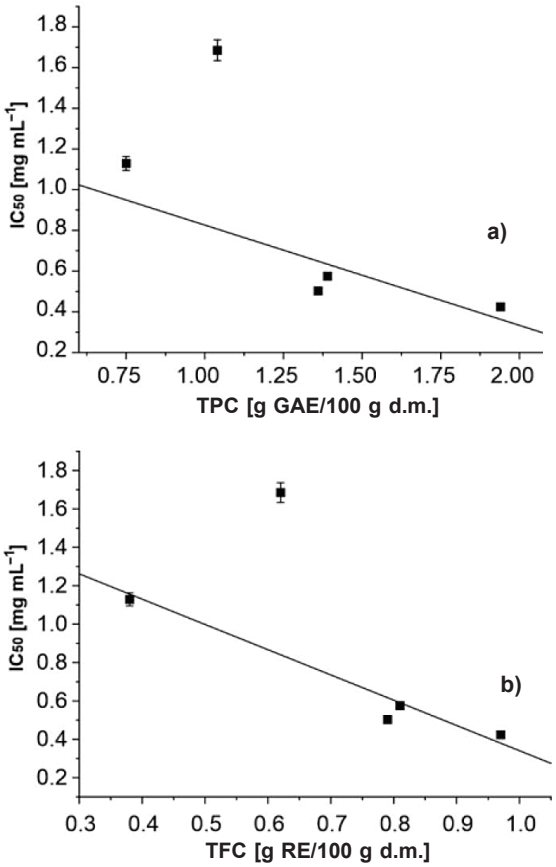

Fig. 2 – *Correlation analysis of black locust flower extracts: (a) total phenolic content (TPC) vs. IC_{50} (R^2 = 0.491), and (b) total flavonoid content (TFC) vs. IC_{50} (R^2 = 0.613). Each value represents the mean ± SD of triplicate measurements (n = 3).*

plant-derived extracts.[40] According to the literature, coefficients of determination above 0.75 are generally considered sufficient to justify predictive models, whereas lower values indicate only moderate associations.[41] The absence of a strong correlation suggests that the antiradical activity of the extracts is not only dependent on TPC and TFC, but is likely influenced by other active secondary metabolites with pronounced antiradical properties. Evaluating the antiradical activity of the extracts requires consideration of the bioactive compounds profile to gain a comprehensive understanding of the factors contributing to their efficiency. In addition, the DPPH assay has certain limitations compared to other antioxidant assays such as ABTS or FRAP. Specifically, it is performed in organic solvents and is therefore less suitable for hydrophilic antioxidants, and it reflects only one mechanism of radical scavenging.[42] In contrast, ABTS and CUPRAC assays provide complementary information by covering both hydrophilic and lipophilic antioxidants and by assessing different antioxidant mechanisms.[43] This highlights the complexity of antiradical mechanisms and emphasizes the need for complementary analyses.

Table 3 – *Mineral composition of black locust flower extracts expressed in mg kg^{-1} d.m.*

Elements	Solvent				
	Water	Ethanol	50 % (v/v) Ethanol	Methanol	50 % (v/v) Methanol
Macroelements					
Na	589.59 ± 17.69^{b}	5.01 ± 0.15^{a}	4.98 ± 0.15^{a}	6.43 ± 0.19^{a}	5.57 ± 0.17^{a}
Mg	279.55 ± 8.39^{c}	31.96 ± 0.96^{b}	5.12 ± 0.15^{a}	35.25 ± 1.06^{b}	5.46 ± 0.16^{a}
K	766.49 ± 22.99^{c}	148.83 ± 4.46^{b}	7.85 ± 0.24^{a}	161.13 ± 4.83^{b}	17.38 ± 0.52^{a}
Ca	393.36 ± 11.80^{b}	9.30 ± 0.28^{a}	8.72 ± 0.26^{a}	9.75 ± 0.29^{a}	6.18 ± 0.19^{a}
Fe	259.94 ± 7.80	n.d.	n.d.	n.d.	n.d.
Microelements					
Al	396.15 ± 11.88^{b}	0.62 ± 0.02^{a}	0.65 ± 0.02^{a}	0.89 ± 0.03^{a}	1.06 ± 0.03^{a}
Cr	283.56 ± 8.51	n.d.	n.d.	n.d.	n.d.
Cu	324.75 ± 9.74	n.d.	n.d.	n.d.	n.d.
Li	670.78 ± 20.12^{b}	0.015 ± 0.003^{a}	0.013 ± 0.002^{a}	0.006 ± 0.001^{a}	0.01 ± 0.001^{a}
Mn	259.37 ± 7.78^{b}	0.135 ± 0.004^{a}	n.d.	0.130 ± 0.004^{a}	n.d.
Zn	213.86 ± 6.42^{b}	2.59 ± 0.08^{a}	0.70 ± 0.02^{a}	1.44 ± 0.04^{a}	6.39 ± 0.19^{a}

n.d. – not detected

Groups sharing the same letter in the rows are not significantly different according to Tukey's post hoc test ($p > 0.05$).

Mineral composition of black locust flower extracts

The content of macro- and microelements in optimal black locust flower extracts (Table 3) was determined using the ICP-OES method. Monitoring heavy metal levels is essential to ensure the safety of the extracts for human applications and their incorporation into innovative product formulations. In all analyzed extracts, K had the highest concentration compared to other detected macroelements, including Ca, Mg, Fe, and Na. These extracts serve as a valuable natural source of this essential macroelement. It is crucial for maintaining optimal physiological functions in the body, such as fluid balance regulation, cardiac function, and nerve activity.[44]

The content of macroelements K, Ca, Mg, and Na was highest in the aqueous extract, indicating their greater solubility in water. The lowest values of these macroelements were recorded in the aqueous-alcoholic extracts, suggesting that alcoholic solvents extract these elements less efficiently from plant material. Fe was detected only in the aqueous extract, with a concentration of 259.94 mg kg^{-1} d.m. This value was the lowest compared to the concentrations of other macroelements in the same extract. The macroelement results indicate that the choice of solvent significantly affects their content in the extracts. Among microelements, Li was the most abundant in the aqueous extract (670.78 mg kg^{-1} d.m.), while its content in the alcoholic extracts was almost undetectable. Cr and Cu were not detected in the alcoholic extracts, but they were present in significant quantities in the aqueous extract. The trace element Mn was most abundant in the aqueous extract. In the extracts prepared with pure ethanol or methanol, its content was significantly lower, around 0.13 mg kg^{-1} d.m. It was not detected in the 50 % (v/v) ethanol and 50 % (v/v) methanol extracts. Among the microelements, Zn had the lowest content of 213.86 mg kg^{-1} d.m. in the aqueous extract. Its concentrations ranged from 0.70 to 6.39 mg kg^{-1} d.m. in the alcoholic extracts. The extracts can be considered safe for use, as the presence of heavy metals, such as Pb and Cd was not detected. The aqueous extracts have potential for applications in the development of dietary supplements and products aimed at supporting cardiovascular health, as well as for treating electrolyte imbalances. Additionally, they could be of interest in agriculture as a natural source of K for fertilizers, contributing to more sustainable food production. Previous study has shown that black locust flower extracts prepared by UAE represent a significant source of K.[36] Significant variations in mineral content highlight the extraction method as a critical determinant of extract quality and composition. Tian *et al.*[18] obtained Fe as the most abundant element in the 70 % (v/v) ethanolic extract of black locust flowers. In our study, Fe was not detected in the ethanolic extracts. Furthermore, the contents of K, Ca, and Mg were significantly lower compared to the values obtained in our study. These differences suggest that the mineral composition of the extracts

is influenced not only by the preparation methodology but also by environmental factors, including geographical, climatic, and seasonal conditions.

Techno-economic analysis of microwave-assisted extraction of polyphenols

A techno-economic analysis was performed to evaluate the energy demand and operating costs associated with the MAE of phenolic compounds from black locust flowers under the experimentally applied conditions. The efficiency of this innovative technology was compared with our previously developed procedures using UAE, Soxhlet extraction, and maceration techniques.[23] It should be noted that these methods employed nearly the same volume and concentration of ethanol per batch. Therefore, the solvent cost was not included in the final financial calculation. The analysis was conducted at laboratory scale for batch processes and focused on energy consumption and extraction efficiency. These parameters are key for assessing the feasibility of green extraction technologies. Specific energy consumption (SEC) was calculated relative to both the processed biomass and the extracted polyphenols (Table 4). On a biomass basis, the SEC for MAE technology was only 9.31 kJ kg^{-1} of dry plant material, underscoring its outstanding energy efficiency. However, when normalized to the mass of extracted phenolic compounds, the SEC increased to approximately $4.80 \cdot 10^5$ kJ kg^{-1}. This reflects the relatively low yield obtained under the applied conditions. This dual perspective highlights both the sustainability advantage of MAE in terms of energy input per biomass and the need for further optimization to improve phenolic compounds recovery. Despite the fact that maceration does not require energy consumption for the extraction of phenolic compounds, its use is limited due to the long duration of the batch process.

The financial cost for production of phenolic compounds according to the current average prices in the EU was the highest using Soxhlet extraction, followed by UAE technology. Despite the higher cost of the final product in UAE, this technology enables the production of phenolic compounds within a relatively short extraction time.

In summary, the proposed MAE technology required a small cost of approximately € 37.5/kg of

Table 4 – *Comparative analysis of energy and cost efficiency of polyphenol production from black locust flowers*

Parameter	Value			
	MAE	UAE[23]	Soxhlet extraction[23]	Conventional extraction[23]
Dry biomass (g)	5	5	5	2
Solvent type	50 % (v/v) ethanol–water	50 % (v/v) ethanol–water	60 % (v/v) ethanol–water	60 % (v/v) ethanol–water
Solvent volume (mL)	50	50	500	20
Liquid-to-solid ratio (mL g^{-1})	10	10	10	10
Power	462 W	Ultrasound 300 W Heater 800 W	150 W	/
Temperature (°C)	Uncontrolled system	59	80	25
Extraction time	90 s	30 min	6 h	24 h
Moisture content in plant material (%, w/w)	10.6	10.6	10.6	10.6
Water heating time in ultrasonic bath (min)	/	23	/	/
Total energy input (kJ)	41.6	540 + 1,104	3,240	0
Polyphenol yield (g GAE/100 g d.m.)	1.94	3.12	3.22	2.54
Polyphenols extracted per batch (mg)	86.7	139.5	143.9	113.5
Specific energy consumption (kJ kg^{-1} dry matter)	9.31	367.8	724.8	0
Specific energy consumption (kJ kg^{-1} polyphenols)	$4.80 \cdot 10^5$	$1.00 \cdot 10^7$	$2.25 \cdot 10^7$	0
Electricity consumption per batch (kWh)	0.0116	0.150 + 0.307	0.900	0
Electricity cost per batch* (€)	0.0032	0.1280	0.252	0
Electricity cost per kg of polyphenols* (€)	37.5	917.3	1,751.2	0

*Average electricity price of 0.28 €/kWh for household consumers in the EU: https://ec.europa.eu/eurostat/web/products-eurostat-news/w/ddn-20251029-2 (Available 9th February, 2026)

phenolic compounds, which was almost the same as in the case of maceration. Considering techno-economic aspects, especially process duration and energy consumption, MAE demonstrates clear advantages. Specifically, MAE enables significantly shorter extraction times with minimal energy consumption, which is crucial for industrial applications and mass production.[45] It is particularly useful when combined with solvent recycling and continuous-flow microwave systems. Furthermore, due to its simpler technical requirements, MAE can be considered a more economically viable option compared to UAE, which necessitates specialized equipment and tends to be more costly to implement.

Conclusion

The impact of solvent polarity on the MAE kinetics of TPC was analyzed. The MAE kinetics was simulated using both Ponomarev's model and the unsteady-state diffusion model. Ponomarev's model provided a slightly better fit to the experimental data, ensuring reliable description and prediction of extraction kinetics. In contrast, the unsteady-state diffusion model elucidated the role of solvent polarity and cell wall permeability in controlling phenolic release. Increasing the water ratio in the alcoholic solution enabled the dissolution of a higher amount of hydrophilic phenolic compounds. The 50 % (v/v) ethanolic solution was identified as the solvent of choice for TPC extraction.

The extraction time of only 90 s using the open-vessel MAE system achieved the ED of 53.15 % of phenolics. Mineral composition analysis demonstrated the safety of the extract, due to the absence of detectable heavy metal ions. The predominant potassium content suggests potential cardioprotective properties of the extract. The results indicated that solvent polarity significantly influenced TPC, TFC, and the antiradical activity of the extracts. Correlation analysis revealed that the antiradical activity of the extracts depended more strongly on TFC than on TPC. More detailed profiling of individual phenolics and flavonoids is required to fully elucidate the origin of antiradical activity.

The findings of this research have practical implications for the development of efficient and sustainable extraction processes for bioactive compounds. Future studies will focus on optimization and evaluation of additional extraction parameters affecting TPC. Furthermore, the implementation of closed-vessel MAE systems will be considered, as they allow precise temperature control. Since extraction efficiency is strongly temperature-dependent, the absence of temperature profiles in this study limits the mechanistic interpretation of the kinetic results.

ACKNOWLEDGEMENT

This work was supported by the Ministry of Science, Technological Development, and Innovation of the Republic of Serbia (Contract No. 451-03-34/2026-03/ 200133).

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

SUPPLEMENT

Table S1 – *Calibration parameters of elements analysed in the ICP-OES*

Element	Wavelength (λ, nm)	Coefficient of determination (R^2)	Limit of detection (μg L^{-1})	Linearity range (mg L^{-1})
As	197.2	0.999	2.55	0.002–3.600
Ag	328.1	0.999	0.39	$3\cdot10^{-4}$–12.000
Al	394.4	0.999	$7.60\cdot10^{-2}$	0.002–12.000
B	249.7	0.999	6.43	0.006–12.000
Ba	233.5	0.995	0.18	$1.83\cdot10^{-4}$–12.000
Bi	223.1	0.999	3.53	0.004–12.000
Ca	396.8	0.999	2.14	0.002–2.410
Cd	214.4	0.999	0.13	$1.27\cdot10^{-4}$–12.000
Co	228.6	0.999	0.33	$3.27\cdot10^{-4}$–12.000
Cr	283.5	0.999	0.44	$4.35\cdot10^{-4}$–12.000
Cu	324.7	0.999	0.26	$2.59\cdot10^{-4}$–12.000
Fe	239.9	0.999	0.12	$1.18\cdot10^{-4}$–12.000
Ga	417.2	0.999	1.70	0.002–12.000
Ge	265.1	0.999	1.00	0.001–12.000
Hg	184.9	0.999	0.19	$1.92\cdot10^{-4}$–12.000
In	325.6	1.000	4.00	0.004–12.000
K	766.4	0.999	0.38	$3.78\cdot10^{-4}$–1.200
Li	670.7	0.999	$5.75\cdot10^{-2}$	$5.75\cdot10^{-5}$–1.200
Mg	285.2	0.999	0.12	4.030–120.000
Mn	257.6	0.999	$3.57\cdot10^{-2}$	$3.570\cdot10^{-5}$–12.000
Na	588.5	0.999	4.75	0.005–12.000
Ni	231.6	0.999	0.47	$4.74\cdot10^{-4}$–12.000
P	214.9	1.000	1.59	0.002–120.000
Pb	220.3	0.999	1.78	0.002–12.000
Sb	187.1	0.999	2.44	0.002–12.000
Si	251.6	0.999	1.60	0.002–60.000
Sr	407.7	0.999	$6.23\cdot10^{-3}$	$6.23\cdot10^{-6}$–2.410
Tl	190.8	0.999	1.72	0.002–12.000
Zn	213.8	0.999	$8.20\cdot10^{-2}$	$8.2\cdot10^{-5}$–12.000

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