MJFAS
Malaysian Journal of Fundamental and Applied Sciences

Zhu *et al.* | Malaysian Journal of Fundamental and Applied Sciences, Vol. 22 (2026) 1043–1054

RESEARCH ARTICLE

Extraction of Flavonoids from *Angelica keiskei* Leaves Using Ultrasound-Assisted Deep Eutectic Solvent Extraction: Optimization and Bioactivity Evaluation

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Abstract *Angelica keiskei* is a medicinal and edible herb rich in bioactive flavonoids, yet green and efficient extraction methods for these compounds remain underexplored. This study aimed to establish an ultrasound-assisted deep eutectic solvent extraction method for total flavonoids from the plant. Response surface methodology (RSM) was employed to optimize the extraction process for *A. keiskei* leaves. Single-factor and Box-Behnken design experiments were conducted to evaluate the influence of DES water content, ultrasonic time, liquid-solid ratio, and DES molar ratio on extraction efficiency. Results demonstrated that a total flavonoid content of 13.02% ± 0.15% was achieved under the optimal conditions of 50% DES water content, 100 min ultrasonic time, 50:1 liquid-solid ratio (w/v), and 4:1 DES molar ratio (1,2-ethanediol: choline chloride). Notably, the obtained extract exhibited strong antioxidant activity in vitro, with a DPPH· radical scavenging IC_{50} value of 31.33 ± 1.35 μg/mL. Antibacterial tests revealed that the extract exhibited moderate sensitivity against *Escherichia coli*, producing inhibition zones exceeding 12 mm at an EC_{50} of 2.20 mg/mL. These findings demonstrate that UAE-DES is a green and efficient approach for flavonoid extraction from *A. keiskei*, and the resulting extract have potential to be used as natural functional additives in the field of food, cosmetics, and pharmaceuticals.

Keywords: *Angelica keiskei*, flavonoids, deep eutectic solvents, optimization.

Introduction

Conventional extraction approaches, including percolation, decoction, maceration, reflux, and Soxhlet extraction, often involve the use of substantial amounts of organic solvents such as methanol, ethanol, and petroleum ether [1]. The volatility, toxicity and poor biodegradability of these solvents can result in considerable harm to health and environment [2]. Recently, deep eutectic solvents (DESs) have emerged as a capable alternative to classical organic solvents with the features of low toxicity, good biodegradability, low cost and adaptable properties [3]. DESs are defined as a mixture comprising a hydrogen bond acceptor (HBA) and one or more hydrogen bond donors (HBD) in an appropriate ratio. The strong hydrogen bonding interactions between HBA and HBD can result in a eutectic mixture that shows a deviation from the ideal thermodynamic behavior. These interactions form a dense molecular network in the DESs, endowing them with tunable physicochemical characteristics consisting of a low melting point, negligible volatility, high thermal stability, excellent extractability for a wide range of compounds [4]. Common examples of HBAs include choline chloride, which is widely regarded as safe and biocompatible and is extensively used in food and pharmaceutical applications [5], as well as

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Received: 03 June 2026
Accepted: 03 September 2026

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naturally occurring uncharged compounds like carboxylic acids, amines, and sugars. Regarding HBD, common examples include glycerol, propanediol, urea, citric acid, alanine, and sugars. The selection of HBA and HBD significantly affects their performance in extraction [6].

Ultrasound-assisted extraction (UAE) is considered a powerful and sustainable approach for extraction that enhances compound release to the solvent by using cavitation forces to rupture plant tissues via explosive bubble collapse [7,8]. Recently, the combination of DESs with UAE has gained considerable attention for its ability in achieving higher extraction yield with reduced energy and financial cost. Shu et al. [9] demonstrated that a ternary DES system combined with ultrasound enables efficient and eco-friendly extraction of flavonoids from *Cercis chinensis* flowers, significantly surpassing that of conventional methods; For polysaccharides from *Viburnum sargentii*, Zhao et al. [10] found that ultrasound-assisted DES extraction gave substantially higher yields and purities than that of hot water.

A. keiskei is a medicinal and edible herb widely distributed across East Asia. In daily life, its leaves are consumed as a health-promoting food, prepared either as a tea or a cooked vegetable [11]. Traditionally, it has long been utilized as a medicinal herb for its anti-hypertensive, anti-diabetic, hepatoprotective, tonic, and detoxifying effects [12]. Phytochemical investigations of the herb have focused on the identification and quantification of its bioactive constituents, with numerous studies confirming that the leaves are rich in flavonoids [13-15]. However, research on the optimization of extraction processes for these compounds remains comparatively limited. The few available studies have mainly employed conventional extraction methods such as ethanol extraction [16], ultrasound-assisted extraction with ethanol [17]. Nevertheless, no systematic optimization study has yet been reported on the ultrasonic-assisted extraction of total flavonoids from *A. keiskei* leaves using DES. Accordingly, this study seeks to establish an ultrasound-assisted DES method for the extraction of flavonoids from *A. keiskei* leaves. This work is expected to provide a green and efficient alternative to facilitate the further development of *A. keiskei* as a medicinal and edible herb.

Materials and Methods

Plant material

A. keiskei leaves collected from the Damaon herb company (Yeongcheon, Gyeongsangbuk-do, Korea) were authenticated, and a voucher specimen (AK2026) was deposited at the Herbarium of the College of Life Science and Technology, Wuhan Polytechnic University (Wuhan, China).

Chemicals and reagents

L-Ascorbic acid was obtained from Solarbio Co., Ltd. (Beijing, China). Rutin, 2,2-diphenyl-1-picrylhydrazyl (DPPH), choline chloride, L-Proline, 1,2,3-propanetriol, and 1,2-ethanediol were purchased from Aladdin Biochemical Technology Co., Ltd. (Shanghai, China). Sodium nitrite, aluminum nitrate nonahydrate, sodium hydroxide, and absolute ethanol were supplied by Macklin Biochemical Co., Ltd. (Shanghai, China).

Main instruments

A T9S UV-Vis spectrophotometer (AOELAB, China) and an EnSpire Multimode Plate Reader (PerkinElmer, Waltham, MA, USA) were used for absorbance measurements. An ultrasonic bath (20 kHz, 500 W) equipped with a standard titanium alloy probe (Beijing Jiumen Ultrasonic Technology Co., Ltd., Beijing, China) was used for extraction. A GNP-9080 water-jacketed electric incubator was used for incubation (Shanghai Jinghong Experimental Equipment Co., Ltd., Shanghai, China).

Preparation of standard solutions and standard curve

A 0.2 mg/mL rutin stock solution was prepared by dissolving precisely weighed 5.0 mg of rutin in 5 mL of absolute ethanol and subsequently diluting with 50% ethanol in a 25 mL volumetric flask. A series of 0 to 5.0 mL aliquots of the standard solution were respectively added in 10 mL volumetric flasks. After adding 0.3 mL of 5% $NaNO_2$, the mixture was allowed to react for six minutes. Subsequently, the reaction mixture was supplemented with 0.3 mL of 10% $Al(NO_3)_3$ and left for another six minutes. After that, 4 mL of 4% NaOH was introduced. Finally, each flask was diluted to a final volume of 10 mL with 50% ethanol. Each standard solution's absorbance was recorded at 510 nm after a fifteen minutes incubation. The linear regression analysis generated the calibration equation $Y = 1.4088X + 0.0653$, with an R^2 value of 0.9991, indicating excellent linearity.

Preparation of DES

Based on the molar ratios listed in Table 1, precise amounts of HBAs and HBDs were measured and

transferred into a 25 mL flask. The mixture was heated to 60 ℃ under magnetic stirring until it became homogeneous. After preparation, the DES was cooled to room temperature for subsequent use.

Table 1. Formulations of DES.

No.	HBA	HBD	Molar ratio
1	L-Proline	1,2,3-Propanetriol	1:3
2	L-Proline	1,2-Ethanediol	1:3
3	Choline chloride	1,2-Ethanediol	1:3
4	Choline chloride	1,2,3-Propanetriol	1:3
5	50% Ethanol		-

Preparation of test solutions and determination of total flavonoids

Dried *A. keiskei* leaves powder (0.100 g) was transferred to a 10 mL flask, and a preset solid-liquid ratio was achieved using either aqueous DES or ethanol. The resulting mixture was then subjected to ultrasonication in an ultrasonic bath with a rated ultrasonic power of 180 W and a frequency of 28 kHz at a defined temperature for a specified duration. After centrifugation of the mixture at 4000 rpm for five minutes, the resulting supernatant was collected by filtration using a 0.22 μm polyethersulfone membrane. The filtrate (1 mL) was subjected to sequential reactions with $NaNO_2$, $Al(NO_3)_3$, and NaOH following the earlier procedures with the absorbance read at 510 nm.

The standard curve was used to quantify the total flavonoid concentration, while the equation given below was applied to estimate the extraction yield.

$$Total\ flavonoid\ yield = \frac{X \times V \times C}{m} \times 100\%$$

X: dilution factor; V: final volume (mL); C: concentration (mg/mL), m: weight of dry leaf powder (mg).

Effect of water content on flavonoid yield

Precisely 0.100 g dried *A. keiskei* leaves powder was weighed into a 10 mL flask. DES aqueous solutions with water contents of 20%, 30%, 40%, 50%, and 60% (v/v) were added at a liquid-solid ratio of 40:1 (v/w). The flasks were sealed with plastic wrap and sonicated at 180 W, 25 ℃ for 30 min. After sonication, the mixture was filtered, and the solution was centrifuged at 4000 rpm for 5 min. A 3 mL aliquot was taken for absorbance measurement and yield calculation according to the formula above.

Effect of ultrasonic time on flavonoid yield

Exactly 0.100 g dried *A. keiskei* leaves powder was extracted with DES at a liquid-solid ratio of 40:1 (v/w). Ultrasonication was carried out at 180 W and 25 ℃ for durations of 30, 60, 70, 90, 110 min. The resulting extract was filtered and centrifuged. A 3 mL aliquot was used for absorbance measurement, and the total flavonoid yield was determined using the formula above.

Effect of liquid-solid ratio on flavonoid yield

Dried *A. keiskei* leaves powder (0.100 g) were mixed with DES at a liquid-solid ratio of 30:1, 40:1, 50:1, 60:1, and 70:1 (v/w). After sonication for 30 min at 180 W and 25 ℃, the blend was filtered and centrifuged. The resulting supernatant was analyzed for flavonoid yield as described above.

Effect of DES molar ratio on flavonoid yield

The effect of DES molar ratios (choline chloride:1,2-ethanediol, 1:2, 1:3, 1:4, 1:5, 1:6, mol/mol) was investigated by 0.100 g dried *A. keiskei* leaves powder. The extraction was conducted via sonication at 180 W and 25 ℃ for 30 min. Thereafter, the mixture was filtered, centrifuged, and analyzed as described above.

Response surface optimization for flavonoid yield

Using the single-factor results, optimization of the extraction process was carried out using a Box-Behnken design generated by Design-Expert 13.0.5 [17]. The four factors were water content (A), ultrasonic time (B), liquid-solid ratio (C), DES molar ratio (D), each at three coded levels (-1, 0, 1). The flavonoid yield (Y) was taken as the response variable (Table 2).

Table 2. Experimental factors and levels for RSM optimization.

Level	A: Water content (%)	B: Ultrasonic time (min)	C: Liquid-solid ratio (w/v)	D: DES molar ratio
-1	40	70	1:40	1:4
0	50	90	1:50	1:5
1	60	110	1:60	1:6

DPPH radical scavenging assay

After mixing 100 µL of each sample (12.5-100 µg/mL) with 100 µL of 0.1 mM ethanolic DPPH solution, the mixture was kept in the dark for 30 minutes, followed by reading the absorbance at 517 nm, where Ai represents the absorbance of the sample solution after reaction with DPPH [18]. Measurements were also taken for a blank (ethanol, Aj) and a DPPH-only control (A0). Blank (ethanol, Aj) and DPPH-only control (A0) measurements were also performed, with L-ascorbic acid used as the positive control. The following equation was employed to calculate the DPPH clearance rate.

$$DPPH = \left[1 - \frac{A_i - A_j}{A_0}\right] \times 100\%$$

Antibacterial activity

Escherichia coli was cultured on LB agar plates at 37 °C. After suspending the bacterial cells in sterile peptone saline, a 10-fold dilution was prepared and spread on agar plates [19]. Thereafter, 20 µL of each tested sample was added to sterile 6 mm paper discs laid onto the inoculated plates. Following a 24 h incubation at 37 °C, the inhibition zone diameters were determined. According to the literature [20], zones exceeding 20 mm were classified as highly active, 14–20 mm as active, 8–14 mm as fairly active, and those below 8 mm as inactive.

Data processing

All assays were carried out three times, with results reported as mean ± standard deviation (SD). For single-factor experiments, independent samples t-test was used to compare specific pairs of groups, and the results are indicated in the corresponding figures. For the RSM model analysis, one-way ANOVA in conjunction by Tukey's honestly significant difference (HSD) post-hoc test was employed, adopting a significance criterion of $p<0.05$. Data visualization and modeling were performed with GraphPad Prism 9 and Design-Expert 13.0.5.

Results and Discussion

DES selection

Four DES formulations (DES-1, DES-2, DES-3, DES-4) were preliminarily screened based on their reported effectiveness in flavonoid extraction [21]. The flavonoid extraction yield of various DESs from *A. keiskei* leaves (Figure 1) was compared under fixed conditions (180 W, 30 min, 50% v/v water content, 40 mL/g, 25 ºC). DES-3 showed the highest performance, giving a yield of 5.54 ± 0.39%. The notable superiority indicates that DES-3 is a promising extraction medium.

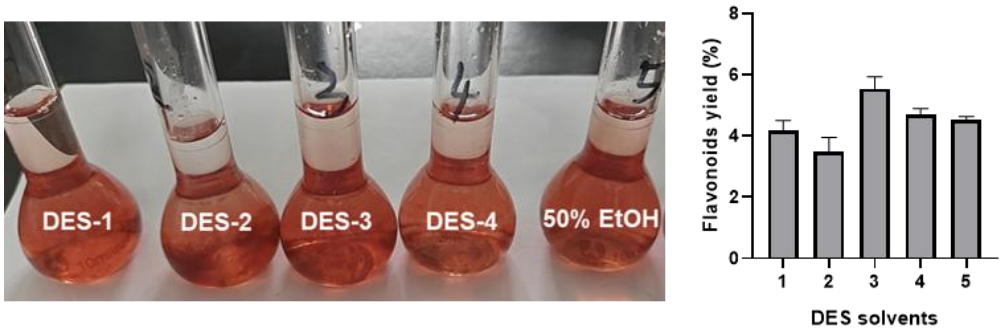


Figure 1. DES performance in flavonoid extraction in comparison with 50% ethanol extraction.

Effect of water content on total flavonoid extraction yield

According to Figure 2, the extraction efficiency of total flavonoid from *A. keiskei* leaves increased alongside the DES water content up to a threshold of 50%, beyond which it began to decline. It might be attributed to the fact that an appropriate amount of water at lower water content levels effectively reduces system viscosity, thereby decreasing mass transfer resistance and promoting flavonoid dissolution [22]. However, when the water content was further increased to 60%, the extraction yield showed a slight decrease. An independent samples t-test revealed that the difference between the 50% and 60% water content groups was not statistically significant (P = 0.106, > 0.05), suggesting that the decline beyond 50% was marginal rather than a pronounced detrimental effect within the tested range. Nonetheless, exceeding the optimal water content range causes water molecules to disrupt the hydrogen bond network of the DES system, significantly weakening its interaction with flavonoids and thus lowering the extraction yield [23]. Accordingly, a DES water content of 50% was chosen as the central value for the subsequent RSM optimization.

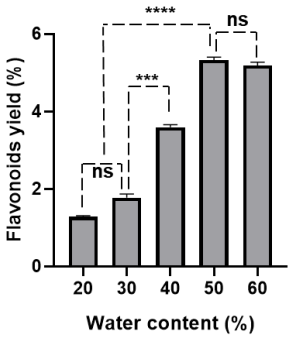


Figure 2. Effect of water content on total flavonoid extraction yield. Values are expressed as mean ± SD (n = 3). One-way ANOVA followed by Tukey's honestly significant difference (HSD) post-hoc test was used for multiple comparisons. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001; ns, not significant.

Effect of ultrasonic time on total flavonoid extraction yield

Based on Figure 3, it demonstrates that the total flavonoid yield from *A. keiskei* initially increased with prolonged ultrasonication until 90 minutes, beyond which the yield marginally declined. A brief and slight decline in extraction yield was observed between 50 and 70 minutes, which may be explained by the dissolution of readily accessible flavonoids having already reached saturation, while the deep compact tissue was not yet been adequately disrupted by ultrasound. After 90 minutes, a marginal decline in extraction yield was observed. This is presumable because that total flavonoid extraction had already reached its maximum, while prolonged ultrasonication generated considerable localized heat, promoting oxidation and structural degradation of heat-sensitive flavonoids. Taken together, the optimal ultrasonic time of 90 minutes was adopted as the central level for the RSM experiments.

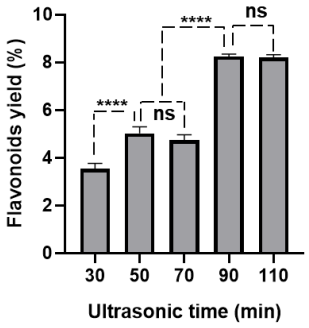


Figure 3. Effect of ultrasonic time on total flavonoid extraction yield. Values are expressed as mean ± SD (n = 3). One-way ANOVA followed by Tukey's honestly significant difference (HSD) post-hoc test was used for multiple comparisons. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001; ns, not significant.

Effect of liquid-solid ratio on total flavonoid extraction yield

The liquid-solid ratio represents a significant variable during extraction, as it significantly affects both the extraction yield and the material cost of the process. It reveals that the extraction yield of total flavonoids from A. keiskei increased with increasing liquid-solid ratio from 30:1 to 50:1, reaching a maximum at 50:1, beyond which the yield showed slight fluctuations (Figure 4). A temporary decline was observed at 60:1, followed by a partial recovery at 70:1. This phenomenon may be attributed to the competing effects of two opposing factors: on one hand, increasing solvent volume enhances the concentration gradient and facilitates flavonoid dissolution; on the other hand, excessive solvent may result in the co-extraction of various impurities, which could compete with flavonoids for the solvent or alter solvent properties [24,25], thereby reducing the solubility of flavonoids, leading to a temporary decline at 60:1. When the ratio was further increased to 70:1, the dominant driving force of mass transfer outweighed the interference, resulting in a partial recovery of the yield. Considering the extraction efficiency, reagent cost, and stability, the optimal liquid-solid ratio under single-factor conditions was determined to be 50:1.

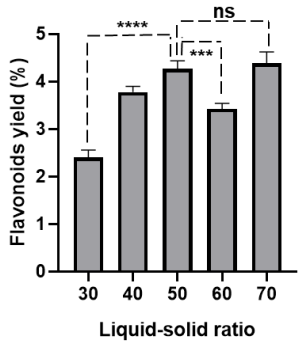


Figure 4. Effect of liquid-solid ratio on total flavonoid extraction yield. Values are expressed as mean ± SD (n = 3). One-way ANOVA followed by Tukey's honestly significant difference (HSD) post-hoc test was used for multiple comparisons. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001; ns, not significant.

Effect of DES molar ratio on total flavonoid extraction yield

As shown in Figure 5, DES molar ratio significantly influenced total flavonoids extraction efficiency. Following an increment in DES molar ratio, the extraction yield generally increased initially and then decreased. Peak of total flavonoid extraction rate occurred at a 1:5 molar ratio of 1,2-ethanediol to choline chloride. This trend can be attributed to the fact that the proportion of HBD to HBA directly determines the system viscosity, intermolecular interaction strength, and hydrogen bond stability, which in turn significantly affects mass transfer capacity and governs the extraction capability of the DES system for total flavonoids [26]. When the DES molar ratio reached 1:6, an excessively high molar ratio disrupted the hydrogen bond network and led to excessive viscosity, which weakens the mass transfer capacity of the system and induces site competition, consequently reducing the extraction capacity of the DES system for flavonoids. Therefore, the central level for RSM experiment was defined as a DES molar ratio of 1:5.

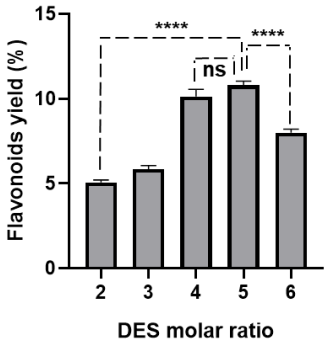


Figure 5. Effect of DES molar ratio on total flavonoid extraction yield. Values are expressed as mean ± SD (n = 3). One-way ANOVA followed by Tukey's honestly significant difference (HSD) post-hoc test was used for multiple comparisons. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001; ns, not significant.

Response surface analysis

The results derived from the response surface methodology experiments and their corresponding analysis are presented in Tables 3 and 4. Using the software Design-Expert 13.0.5, a variance analysis of the regression model was performed on the dataset, Table 4 displays the regression fitting results for each factor, generating the following Eq.1 in terms of actual factors:

$$Y= 26.4486A + 9.10692B + 24.4067C + 266.367D + 0.018625AB + 0.0047AC -1.2415AD - 0.00875BC - 0.5215BD - 1.0695CD - 0.217975A^2 - 0.0373875B2 - 0.178225C^2 - 10.54D^2 - 2257.85 \quad \text{Eq.1}$$

According to Table 4, the model shows statistical significance, indicated by a P-value below 0.01 and R^2 value of 0.9470, reflecting an adequate fit of the regression model. Based on the P-values, the quadratic terms A^2, B^2, C^2, and D^2 exerted highly significant effects on the response ($P < 0.01$), while the linear term D showed a significant effect ($P < 0.05$).

Based on the F-values obtained from the single-factor analysis, the order of influence of the four factors on the extraction yield of total flavonoids from *A. keiskei* leaves is ranked as follows: C (liquid- solid ratio) > B (ultrasonic time) > A (water content) > D (DES molar ratio) (Table 4). Among these, water content, ultrasonic time, and liquid-solid ratio all showed significant effects on the extraction yield ($P < 0.05$), whereas the effect of DES molar ratio was not significant ($P > 0.05$), indicating that the molar ratio alone does not significantly affect the extraction yield. However, the interaction terms between the DES molar ratio and each of the other three factors all reached extremely significant levels ($P < 0.01$) (Figure 6), demonstrating that the DES molar ratio exhibits a clear interactive effect with the other factors.

Hence, the DES molar ratio influences the total flavonoid yield from *A. keiskei* by influencing factors such as the viscosity of the DES and the strength of intermolecular interactions. This model can be applied to analyze the extraction yield and to predict the experimental results under the optimal processing parameters.

Table 3. Results of Box-Behnken experiment.

Group	A: Water content (%)	B: Ultrasonic time (min)	C: Liquid-solid ratio (w/v)	D: DES molar ratio	Extraction rate of total flavonoids (%)
1	40	110	50	5	8.54
2	40	90	50	4	7.80
3	60	90	50	6	7.94
4	40	90	60	5	8.35
5	50	90	40	6	9.56
6	60	90	50	4	10.61
7	40	70	50	5	8.22
8	50	90	50	5	10.81
9	50	90	50	5	12.67
10	50	90	50	5	12.49
11	50	90	60	6	8.97
12	50	110	40	4	11.07
13	40	90	50	6	10.09
14	50	110	60	5	10.32
15	40	90	40	5	7.09
16	50	70	60	5	9.01
17	50	70	50	4	8.54
18	50	110	50	6	8.80
19	50	70	50	6	10.45
20	50	90	50	5	12.78
21	50	70	40	5	7.47
22	50	110	40	5	9.47
23	60	90	60	5	9.61
24	60	70	50	5	8.12
25	50	90	40	4	7.97
26	50	90	60	4	11.66
27	60	110	50	5	9.93

28	50	90	50	5	12.89
29	60	90	40	5	8.16

Table 4. Analysis of variance.

Source of variance	Sum of squares	df	Mean square	*F* value	*P* value
Model	7577.73	14	541.27	17.86	＜0.0001
A	151.09	1	151.09	4.99	0.0424
B	333.06	1	333.06	10.99	0.0051
C	561.70	1	561.70	18.54	0.0007
D	27.66	1	27.66	0.9131	0.3555
AB	55.50	1	55.50	1.83	0.1974
AC	0.8836	1	0.8836	0.0292	0.8668
AD	616.53	1	616.53	20.35	0.0005
BC	12.25	1	12.25	0.4043	0.5351
BD	435.14	1	435.14	14.36	0.002
CD	457.53	1	457.53	15.10	0.0016
A^2	3081.93	1	3081.93	101.72	＜0.0001
B^2	1450.72	1	1450.72	47.88	＜0.0001
C^2	2060.38	1	2060.38	68.00	＜0.0001
D^2	720.59	1	720.59	23.78	0.0002
Residual	424.17	14	30.30		
Lack of fit	128.96	10	12.90	0.1747	0.9884
Pure error	295.21	4	73.80		
Cor total	8001.89	28			

Note: P＜0.001 denotes extremely significant difference, P＜0.05 denotes significant difference.

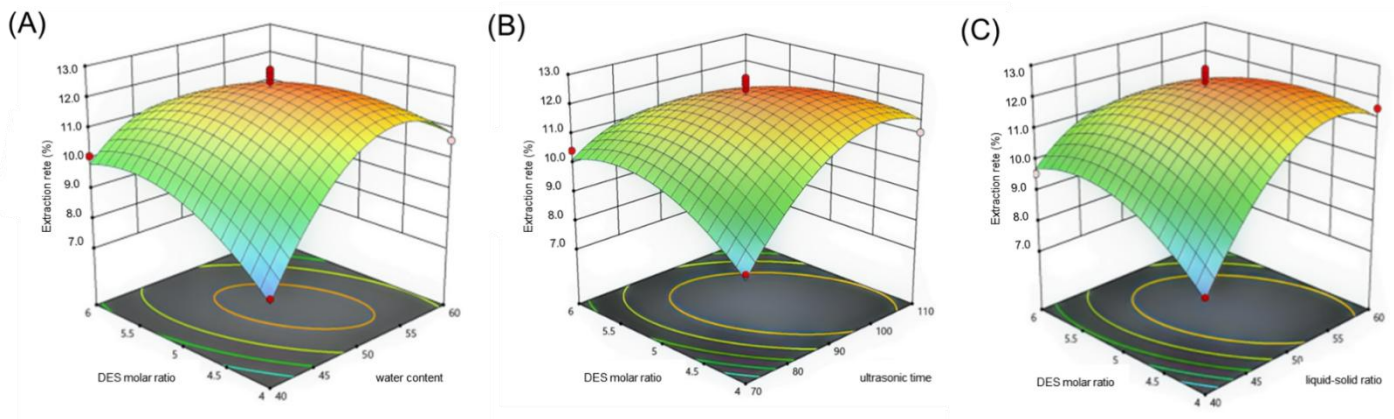


Figure 6. Response surface graphs illustrating the interactions of other factors with molar ratio on total flavonoid extraction rate; (A) Interaction between DES molar ratio and water content; (B) Interaction between DES molar ratio and ultrasonic time; (C) Interaction between DES molar ratio and liquid-solid ratio.

Optimization of extraction conditions and model verification

Using Design-Expert 13.0.5 software, the experimental data was applied for optimization and prediction. The optimal conditions for extracting total flavonoids from *A. keiskei* were identified as 53.0529% DES water content, 98.3493 min ultrasonication, 53.6953 mL/g liquid-solid ratio, and a 4.3546:1 molar ratio of 1,2-ethanediol to choline chloride, under which the predicted flavonoid yield was 12.67%. To ensure practical feasibility, the operational conditions were rounded to 50% DES water content, 100 min ultrasonic treatment, 50 mL/g liquid-solid ratio, and a 4:1 molar ratio. Under these adjusted conditions, the experimental yield was 13.02% ± 0.15% (n=3), surpassing the predicted value by 0.35% ± 0.15%, which substantiates the reliability and efficacy of the model for total flavonoid extraction from *A. keiskei*. The optimized extraction yield (13.02% ± 0.15%) obtained in this study compares favorably with

previously reported values for *A. keiskei* flavonoid extraction. For instance, Zhang et al. [17] reported an optimized yield of 8.79% using ultrasound-assisted ethanol extraction, while combined ultrasound-mircowave-assisted extraction method achieved a total flavonoid content of 4.17% [27]. These comparisons clearly demonstrate the superiority of the DES-based ultrasonic extraction method developed in this work. These findings collectively demonstrate that UAE-DES is an effective and mild method for extracting flavonoids, making it a promising technique for the sustainable utilization of *A. keiskei* leaves in functional food and nutraceutical applications.

Radical scavenging activity of *A. keiskei* leaves flavonoids against DPPH·

As depicted in Figure 7, the flavonoids showed a concentration-dependent increase in free radical scavenging activity, indicating strong DPPH· radical scavenging effects. Flavonoid concentration and scavenging yield showed a positive correlation. The DES extract demonstrated potent scavenging even at low concentrations, with an IC_{50} of 31.33 ± 1.35 µg/mL. The strong DPPH· radical scavenging effects of the *A. keiskei* flavonoid extract can be explained by the structural features of its major flavonoid constituents, particularly chalcones and flavanones, which are known to be abundant in this plant [28,29]. These compounds possess phenolic hydroxyl groups that can act as efficient electron or hydrogen donors to neutralize DPPH· radicals and potentially reducing oxidative stress [30].

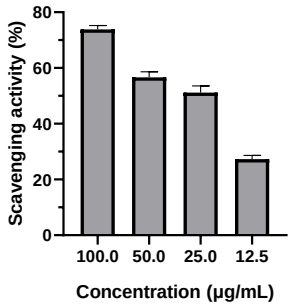


Figure 7. Scavenging activity on DPPH· by *A. keiskei* leaves flavonoids.

Antibacterial activity of *A. keiskei* leaves flavonoids

As shown in Figure 8 and Table 5, the total flavonoids extracted from *A. keiskei* leaves exhibited antibacterial effects against *Escherichia coli*. The minimum inhibitory concentration (MIC) was 1.15 mg/mL. At a concentration of 2.2 mg/mL, the inhibition zone diameter was 12.33 ± 0.58 mm, which was comparable to that of rutin. Additionally, flavonoid concentration correlated positively with antibacterial activity as evidenced by the increase in inhibition zone diameter with rising concentration. These findings are consistent with earlier reports on plant-derived flavonoids, which have been shown to exert antimicrobial effects through mechanisms such as disrupting bacterial cell membranes, inhibiting protein synthesis, or interfering with DNA replication [31]. Flavonoids such as xanthohumol and related chalcones identified from *A. keiskei* are known for their antimicrobial effects by multiple mechanisms. The membrane-targeting ability of these compounds enables them to bind to phospholipids in bacterial membranes, causing increased permeability and leakage of intracellular contents [32]. Additionally, they may exert antibacterial activity by chelating metal ions essential for bacterial metabolism or by interfering with key enzymes such as DNA gyrase [33,34]. All these indicate that the *A. keiskei* flavonoid extract obtained by UAE-DES has promising potential as a natural antimicrobial ingredient.

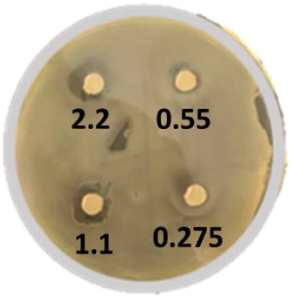


Figure 8. Inhibition zone of *A. keiskei* leaves flavonoids against *Escherichia coli*.

Table 5. Antibacterial effect of *A. keiskei* leaves flavonoids on *Escherichia coli*.

Tested sample	Concentration (mg/mL)	Antibacterial diameter (mm)	Sensitivity
Total flavonoids from *A. keiskei*	2.2	12.33 ± 0.58	+
	1.1	9.33 ± 0.58	+
	0.55	7.00 ± 0.00	-
	0.275	6.33 ± 0.58	-
Rutin	5	13.33 ± 0.58	+
	2.5	12.67 ± 0.58	+
	1.25	8.00 ± 0.00	-
	0.625	7.00 ± 0.00	

Note: "-" indicated insensitivity; "+" indicates moderate sensitivity.

Conclusions

Angelica keiskei, an important edible and medicinal **herb**, has potential applications in diverse fields, for instance food, cosmetics, and pharmaceuticals, possessing significant industrial value. In recent years, with the deepening research on the biological activities of *A. keiskei*, how to extract flavonoids from *A. keiskei* in a green and efficient manner has gradually become a research hotspot. Deep eutectic solvents (DES), as novel green solvents, have drawn considerable attention in the field of natural product extraction. However, their use for extracting total flavonoids from *A. keiskei* remains unexplored. Therefore, in this study, *A. keiskei* leaves were selected as the raw material. Systematic investigation of main parameters on total flavonoid yield was conducted via single-factor experiments and RSM. Optimal extraction was achieved with 50% DES water content, 100 min ultrasonic treatment, a 50 mL/g liquid-solid ratio, and a 4:1 molar ratio of 1,2-ethanediol to choline chloride, yielding a total flavonoid content of 13.02% ± 0.15%. Previous studies on the extraction of *A. keiskei* using traditional methods have reported flavonoid yields ranging from 4.17 to 8.79% [17,27]. These results clearly demonstrate the superiority of the DES-based ultrasonic extraction method developed in this work. The good agreement between experimental results and model predictions confirmed that the RSM model is highly reliable, offering a methodological basis for the industrial-scale extraction of *A. keiskei* flavonoids. Furthermore, the flavonoid extract was tested for antioxidant and antibacterial activities. It demonstrated strong in vitro antioxidant activity, with a DPPH radical scavenging IC_{50} value of 31.33 ± 1.35 μg/mL, and showed moderate antibacterial activity against E. coli, producing inhibition zones over 12 mm at 2.2 mg/mL. Thus, the total flavonoids extracted from *A. keiskei* have potential to be used as health-related products or functional food for further application. Future work will focus on elucidating the underlying mechanisms responsible for the antioxidant and antibacterial activities, as well as identifying the specific flavonoid profiles in the optimized extract using advanced analytical techniques such as UPLC-MS/MS to establish structure-activity relationships.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

Acknowledgment

This study was supported by the Research and Innovation Initiatives (2024Y16) and the Research Funding of Wuhan Polytechnic University (2024RZ027). This research was also funded by Liaocheng University (grant number 318052337). In addition, Yuchen Zhu, Anbo Liu, and Jianghao Zhou have contributed equally to this manuscript.

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