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Ultrasound-assisted deep eutectic solvent extraction of flavonoids from Arundina graminifolia: Process optimization, extraction kinetics, and molecular interactions

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Ultrasound-assisted deep eutectic solvent extraction of flavonoids from *Arundina graminifolia*: Process optimization, extraction kinetics, and molecular interactions

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Abstract

This study investigated ultrasound-assisted extraction of flavonoids from *Arundina graminifolia* using deep eutectic solvents (DESs). The study focused on process optimization, extraction kinetics, and possible molecular interactions. The contribution of ultrasound was also examined by comparing extraction yields with and without ultrasonic treatment using DES15 and 80% ethanol. The extraction process and antioxidant activity of the extract were also studied. Sixteen DESs were screened, and single-factor experiments and response surface methodology

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(RSM) were used for process optimization. L-proline–acetic acid (1:2, molar ratio) showed the best extraction performance. The optimized conditions included 14.5% water, a liquid-to-solid ratio of 56mL/g, and an ultrasonic temperature of 66 °C. The total flavonoid yield reached 25.738mg/g, which was 1.693 times that obtained with 80% ethanol. The comparison of ultrasonic and non-ultrasonic extraction showed a more pronounced increase in flavonoid yield with DES15 than with 80% ethanol under the tested conditions. The RSM model was highly significant ($P<0.0001$), and the lack-of-fit was not significant ($P=0.3271$). Scanning electron microscopy, Fourier transform infrared spectroscopy, extraction kinetics, and density functional theory calculations were used to study the extraction mechanism. The apparent activation energy was 10.50kJ/mol. UHPLC-QTOF-MS tentatively identified 27 flavonoid compounds in the DES extract. The DES extract showed stronger DPPH radical-scavenging activity than the 80% ethanol extract, with IC_{50} values of 0.4761 and 0.9624mg/mL, respectively. The AGREE score was 0.51. These findings show that extraction performance depends on both solvent composition and extraction conditions. The results also highlight the importance of evaluating ultrasound-assisted extraction against non-ultrasonic controls. Further work is needed to clarify the contribution of cavitation and to improve solvent recovery and energy efficiency.

Keywords: *Arundina graminifolia*; Deep eutectic solvents; Ultrasound-assisted extraction;density functional theory calculations; Green chemistry

1. Introduction

Arundina graminifolia is a medicinal species from the Orchidaceae family and has a long history of use in traditional Dai medicine. Previous studies have identified several types of natural compounds in this plant, such as flavonoids, stilbenes, and phenolic substances[1]. Flavonoids

have attracted considerable attention due to their potential biological activities. Effective extraction of these compounds is essential for further research and practical applications of *A. graminifolia*. However, the effects of different solvent systems and extraction conditions on flavonoid recovery remain unclear. Further studies are therefore needed to establish suitable extraction methods and improve the use of flavonoid compounds from this medicinal plant. Flavonoids are a major group of naturally occurring compounds found in many plant species. They exhibit a range of biological properties, particularly antioxidant activity, and show promise for use in functional foods, natural products, and cosmetic formulations[2]. Developing an efficient approach to recover total flavonoids from A. graminifolia could support the better use of this medicinal plant. In addition, further assessment of the biological activities of the obtained extracts is necessary to explore their potential applications.

Conventional organic solvents are widely applied to recover total flavonoids from *A. graminifolia*. However, their use may lead to harmful solvent residues, environmental concerns, and limited extraction yields. These drawbacks can restrict the application of the resulting extracts in food and cosmetic products [3]. Deep eutectic solvents (DESs) have emerged as a possible alternative to conventional solvents. These solvents are generally prepared by combining a hydrogen-bond acceptor with a hydrogen-bond donor at a specific molar ratio. The interactions between these components reduce the melting temperature of the resulting mixture[4, 5]. Earlier research has reported that DESs can increase flavonoid solubility and promote their extraction. Hydrogen-bond interactions between DES components and flavonoid molecules may contribute to these improvements[4]. Nevertheless, the mechanisms responsible for these effects remain unclear. Further research is also required to understand how extraction parameters influence flavonoid

yield and the biological properties of the extracts[6].

Ultrasound-assisted extraction has been widely used to improve the recovery of bioactive compounds from plant materials. Acoustic cavitation can generate local shear forces, microjets, and fluid motion, which may improve solvent penetration and mass transfer. However, the effectiveness of ultrasound may depend on the properties of the extraction medium. In DES-based systems, solvent composition may influence both the dissolution of target compounds and the response of the plant matrix to ultrasonic treatment. Therefore, comparing extraction with and without ultrasound is important for distinguishing the contribution of ultrasonic treatment from the effect of solvent selection[7].

Despite growing interest in DES-based extraction, the reasons for the varying efficiencies of different DES formulations are not yet fully clear. In particular, the role of DES composition in flavonoid recovery from *A. graminifoli*a requires further study. The relationships among solvent properties, changes in plant tissues, and interactions at the molecular level also need to be explored. This study investigated DES-based ultrasound-assisted extraction of flavonoids from *A. graminifolia*, using total flavonoid yield as the primary indicator of extraction performance. A direct comparison between ultrasonic and non-ultrasonic extraction was conducted using DES15 and 80% ethanol to evaluate the effect of ultrasound in different solvent systems. The physical and chemical properties of selected DES formulations were investigated to help explain differences in their extraction abilities. The extraction conditions were improved by screening different DESs, conducting single-factor tests, and applying response surface methodology. The flavonoid constituents of the extract obtained under the optimal conditions were characterized using UHPLC-QTOF-MS. To better understand the extraction process, scanning electron microscopy

(SEM), kinetic modeling, Fourier transform infrared spectroscopy (FT-IR), and density functional theory (DFT) calculations were employed. These approaches were used to examine changes in plant tissue morphology, extraction kinetics, and possible molecular interactions. Together with the comparison of ultrasonic and non-ultrasonic extraction, they provided complementary evidence for interpreting the observed extraction performance. The antioxidant capacity of the optimized extract was assessed using the DPPH radical-scavenging assay and compared with an extract prepared using 80% ethanol. The environmental performance of the extraction procedure was also assessed. Overall, this research helps explain the behavior of DESs during flavonoid extraction and provides useful guidance for developing more effective extraction methods for *A. graminifolia*.

2. Materials and methods

2.1. Materials

The *A. graminifolia* (D. Don) Hochr. samples were collected from cultivated plants at the Xishuangbanna Southern Medicinal Plant Garden in Jinghong, Yunnan, China, on September 10, 2021. Guang Li collected the samples, and Lixia Zhang identified the plant species. A voucher specimen (No. YNFS-LG-2021003; barcode IMDY 0052737) was stored at the Herbarium of the Yunnan Branch, Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences (IMDY). The plant materials were dried, ground, passed through a 60-mesh sieve, and stored at 4°C until use. All DES components, antioxidant assay reagents, and other extraction reagents were of analytical grade. Shanghai Titan Scientific Co., Ltd. supplied these reagents.

2.2. Analytical Instrument

The analytical instruments used in this study included a Velocity 18R refrigerated centrifuge

(Dynamica, Singapore); an XM-P30H ultrasonic cleaner (Xiaomei Ultrasonic Instrument (Kunshan) Co., Ltd., China); a DF-101S heat-collecting, constant-temperature magnetic stirrer (Gongyi Yuhua Instrument Co., Ltd., China); a SpectraMax i3 multi-mode microplate reader (Molecular Devices, USA); an S-4800 cold field-emission high-resolution scanning electron microscope (Hitachi, Japan); and a Fourier transform infrared (FT-IR) spectrometer (Nicolet iS50, Thermo Fisher Scientific, USA).A Nexera UHPLC system (Shimadzu, Japan) coupled with an X500B quadrupole time-of-flight mass spectrometer (SCIEX, USA). a DDS-307 conductivity meter (REX, China); a PHS-3Cb pH meter (Shanghai Yueping, China); a DVNXHBCBG viscometer (Brookfield, USA); and a TH2830 digital LCR meter (Tonghui Electronic, China).

2.3. Experimental Methods

2.3.1. Preparation and Screening of DES

A rutin standard solution was diluted with 80% ethanol to obtain standard solutions at 20, 40, 60, 80, and 100 μg/mL. The total flavonoid content was measured by the sodium nitrite–aluminum nitrate colorimetric method[8]. A standard calibration curve was then prepared.

The DESs were prepared according to a reported method with slight changes[9]. The hydrogen-bond acceptor (HBA) and hydrogen-bond donor (HBD) were weighed according to the molar ratios listed in Table 1. The components were placed in a 100 mL beaker. The initial water content was set at 20%. The mixture was heated in a 70°C water bath with magnetic stirring until a clear solution formed. After the mixture cooled to room temperature, water was added to reach the required water content. The mixture was stirred again until a clear and uniform solution was obtained. For extraction, 0.5g of powder was placed in a 50mL centrifuge tube. The selected DES was then added according to the composition and molar ratio in Table 1. The initial conditions

were a liquid-to-solid ratio of 30ml/g, an ultrasonic temperature of 40°C, and an extraction time of 40min. The mixture was first soaked for 1h and then treated by ultrasound. The extract was filtered and centrifuged at 10,000rpm for 10min. The supernatant was collected as the sample solution. The control group used 80% aqueous ethanol as the extraction solvent. The other steps were kept the same. The total flavonoid content was measured by the sodium nitrite–aluminum nitrate colorimetric method. The sample solutions were diluted ten times with 80% ethanol. The absorbance was measured at 510nm. The absorbance of the corresponding blank DES or 80% ethanol was subtracted to remove background interference[8]. The prepared DESs showed good stability. The solutions remained clear after two months of storage.

Table 1 Compositions of DES

DES	Abbreviation	HBA	HBD	Molar ratio
DES1	ChCl:EG	Choline chloride	Ethylene Glycol	1:2
DES2	ChCl:AcOH:EG	Choline chloride	Acetic Acid、Ethylene Glycol	1:2:1
DES3	ChCl:Gly	Choline chloride	Glycerol	1:2
DES4	ChCl:Laa	Choline chloride	Lactic Acid	1:2
DES5	ChCl:Ure	Choline chloride	Urea	1:2
DES6	ChCl:AcOH	Choline chloride	Acetic Acid	1:2
DES7	ChCl:Cia	Choline chloride	Citric Acid	1:1
DES8	Bet:EG	Betaine	Ethylene Glycol	1:2
DES9	Bet:AcOH:Eg	Betaine	Acetic Acid、Ethylene Glycol	1:3:1
DES10	Bet:Gly	Betaine	Glycerol	1:2

DES11	Bet:Laa	Betaine	Lactic Acid	1:3
DES12	Bet:Ure	Betaine	Urea	1:2
DES13	Bet:AcOH	Betaine	Acetic Acid	1:3
DES14	L-Pro:EG	L-Proline	Ethylene Glycol	1:2
DES15	L-Pro:AcOH	L-Proline	Acetic Acid	1:2
DES16	L-Pro:AcOH:EG	L-Proline	Acetic Acid、Ethylene Glycol	1:2:1

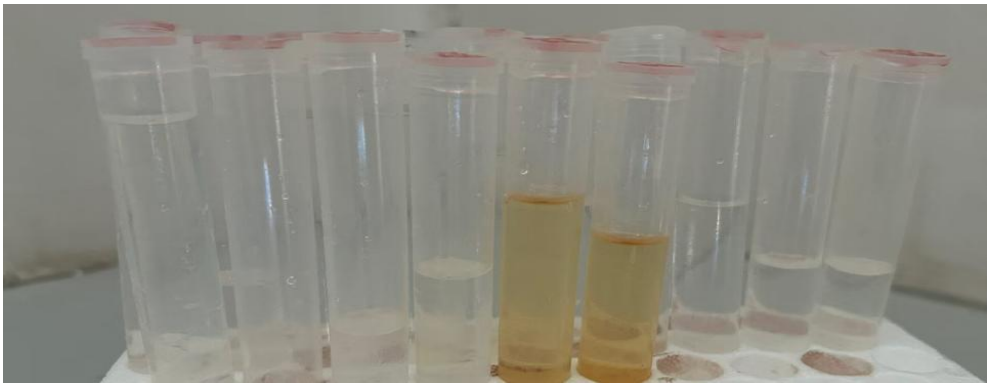


Figure 1 16 types of DES solvents

2.3.2. Physicochemical characterization of representative DESs

Seven representative DESs (DES1, DES3, DES5, DES7, DES9, DES15, and DES16) were selected based on the initial solvent screening. The viscosity, electrical conductivity, apparent pH, density, and relative permittivity of each DES were measured to describe its physicochemical properties. The measurements were carried out on the prepared DES solutions. The results were summarized for each solvent and compared with the total flavonoid yields obtained under the initial screening conditions. Pearson correlation coefficients (r) and corresponding p values were calculated to examine the relationships between each physicochemical property and extraction yield. Statistical significance was assessed at $p < 0.05$. These results show that DES composition

affects flavonoid extraction. However, the reasons for the differences among DESs remain unclear. Therefore, the physicochemical properties of selected DESs were further examined.

2.3.3. Optimization of Flavonoid Extraction Using DES

Precisely 0.5 g of powder was placed in a 50 mL centrifuge tube. Ultrasound-assisted extraction was carried out under different conditions. The tested water contents were 10%, 15%, 20%, 30%, and 40%. The tested liquid-to-solid ratios were 10, 20, 30, 40, 50, and 60mL/g. The ultrasonic temperatures were 30, 40, 50, 60, and 70°C. The extraction times were 20, 30, 40, 50, and 60min. After sonication, the samples were centrifuged and filtered. The supernatants were collected for total flavonoid determination.

2.3.4. Response surface optimization experiment

The single-factor results were used to select the variables for response surface analysis. A three-factor, three-level Box–Behnken design was established with Design-Expert 13.0. Water content, liquid-to-solid ratio, and ultrasonic temperature were selected as the independent variables. Total flavonoid content was used as the response value[10]. Response surface methodology was then used to obtain the best combination of extraction conditions. Validation experiments were performed under the optimized conditions.

2.3.5. Comparison of ultrasonic and non-ultrasonic extraction

Four experimental groups were established to evaluate the effect of ultrasound on flavonoid extraction[11]. DES15 and 80% ethanol were used as extraction solvents. Each solvent was tested with and without ultrasonic treatment. For each experiment, 0.5g of *A. graminifolia* powder was mixed with the extraction solvent at a liquid-to-solid ratio of 56 mL/g. The extraction temperature was 66°C, and the extraction time was 40min. The DES15 water content was 14.5%. The

non-ultrasonic groups were extracted in a water bath at the same temperature. All samples were pre-soaked for 1h before extraction. The extracts were centrifuged, and the total flavonoid yields were determined. Each experiment was performed in triplicate.

2.3.6. Study on extraction kinetics

The effects of extraction temperature and time were further examined under the optimal conditions obtained from the response surface analysis. The experiment used 0.5 g of dried *A. graminifolia* powder in a 50 mL centrifuge tube. DES15 was added at a liquid-to-solid ratio of 56 mL/g. Ultrasound-assisted extraction was performed at 30, 40, 50, 60, and 70°C. The total extraction period was 80 min. Samples were collected every 10 min at 10, 20, 30, 40, 50, 60, 70, and 80 min. After extraction, the samples were cooled to room temperature and centrifuged. The supernatants were collected for total flavonoid analysis. Each experiment was performed in triplicate.

The experimental data were fitted with a pseudo-first-order kinetic model. The model was described by the following equation:

$$Ct = Ce\ （1 - e^{-k1t}）$$

where Ct is the total flavonoid yield (mg/g) at extraction time t (min), Ce is the equilibrium total flavonoid yield (mg/g) estimated by nonlinear fitting, and k1 is the pseudo-first-order extraction rate constant (min^{-1}).

2.3.7. SEM observation

SEM was used to examine changes in the plant residue after different extraction treatments. The images were collected from randomly selected areas at magnifications of 2000× and 5000×. The accelerating voltage was 5.0 kV. The extraction effects were evaluated by comparing surface

pores, cracks, and structural loosening. These observations provided morphological information on the extraction process[9].

2.3.8. FT-IR Analysis

Attenuated total reflection Fourier transform infrared (ATR-FTIR) spectroscopy was used to study the intermolecular interactions involved in DES15 formation. The spectra of L-Pro, AcOH, and DES15 were recorded from 4000 to 400 cm^{-1}. The resolution was 4 cm^{-1}, and 32 scans were collected for each sample. Air was used as the background. Changes in the position and shape of the characteristic O–H and C=O stretching peaks were examined to evaluate possible hydrogen-bond interactions in DES15[12].

2.3.9. Computational analysis of molecular interactions

Density functional theory calculations were performed to study the interaction between DESs and flavonoids from *A. graminifolia*. Quercetin was selected as a representative flavonoid. The quercetin molecule was paired separately with the DES showing the highest extraction efficiency and the DES showing the lowest extraction efficiency[13]. The resulting DES–flavonoid systems were used for molecular interaction analysis. For each molecular system, 500 initial configurations were generated using the genmer module in Molclus. These configurations were first optimized using the GFN2-xTB semiempirical method implemented in xtb. The optimized structures were then ranked according to their calculated energies. The ten lowest-energy configurations were selected for further geometry optimization using Gaussian 16. The DFT calculations were performed at the B3LYP/6-31G* level with D3(BJ) dispersion correction. The final low-energy configurations were selected based on their DFT energies and used for subsequent molecular interaction analysis.

2.3.10. Identification of flavonoids in DES15 extract

The flavonoid profile of the DES15 extract was analyzed using a Nexera UHPLC system (Shimadzu, Japan) coupled with an X500B quadrupole time-of-flight mass spectrometer (SCIEX, USA). Chromatographic separation was performed on an ACQUITY HSS T3 column (2.1×100 mm, 1.8μm) at 40°C. The flow rate was 0.3 mL/min, and the injection volume was 5μL. Mobile phase A consisted of water containing 0.1% formic acid, while mobile phase B consisted of acetonitrile containing 0.1% formic acid. The gradient program was set as follows: 0–2min, 5%B; 2–6min, 5–30%B; 6–7min, 30%B; 7–10min, 30–60%B; 10–11.5min, 60%B; 11.5–13min, 60–80%B; 13–13.5min, 80%B; 13.5–16min, 80–90%B; 16–17min, 90%B; 17–17.5min, 90–100%B; 17.5–20min, 100%B; 20–20.5min, 100–5%B; and 20.5–23min, 5%B. Mass spectrometry analysis was performed using both positive and negative electrospray ionization modes. The flavonoid compounds were provisionally characterized by matching their accurate precursor ion masses and MS/MS fragment patterns against spectral records from the SpectraVerse and MoNA databases. These proposed identifications were not verified using authentic reference standards.

2.3.11. Determination of antioxidant activity

The DPPH radical-scavenging assay was used to determine the antioxidant capacity of the DES15 extract. The experiment compared four groups: the DES15 extract, an extract prepared with 80% ethanol, ascorbic acid as the positive control, and a blank control.

The DPPH radical-scavenging rate was calculated using the following formula:

$$DPPH\ 清除率（\%）=[A0-（A1-A2）]/A0*100\%$$

where A_0 is the absorbance of the blank tube, A_1 is the absorbance of the sample test tube, and A_2 is the absorbance of the sample control tube.

2.3.12. Green Assessment

The environmental performance of the DES-based ultrasound-assisted extraction procedure was assessed using the Analytical GREEnness metric (AGREE) and Green Analytical Procedure Index (GAPI)[14]. The assessment included all analytical steps, from sample preparation and DES extraction to color development and visible spectrophotometric measurement.

The AGREE evaluation included several experimental parameters, such as sample weight (0.5g), DES consumption (28mL), ultrasound power (800W), extraction duration (40min), energy consumption (0.533kWh/sample), sample throughput (3samples/h), and waste production (15mL/sample). The evaluation also accounted for the quantities of $NaNO_2$, $Al(NO_3)_3$, and NaOH required for the colorimetric reaction. Both AGREE and ComplexGAPI were applied to provide a visual overview of the analytical procedure, with a focus on sample processing, chemical consumption, energy requirements, waste production, and waste management.

3. Results and Discussion

3.1. Standard curve

The calibration curve was established by plotting rutin concentration against the corresponding absorbance values. Linear regression gave the equation $y = 0.0028x - 0.0106$, with a coefficient of determination (R^2) of 0.9982. This result showed a strong linear relationship within the examined concentration range. All subsequent measurements of total flavonoids were reported as rutin equivalents. The flavonoid yield calculated per unit weight of plant material was then used to assess and compare the efficiency of different extraction conditions.

3.2. Screening of DES

Sixteen DESs with different compositions were tested under the initial conditions. The water

content was 20%. The liquid-to-solid ratio was 30mL/g. The ultrasonic temperature was 40°C, and the extraction time was 40min. The results are shown in Figure 2.

The tested DES formulations exhibited noticeable variations in their ability to recover flavonoids. These results indicate that solvent composition may have a considerable influence on extraction efficiency, although the factors responsible for these differences remain unclear. Among all the tested systems, DES15, consisting of L-Pro and AcOH at a molar ratio of 1:2, achieved the highest flavonoid yield of 21.40 mg/g. This result was greater than the 15.20 mg/g obtained with 80% ethanol. Based on these findings, DES15 was chosen for subsequent single-factor experiments and response surface optimization. The differences in extraction performance may be linked to solvent properties, including polarity, viscosity, and interactions with flavonoid molecules. L-Pro contains functional groups that can act as hydrogen-bond donors and acceptors. These groups may compete with the hydrogen bonds between flavonoid molecules, thereby reducing their aggregation and improving their dissolution in the extraction medium[15]. Meanwhile, AcOH may influence the viscosity of the DES system and facilitate mass transfer. Its carboxyl group may also interact with the phenolic hydroxyl groups of flavonoids through hydrogen bonding. Such interactions could further enhance flavonoid solubility and contribute to improved extraction efficiency[16].However, these proposed mechanisms require further support from experimental observations and molecular-level analysis.

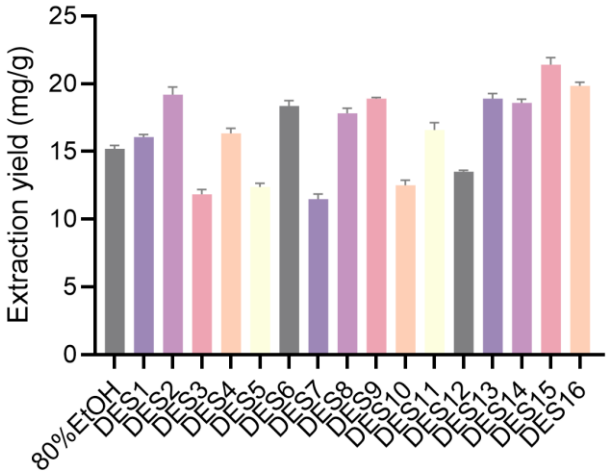


Figure 2 Effect of different DES and 80% aqueous ethanol on the yield of total flavonoids from *A. graminifolia*.

3.3. Physicochemical properties of representative DESs

Seven representative DES formulations were chosen to investigate the relationship between solvent characteristics and flavonoid recovery. The analysis focused on five physicochemical parameters: viscosity, electrical conductivity, apparent pH, density, and relative permittivity. The measured values are summarized in Table 2. Considerable variations were observed among these solvents, possibly due to differences in their components and molar proportions. DES7 (ChCl:Cia, 1:1) exhibited the greatest viscosity of 243.10mPa • s and the lowest flavonoid yield of 11.48mg/g. This relatively high viscosity may limit solvent flow and hinder the transfer of flavonoids from the plant matrix into the extraction medium. In contrast, DES9 showed the lowest viscosity of 10.25mPa • s and achieved a flavonoid yield of 18.90mg/g. However, its extraction performance remained below that of DES15, which produced 21.40mg/g. These findings indicate that low viscosity may favor mass transfer, but extraction efficiency is also influenced by other solvent characteristics.

This study also used Pearson correlation analysis to examine the links between solvent

properties and flavonoid yield (Figure 3). Viscosity (r=−0.494), conductivity (r=−0.455), density (r=−0.504), and relative permittivity (r=−0.550) showed negative correlations with extraction yield. Apparent pH showed a weak positive correlation (r=0.176). However, all five relationships were statistically insignificant (p>0.05). These findings suggest that no single measured property can fully explain the differences in flavonoid recovery among the selected DESs.DES15 (L-Pro:AcOH, 1:2) produced the highest flavonoid yield, although it did not have the lowest viscosity. This result suggests that its extraction performance may depend on several factors rather than one solvent property[17]. These factors may include solvent characteristics and interactions between DES components and flavonoid molecules. These results suggest that DES15 may offer a suitable combination of solvent properties and molecular interactions. Based on its extraction performance, DES15 was selected for further process optimization.

Table 2 Physicochemical properties of DES

DES	Abbreviation	Molar ratio	Viscosity (mPa·s)	Conductivity (mS/cm)	Apparent pH	Density (g/cm³)	Relative permittivity (εr)	Extraction yield (mg/g)
DES1	ChCl:EG	1:2	11.72 ± 0.00	29.40 ± 0.17	5.56 ± 0.05	1.1004 ± 0.0002	17.342	16.08
DES16	L-Pro:AcOH:EG	1:2:1	16.01 ± 0.10	2.13 ± 0.02	3.71 ± 0.00	1.1466 ± 0.0002	2.228	19.84
DES3	ChCl:Gly	1:2	30.38 ± 0.21	12.48 ± 0.02	4.06 ± 0.03	1.1605 ± 0.0001	15.077	11.84

DES5	ChCl:Ure	1:2	11.89 ± 0.06	35.20 ± 0.17	5.65 ± 0.02	1.1576 ± 0.0003	27.627	12.39
DES9	Bet:AcOH:EG	1:3:1	10.25 ± 0.00	2.18 ± 0.01	3.77 ± 0.01	1.1062 ± 0.0000	1.674	18.90
DES15	L-Pro:AcOH	1:2	21.97 ± 0.11	2.26 ± 0.01	3.65 ± 0.01	1.1607 ± 0.0002	1.658	21.40
DES7	ChCl:Cia	1:1	243.10 ± 0.82	2.68 ± 0.02	0.03 ± 0.01	1.2709 ± 0.0001	1.286	11.48

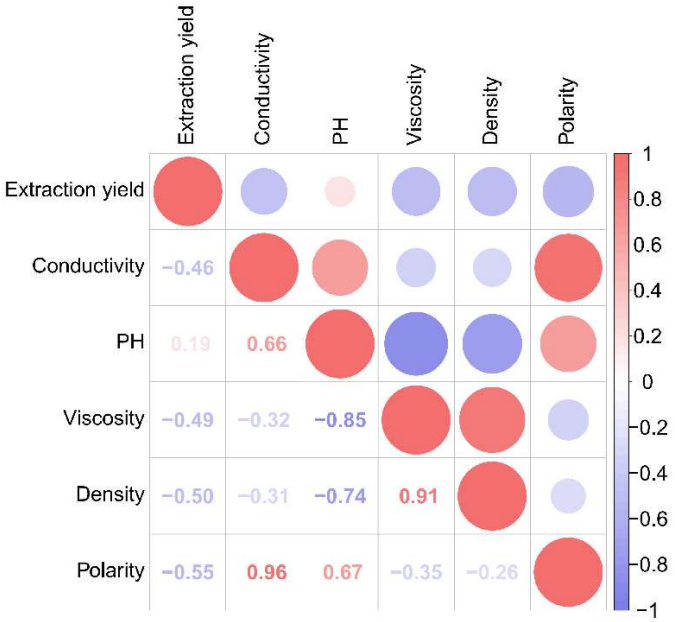


Figure 3 Pearson Correlation Heatmap

3.4. Analysis of single-factor experiments

The effects of water content, liquid-to-solid ratio, ultrasonic temperature, and extraction time were examined using DES15. The results are shown in Figure 4.

When the water content increased, the total flavonoid yield first increased and then decreased. The highest yield was obtained at about 15% water. A suitable amount of water can lower the

316 viscosity of DES15 and improve its contact with the plant material. However, excessive water
317 may alter the hydrogen-bonding environment of the DES system and reduce its extraction
318 performance [18]. Therefore, 15% water was selected for further optimization. The total flavonoid
319 yield also increased with the liquid-to-solid ratio and then reached a relatively stable level. The
320 yield was high at about 50mL/g. A higher solvent volume can provide more contact between
321 DES15 and the plant material. However, excessive solvent does not necessarily produce a further
322 increase in extraction yield. Therefore, 50mL/g was selected as the suitable ratio for the following
323 experiments[9]. The extraction yields increased as the ultrasonic temperature rose from 30 to 60°C.
324 The highest yield was obtained at about 60°C. A higher temperature can reduce the viscosity of
325 DES15 and improve solvent penetration and flavonoid transfer. However, further increases in
326 temperature did not improve the extraction yield, possibly because of changes in solvent–solute
327 interactions or the thermal sensitivity of some extracted compounds. Considering both extraction
328 performance and energy use, 60°C was selected for further optimization[9]. The total flavonoid
329 yield increased as the ultrasonic time increased from 20 to 50min. The yield then remained nearly
330 unchanged. At shorter times, the target compounds were not fully released from the plant matrix.
331 Longer treatment increased the mechanical effects of ultrasonic cavitation and improved contact
332 between the solvent and plant tissue. As extraction proceeded, the release of readily extractable
333 flavonoids may have slowed, resulting in a gradual decrease in the extraction rate. Further
334 extension of the treatment therefore produced only a small improvement[19]. As the total
335 flavonoid yields at 40 and 50 minutes showed negligible difference, and taking green
336 energy-saving factors into account, the 40-minute extraction time was selected for subsequent
337 experiments.

Compared with the other factors, extraction time produced a relatively smaller change in yield. Therefore, water content, liquid-to-solid ratio, and ultrasonic temperature were selected as the three variables for the response surface analysis. These results provided the basis for further optimization of the three selected variables using response surface methodology.

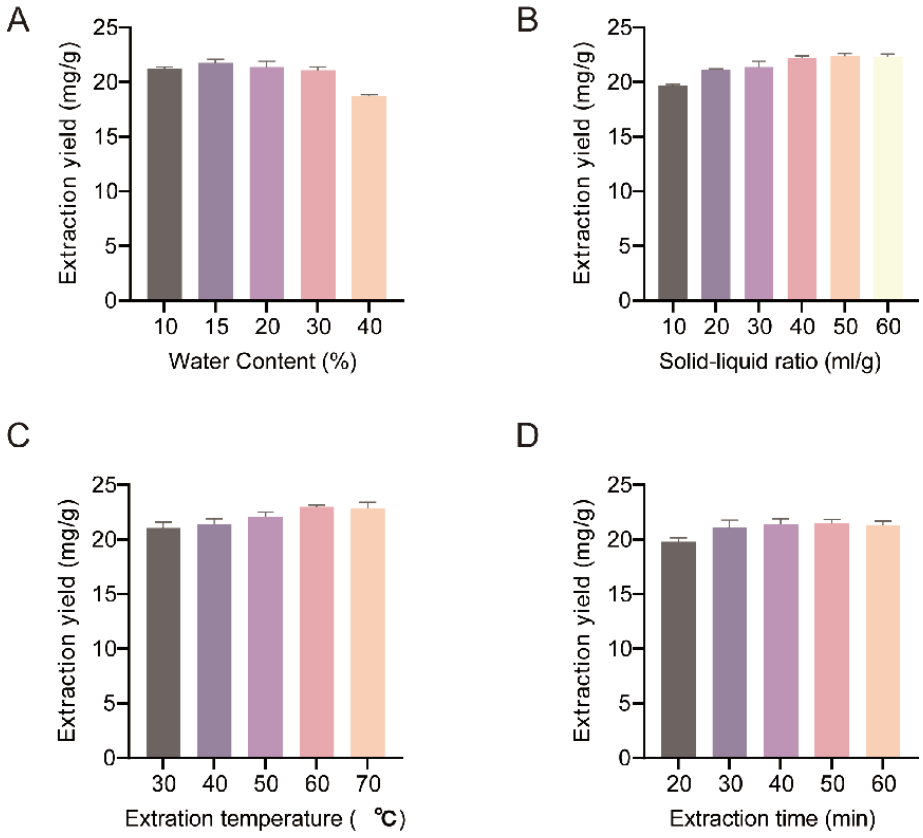


Figure 4 Effects of different extraction parameters on the yield of total flavonoids from *A. graminifolia*: (a) water content, (b) liquid-to-solid ratio, (c) ultrasonic temperature, (d) ultrasonic time.

3.5. Response surface optimization results

The single-factor experiments showed that DES15 gave a high flavonoid yield at 15% water, a liquid-to-solid ratio of 50mL/g, and an ultrasonic temperature of 60°C. A Box–Behnken design was therefore used with water content (A), liquid-to-solid ratio (B), and ultrasonic temperature (C) as the independent variables. Total flavonoid yield (Y) was used as the response value. A

second-order polynomial regression model was established to describe the relationship between the three extraction variables and the total flavonoid yield, as follows:

Y=-40.9765+1.05658A+0.91467B+1.05896C+0.0008335AB-0.005953AC-0.00107125BC-0.0241668A2-0.00842245B2-0.0066967C2

Table 3 Box-Behnken experimental results

std	A:Water content（%）	B:Liquid-to-solid ratio（ml/g）	C:Ultrasonic temperature（℃）	Y：Total flavonoid yield（mg/g）
1	-1	-1	0	23.981
2	1	-1	0	23.981
3	-1	1	0	25.571
4	1	1	0	24.400
5	-1	0	-1	22.833
6	1	0	-1	23.667
7	-1	0	1	25.571
8	1	0	1	25.214
9	0	-1	-1	22.552
10	0	1	-1	23.257
11	0	-1	1	25.124
12	0	1	1	25.400
13	0	0	0	25.452

14	0	0	0	25.691
15	0	0	0	25.571
16	0	0	0	25.691
17	0	0	0	25.571

357 Table 4 ANOVA for quadratic model

Source of variance	Source of Squares	df	Mean square	F-Value	P-Value	Significance
Model	18.07	9	2.01	162.26	<0.0001	significant
A	0.0517	1	0.0517	4.18	0.0803	
B	0.3412	1	0.3412	27.58	0.0012	
C	10.12	1	10.12	818.24	<0.0001	
AB	0.0069	1	0.0069	0.5614	0.4781	
AC	0.3544	1	0.3544	28.64	0.0011	
BC	0.0459	1	0.0459	3.71	0.0955	
A^2	1.54	1	1.54	124.21	<0.0001	
B^2	2.99	1	2.99	241.38	<0.0001	
C^2	1.89	1	1.89	152.60	<0.0001	
Residual	0.0866	7	0.0124			
Lack of Fit	0.0469	3	0.0156	1.58	0.3271	not significant
Pure Error	0.0397	4	0.0099			
Cor Total	18.16	16				

R^2=0.9952, R^2_{Adj}=0.9891, R^2_{Pre}=0.9552

The ANOVA results showed that the model was highly significant, with an F-value of 162.26 and P < 0.0001. The lack-of-fit test was not significant (P=0.3271). The R^2 value was 0.9952, while the adjusted R^2 and predicted R^2 values were 0.9891 and 0.9552, respectively. These results indicated that the model fitted the experimental data well. The difference between the adjusted and predicted R^2 values was less than 0.2, which also supported the stability of the model.

The F-values showed that ultrasonic temperature (C) had the greatest effect on the extraction yield. The liquid-to-solid ratio (B) had the next strongest effect. Water content (A) had the smallest effect among the three main factors. The 17 experimental points were also close to the regression line in Figure 5. This result showed good agreement between the predicted and measured values.

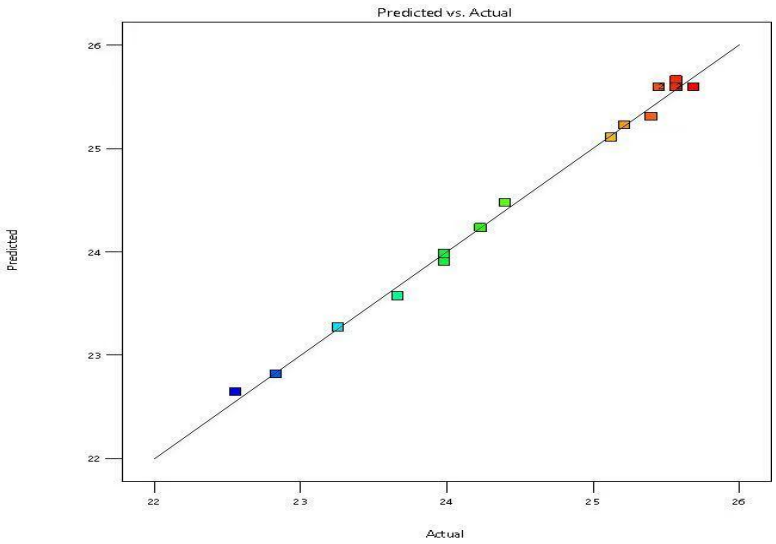


Figure 5 Correlation between predicted and actual values

The response surface plots in Figure 6 illustrate the combined effects of water content, liquid-to-solid ratio, and ultrasonic temperature on flavonoid yield. The significance of their interaction effects was evaluated using the ANOVA results.

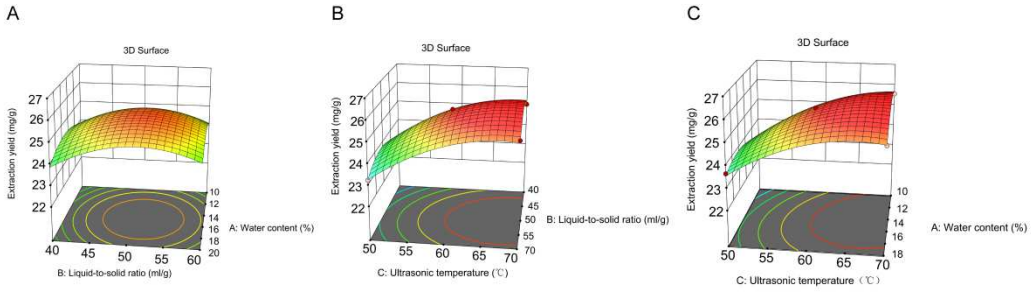


Figure 6 Response surface plots showing the pairwise interactions of different factors on the yield of total flavonoids from *A. graminifolia*.

The response surface model identified the optimal extraction parameters as a water content of 14.477%, a liquid-to-solid ratio of 56.416mL/g, and an ultrasonic temperature of 66.297 °C, with an estimated flavonoid yield of 25.782mg/g. To simplify experimental operation, these parameters were adjusted to 14.5%, 56mL/g, and 66°C, respectively. Three independent validation experiments were conducted under the adjusted conditions, and the resulting flavonoid yield was 25.738mg/g. The experimental result closely matched the model prediction, indicating that the developed model could effectively predict flavonoid recovery within the tested experimental range. These findings also confirmed that the optimized conditions were suitable for improving flavonoid extraction with DES15. However, the extraction yield alone provides limited information about the behavior of flavonoids during the extraction process. Therefore, further kinetic analysis was performed to investigate how extraction temperature and duration affect flavonoid release from the plant material.

3.6. Effect of ultrasound on flavonoid extraction

To evaluate the effect of ultrasound on flavonoid extraction, the total flavonoid yields obtained with and without ultrasound were compared using 80% ethanol and DES15 (Fig.ure 7). For 80% ethanol, ultrasound led to only a slight increase in flavonoid yield. In contrast, DES15

showed a clear increase in yield under ultrasonic conditions. DES15 with ultrasound achieved the highest yield among the four groups. Moreover, DES15 without ultrasound produced a higher yield than both ethanol groups. Previous studies have also reported improved flavonoid extraction using DES-based ultrasound-assisted extraction compared with conventional solvent systems[20]. These results suggest that the effect of ultrasound varied with the extraction solvent. The combination of DES15 and ultrasound improved flavonoid recovery under the tested conditions. These findings also support further investigation of the extraction kinetics[7].

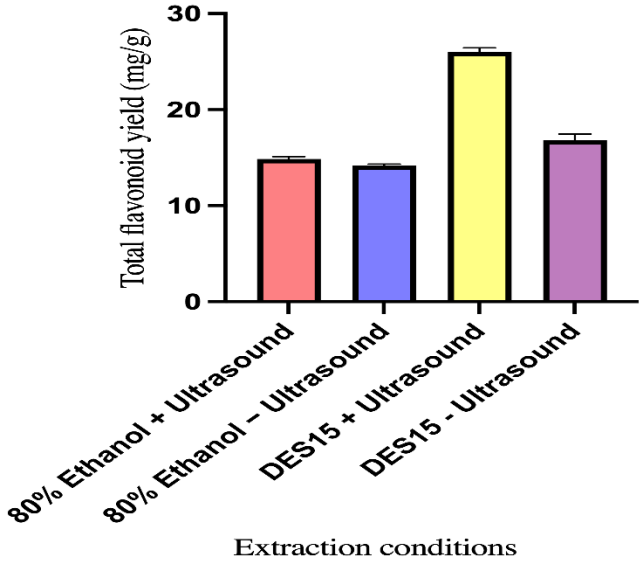


Figure 7 Effect of ultrasound on total flavonoid yield using DES15 and 80% ethanol.

3.7. Study on extraction kinetics

3.7.1. Extract kinetic curves

Figure 8 shows the changes in total flavonoid yield with extraction time at different temperatures. The yield increased as the extraction time increased at all tested temperatures. At 60°C, the yield increased quickly during the first 30 min. The increase then became slower and reached a stable level after about 60 min. This pattern showed that the extraction process gradually approached dynamic equilibrium. Temperature had a clear positive effect on extraction. At the

same extraction time, higher temperatures produced higher flavonoid yields. Higher temperatures can reduce the viscosity of DES15. This change can improve its wettability and penetration into the plant matrix. It can also promote flavonoid dissolution and mass transfer[21]. Increasing temperature may improve flavonoid extraction by influencing solvent properties and facilitating mass transfer between the plant matrix and the DES system.[22]. The curves at all temperatures became almost flat during the later stage of extraction, especially from 60 to 80 min. This result indicated that the system was approaching dynamic equilibrium. Further extension of extraction time would therefore provide only limited improvement. The extraction temperature should be selected by considering both flavonoid yield and energy consumption.

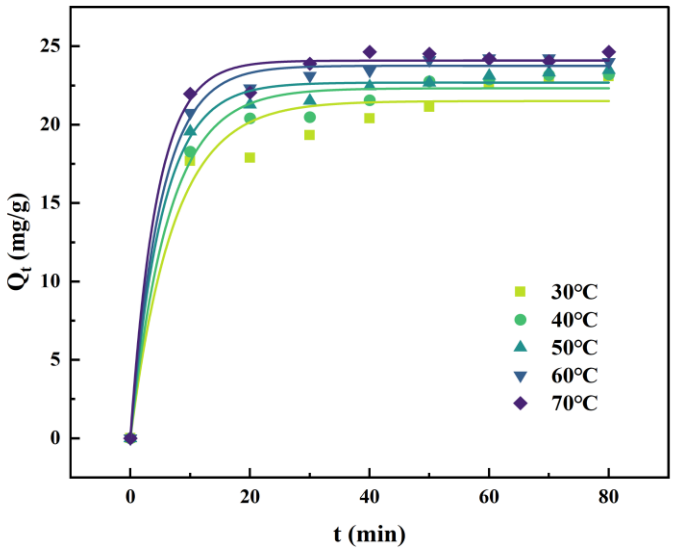


Figure 8 Extraction kinetics curves

3.7.2. Fitting with the pseudo-first-order kinetic model

The total flavonoid yield–time data at each temperature were fitted to the pseudo-first-order model using the FirstOrder function in Origin. Nonlinear fitting provided the equilibrium yield (Ce) and extraction rate constant (k1). Table 5 summarizes the rate constants and R^2 values at different temperatures. The increase in the fitted rate constant with temperature suggests that

higher temperatures accelerated flavonoid extraction within the investigated range.

Table5 Kinetic parameters of pseudo-first-order model for the extraction of total flavonoids from *A. graminifolia* at different temperatures.

Temperature（K）	R^2	k（min^{-1}）
303	0.95601	0.13874
313	0.98515	0.1565
323	0.99257	0.18777
333	0.99567	0.19725
343	0.99179	0.22652

3.7.3. Calculation of apparent activation energy

Based on the Arrhenius equation, the relationship between the rate constant k1 and temperature T is as follows:

$$lnK1_{=lnA-}\frac{Ea}{RT}$$

Linear regression of lnK1 against 1000/T was performed, and the fitting results are shown in Figure 9. The fitted equation is:

$$lnK1=2.19-1262.38（1000/T）\quad（R^2=0.98183）$$

The apparent activation energy calculated from the slope was 10.50 kJ/mol, which is significantly lower than the activation energy typically reported for conventional thermal reflux extraction (usually >20 kJ/mol) and falls within the range characteristic of diffusion-controlled processes (<40 kJ/mol)[23].

The relatively low activation energy suggests that the extraction rate was moderately affected

by temperature within the tested range. Physical mass transfer may play an important role in this process. Ultrasound can promote solvent penetration and the release of compounds from plant tissues. The DES system may also affect flavonoid dissolution through molecular interactions[24, 25].However, the activation energy alone cannot confirm that diffusion is the only rate-controlling step. The extraction process may involve several effects, including solvent penetration, dissolution, and mass transfer. To further understand the extraction process, SEM was used to examine the structural changes in plant tissues after different extraction treatments.

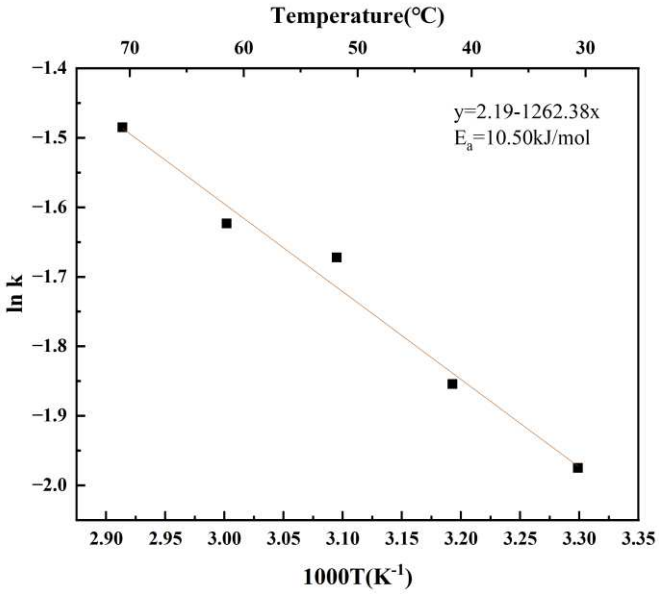


Figure 9 Arrhenius plot.

3.8. SEM observation

SEM was used to compare the surface morphology of the plant residues after different extraction treatments. The untreated sample showed a relatively compact structure. The cell walls remained intact, and only a small number of pores were visible. This structure may limit solvent penetration and the release of flavonoids.

The plant residue extracted with 80% ethanol exhibited minor surface contraction and a few

localized cracks, but its overall structure remained relatively compact. These limited morphological changes may have hindered solvent entry and flavonoid diffusion, partly explaining the lower extraction yield of 15.20mg/g. In contrast, DES15 treatment caused more noticeable surface damage, including numerous wrinkles, small pores, and partially broken regions. The plant tissue also appeared less compact, suggesting greater structural disruption. These changes may be attributed to the combined action of DES15 and ultrasonic treatment. The DES system may weaken the plant cell wall structure, while ultrasonic cavitation can produce microjets and shock waves that further disrupt the tissue. This combined effect could facilitate solvent penetration and create additional pathways for flavonoid release. The greater structural changes observed after DES15 treatment were consistent with its higher flavonoid yield of 21.40mg/g.[9]. The residue obtained under the optimized DES15 conditions showed the greatest structural changes. The surface had a highly loose structure with obvious layered separation and many pores. The cell walls were strongly disrupted, and fragmented particles were widely distributed. The total flavonoid yield under these conditions reached 25.74 mg/g.

SEM mainly shows changes in the physical structure of the plant matrix. SEM alone cannot prove chemical degradation of cell wall components. These morphological changes may facilitate solid–liquid mass transfer and contribute to the improved flavonoid recovery observed with DES15. In addition to changes in plant tissues, interactions within the DES system may also affect extraction performance. Therefore, ATR-FTIR analysis was used to examine the hydrogen-bonding environment of DES15.

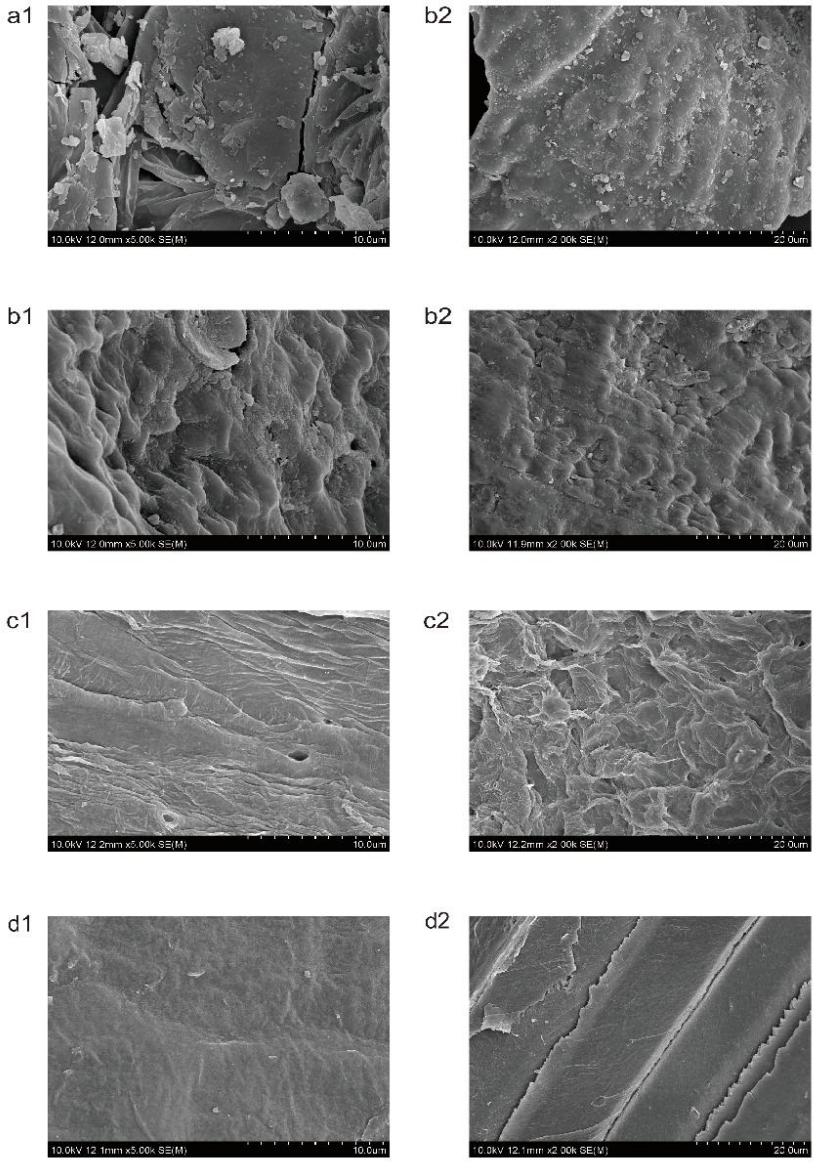


Figure 10 SEM micrograph of the extraction residue.

3.9. ATR-FTIR analysis

ATR-FTIR was used to examine the interactions involved in the formation of DES15. The spectra of L-Pro, AcOH, and DES15 were compared. Pure L-Pro showed a broad O–H/N–H stretching band at 3445 cm^{-1}. It also showed characteristic COO^- peaks at 1616 and 1559 cm^{-1}. Pure AcOH showed a strong C=O stretching peak at 1708 cm^{-1}.

After DES15 formation, changes were observed in the broad absorption region associated with O–H and N–H stretching vibrations. These spectral changes may reflect a rearrangement of

the hydrogen-bonding environment between L-Pro and AcOH. In pure AcOH, the carboxyl groups mainly form cyclic dimers through strong hydrogen bonding[26]. The addition of L-Pro may alter the hydrogen-bonding interactions among AcOH molecules and introduce new interactions between the two DES components[27]. The C=O stretching peak also changed slightly from 1708 to 1705 cm^{-1}. This red shift provided additional evidence that the hydrogen-bond environment had changed after DES15 formation.

However, FTIR alone cannot determine the exact positions, numbers, or arrangements of hydrogen bonds. These results suggest that hydrogen-bond interactions contribute to the formation of DES15. However, ATR-FTIR cannot directly explain how DES15 interacts with flavonoid molecules. Therefore, DFT calculations were further used to examine possible interactions between representative DES components and quercetin.

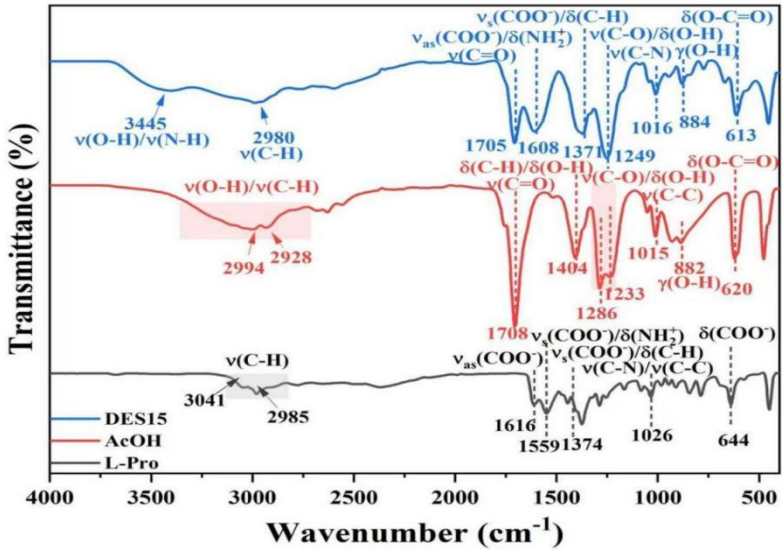


Figure 11 FTIR spectra of L-Pro, AcOH, and the DES15 formed from them.

3.10. Analysis of noncovalent interactions

DES15 and DES7 were selected for comparison because they showed high and low extraction performance, respectively. Quercetin was used as a representative flavonoid molecule.

Density functional theory calculations were performed to examine the interactions between quercetin and the two DES systems.

The three-dimensional isosurface plots in Figures 12a1 and 12a2 showed clear non-covalent interaction regions in both systems. Green isosurfaces were observed in the L-Pro (1:2)–Quercetin and ChCl acid (1:1)–Quercetin systems. These results suggest that noncovalent interactions, including van der Waals interactions and possible hydrogen bonding, contribute to the association between quercetin and the DES components. The corresponding scatter plots in Figures 12b1 and 12b2 provided further information on the interaction types. The L-Pro system showed a wider distribution of strong interactions with quercetin. The ChCl acid system showed fewer strong interactions and a weaker interaction pattern. The DFT calculations yielded a binding energy of −65.36 kcal/mol for the L-Pro‑AcOH‑quercetin complex and −56.45 kcal/mol for the ChCl‑citric acid‑quercetin complex. The calculated interaction differences provide a possible molecular-level explanation for the different extraction performances of DES15 and DES7, although extraction efficiency is also influenced by solvent properties and mass transfer. Together with the physicochemical and extraction results, these calculations suggest that the performance of DES15 may be related to both solvent properties and molecular interactions. The chemical composition of the DES15 extract was then examined to provide further information on the extracted flavonoids.

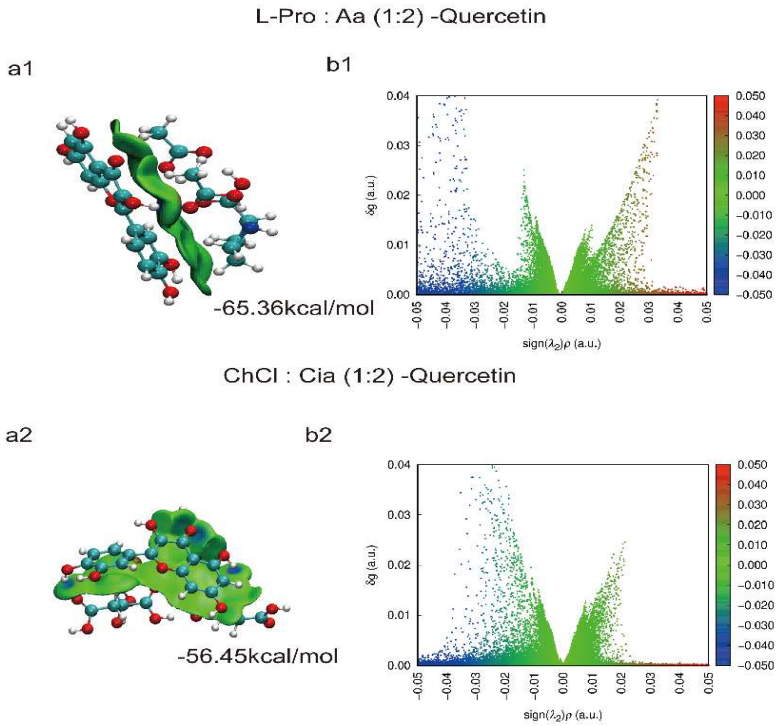


Figure 12 Analysis of non-covalent interactions between Quercetin molecules and different solvent systems.

3.11. UHPLC-QTOF-MS analysis

UHPLC-QTOF-MS was used to characterize the flavonoid profile of the DES15 extract. Figure 13 shows the total ion chromatograms obtained in positive and negative ion modes. The data were compared with spectral databases, including SpectraVerse and MoNA. A total of 27 compounds were tentatively assigned as flavonoids based on accurate mass measurements and spectral library matching, as summarized in Table 6. The compound identification was based only on high-resolution mass data and spectral library matching. The study did not use reference standards to confirm retention times or fragmentation patterns. Therefore, these compound assignments should be considered tentative[9]. The presence of these tentatively identified flavonoids provides a chemical basis for further evaluating the antioxidant activity of the DES15 extract.

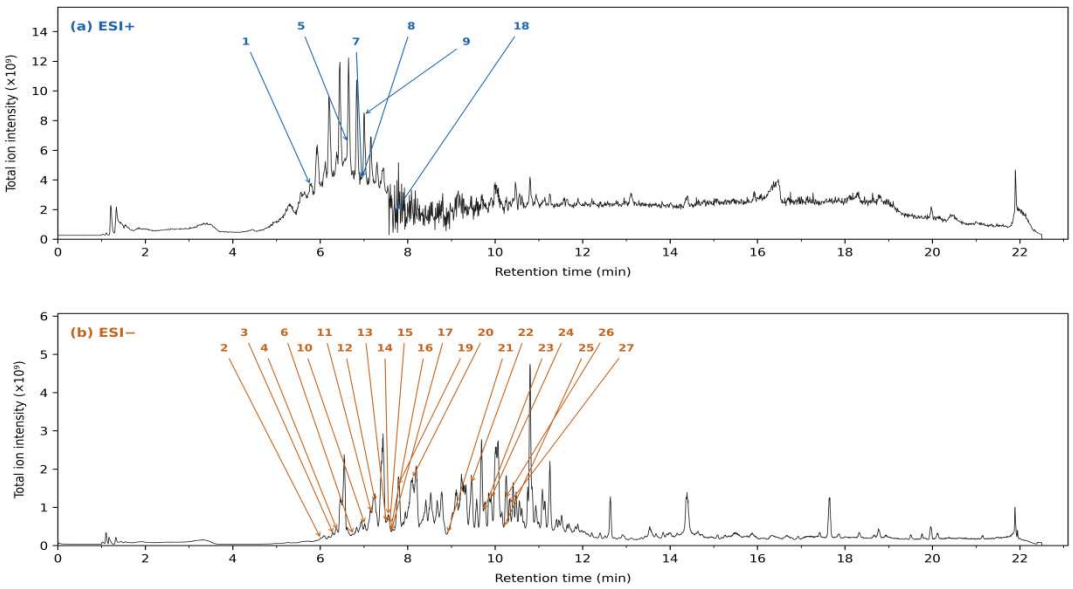


Figure 13 Characterization of flavonoids from *A. graminifolia* by UHPLC-QTOF-MS

Table 6 Characterization of flavonoids from *A. graminifolia* by UHPLC-QTOF-MS

peak	RT (min)	Formula	Ion type	m/z	Name
1	5.77	C27H31O16	[M]+	611.16	Cyanidin-3,5-di-O-glucoside
2	6.01	C27H30O15	[M-H]-	593.15	Apigenin-6,8-di-O-galactoside
3	6.30	C27H30O17	[M-H]-	625.14	Quercetin-3,4'-di-O-glucoside
4	6.39	C21H20O11	[M-H]-	447.09	Homoorientin
5	6.62	C27H30O15	[M+H]+	595.17	Datiscin
6	6.75	C27H30O15	[M-H]-	593.15	Kaempferol-7-O-neohesperidoside
7	6.95	C21H20O11	[M+H]+	449.11	Kaempferol-3-O-glucoside
8	6.96	C15H10O6	[M+H]+	287.06	Kaempferol

9	7.01	C15H10O7	[M+H]+	303.05	Quercetin
10	7.02	C21H20O12	[M-H]-	463.09	Isoquercitrin
11	7.17	C27H30O15	[M-H]-	593.15	Kaempferol-3-O-rutinoside
12	7.26	C28H32O16	[M-H]-	623.16	Isorhamnetin-3-O-rutinoside
13	7.51	C22H22O12	[M-H]-	477.10	Isorhamnetin-3-O-glucoside
14	7.56	C23H22O13	[M-H]-	505.10	Quercetin-3-O-(6"-O-acetyl)-glucoside
15	7.59	C21H20O11	[M-H]-	447.09	Kaempferol-7-O-glucoside
16	7.61	C21H22O10	[M-H]-	433.11	Prunin
17	7.62	C20H18O10	[M-H]-	417.08	Kaempferol-3-O-arabinoside
18	7.73	C22H23O12	[M]+	479.12	Petunidin-3-O-beta-glucoside
19	7.78	C21H20O11	[M-H]-	447.09	Quercitrin
20	8.11	C21H20O10	[M-H]-	431.10	Kaempferol-3-O-rhamnoside
21	8.91	C30H26O13	[M-H]-	593.13	Tiliroside
22	9.45	C16H12O6	[M-H]-	299.06	Tectorigenin
23	9.74	C18H16O6	[M-H]-	327.09	Isotectorigenin 7-methyl ether
24	9.90	C15H12O5	[M-H]-	271.06	Naringenin
25	10.20	C15H12O7	[M-H]-	303.05	Taxifolin
26	10.23	C16H12O7	[M-H]-	315.05	Isorhamnetin
27	10.32	C22H22O10	[M-H]-	445.12	Calycosin-7-O-glucoside

533

534 3.12. Evaluation of antioxidant activity

In this study, the DPPH radical-scavenging assay was employed to evaluate and compare the antioxidant activities of the samples.

As shown in Figure 14, ascorbic acid (Vc) exhibited a strong linear relationship over the concentration range of 0.01–0.05 mg/mL, with the DPPH radical-scavenging rate increasing positively with Vc concentration and an IC_{50}value of 0.02611 mg/mL. Figure 15 shows that the DPPH radical-scavenging rates of the different samples increased progressively with increasing sample concentration, indicating that A. *graminifolia* extracts exhibited DPPH radical-scavenging activity. The DES15 extract exhibited a lower IC_{50} value (0.4761 mg/mL) than the 80% ethanol extract (0.9624 mg/mL), indicating a stronger DPPH radical-scavenging response under the tested conditions. The IC_{50} value of the DES15 extract was approximately 49.5% of that of the ethanol extract, further supporting its stronger DPPH radical-scavenging response. These results indicate that, under the experimental conditions and concentration units used in this study, the DES15 extract exhibited a stronger DPPH radical-scavenging response. Although the IC_{50} value of Vc (0.02611 mg/mL) was lower than those of the two plant extracts, indicating that a lower concentration of Vc was required to achieve 50% scavenging, Vc and the plant extracts represent different types of samples. Therefore, Vc served primarily as a positive control in the experimental system, and the results should not be interpreted as a direct comparison of the scavenging potency of the extracts with that of the pure compound.

The DES15 extract showed considerable DPPH radical-scavenging activity, indicating its potential antioxidant properties. Under the optimized extraction conditions, DES15 achieved a total flavonoid yield of 25.738mg/g, which was higher than the 15.20mg/g obtained using 80% ethanol. These results demonstrate that the optimized DES15 system improved flavonoid recovery

while producing an extract with measurable antioxidant activity. The findings suggest that DES-assisted extraction may be a useful approach for obtaining bioactive constituents from *A. graminifolia*. However, further investigation is needed to clarify the relationship between flavonoid content and antioxidant activity.

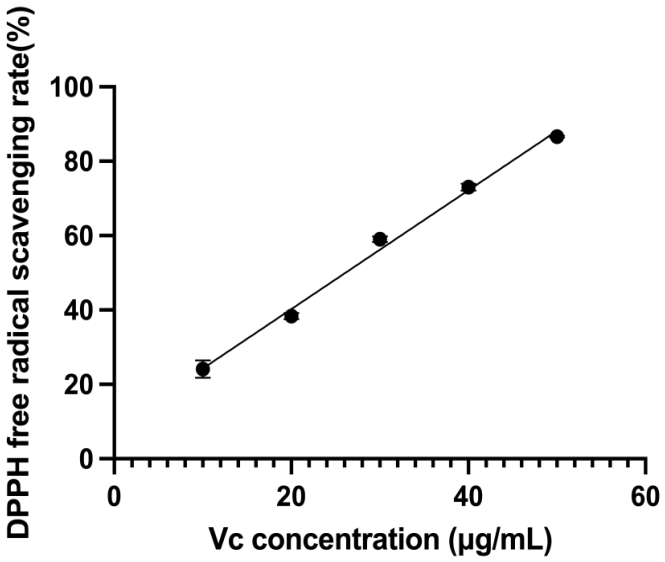


Figure 14 Standard curve for the DPPH radical scavenging assay.

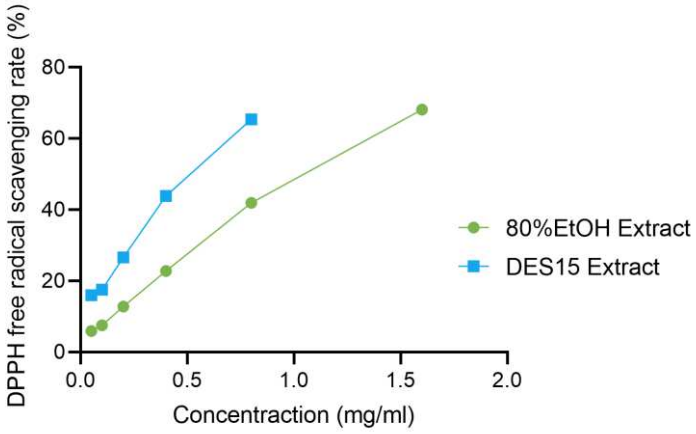


Figure 15 Comparison of DPPH scavenging capacities of total flavonoids from *A. graminifolia* extracted under different conditions.

3.13. Green Assessment

The environmental sustainability of the proposed extraction procedure was assessed using the

AGREE and ComplexGAPI tools. This evaluation considered the entire analytical workflow, including sample preparation, DES-based extraction, colorimetric reaction, and visible spectrophotometric measurement.

The proposed method obtained an AGREE score of 0.51, indicating moderate green performance with opportunities for further improvement. The L-proline-based DES offered an alternative to traditional organic solvents, but the environmental impact of the procedure was also influenced by solvent usage, energy consumption, and waste production. Although the method did not require a derivatization step, several aspects of the analytical workflow limited its overall sustainability. This included sample preparation, the amount of DES required, electricity consumption during ultrasonic extraction, and the waste produced. The AGREE result suggests that further optimization is necessary to improve the environmental performance of the method. Future studies should therefore aim to decrease DES usage, investigate solvent recycling and reuse, and minimize energy requirements and waste generation throughout the extraction process.

Overall, this study combined solvent screening, physicochemical characterization, process optimization, extraction kinetics, structural observations, and molecular interaction analysis to examine DES-assisted flavonoid extraction from A. graminifolia. The chemical profiling, antioxidant assay, and green assessment further provided information on the extract and the analytical procedure.

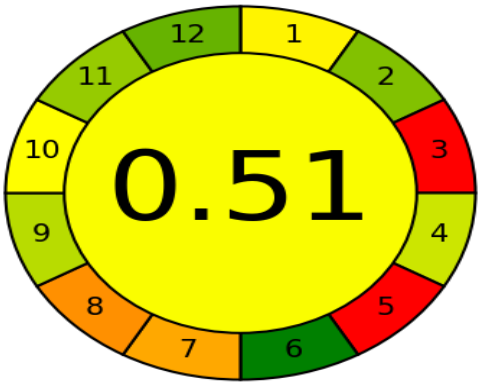


Figure 16 Green analysis evaluation results based on the AGREE model.

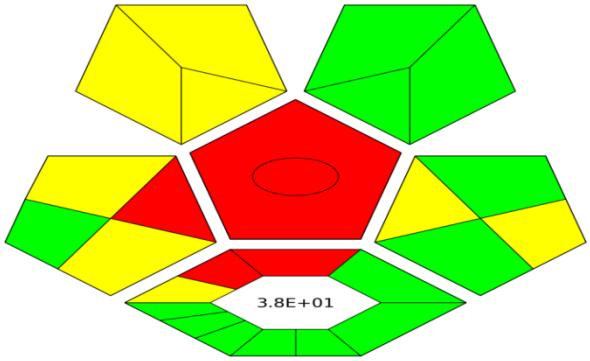


Figure 17 Green evaluation results of the analysis process based on the ComplexGAPI model.

4. Conclusion

This study developed a deep eutectic solvent-based ultrasound-assisted method for recovering flavonoids from *A. graminifolia*. Among 16 DES formulations, DES15 (L-Pro, 1:2) showed the best extraction performance. The extraction parameters were further optimized through single-factor experiments and response surface methodology. The optimal conditions included 14.5% water, a liquid-to-solid ratio of 56mL/g, and an extraction temperature of 66°C. Under these conditions, the total flavonoid yield reached 25.738mg/g, which was 1.693 times that

obtained with 80% ethanol. The additional comparison showed that ultrasound produced a more pronounced increase in flavonoid yield with DES15 than with 80% ethanol under the tested conditions. DES15 also achieved a higher yield than 80% ethanol in the absence of ultrasound. The physicochemical properties of seven representative DESs were also examined, including viscosity, conductivity, apparent pH, density, and relative permittivity. Pearson correlation analysis showed no statistically significant association between flavonoid yield and any single property ($p>0.05$). Although DES15 achieved the greatest extraction yield, it did not exhibit the lowest viscosity. This result suggests that extraction efficiency may depend on the combined influence of multiple solvent characteristics. Further analyses were conducted to better understand the extraction process. The experimental data were well described by a pseudo-first-order kinetic model, with an apparent activation energy of 10.50kJ/mol. SEM observations showed greater disruption of plant tissues following DES15 treatment. ATR-FTIR analysis revealed changes in the hydrogen-bonding environment during DES15 formation, while DFT calculations indicated possible molecular interactions between quercetin and the solvent components. Together, these results offer complementary evidence for understanding the extraction behavior of DES15. UHPLC-QTOF-MS analysis provided tentative assignments for 27 flavonoid compounds in the obtained extract. The DES15 extract also exhibited greater DPPH radical-scavenging activity than the 80% ethanol extract under the tested conditions, with IC_{50} values of 0.4761 and 0.9624mg/mL, respectively. These findings highlight the potential of DES15 for producing flavonoid-rich extracts from A. graminifolia. However, the AGREE score of 0.51 indicates that further efforts are needed to reduce the environmental impact of the analytical procedure.

Overall, this study integrated DES screening, extraction optimization, physicochemical

621 characterization, and experimental and theoretical analyses to investigate flavonoid recovery from
622 *A. graminifolia*. The findings offer a better understanding of the factors that may influence
623 extraction efficiency and provide a basis for further improving DES-assisted extraction methods.
624 Future research should focus on confirming the structures of the tentatively identified flavonoids
625 and investigating DES recovery and reuse. Further studies should also compare extraction kinetics
626 under ultrasonic and non-ultrasonic conditions. Direct measurements of acoustic power and
627 energy consumption would help clarify the role of ultrasound and improve process efficiency. The
628 environmental impact of the complete extraction procedure should also be assessed.

629 **List of abbreviations**

Abbreviation	Full term
AcOH	Acetic acid
AGREE	Analytical GREEnness metric
ANOVA	Analysis of variance
ATR-FTIR	Attenuated total reflection Fourier transform infrared spectroscopy
Bet	Betaine
ChCl	Choline chloride
Cia	Citric acid
ComplexGAPI	Complex Green Analytical Procedure Index
DES	Deep eutectic solvent
DFT	Density functional theory

Abbreviation	Full term
DPPH	2,2-Diphenyl-1-picrylhydrazyl
EG	Ethylene glycol
FTIR	Fourier transform infrared spectroscopy
GAPI	Green Analytical Procedure Index
Gly	Glycerol
HBA	Hydrogen-bond acceptor
HBD	Hydrogen-bond donor
IC_{50}	Half-maximal inhibitory concentration
Laa	Lactic acid
L-Pro	L-Proline
MoNA	MassBank of North America
MS/MS	Tandem mass spectrometry
RSM	Response surface methodology
RT	Retention time
SEM	Scanning electron microscopy
UHPLC	Ultra-high-performance liquid chromatography
UHPLC-QTOF-MS	Ultra-high-performance liquid chromatography–quadrupole time-of-flight mass spectrometry
Ure	Urea

Abbreviation	Full term
Vc	Vitamin C (ascorbic acid)

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding authors on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Junjie Wang and Yunfei Cui: Writing – original draft, Writing – review & editing, Conceptualization, Methodology, Investigation, Formal analysis, Data curation, Visualization, Validation, Software. Jing Su: Writing – original draft, Investigation, Data curation. Guang Li and Lei Wang: Writing – review & editing, Supervision, Project administration, Funding acquisition. Hanqing Liu: Investigation, Resources, Methodology. Chunli Gai: Investigation, Resources, Methodology.

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