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Microwave-assisted extraction of triterpenoid from *Abelmoschus sagittifolius* (Kurz) Merr roots using deep eutectic solvents

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Abstract

A green and efficient method was developed for extracting triterpenoids from *Abelmoschus sagittifolius* roots using microwave-assisted extraction (MAE) combined with a choline chloride–citric acid-based deep eutectic solvent (DES). Optimization by response surface methodology identified optimal conditions (365 W, 46 min, 40 mL/g), yielding 39.8 mg/g of triterpenoids, in close agreement with the predicted value (39.5 mg/g). This yield was significantly higher than those obtained by ethanol-based MAE (32.69 mg/g) and Soxhlet extraction (28.14 mg/g). SEM analysis revealed marked cell wall disruption in DES–MAE-treated samples. The extract exhibited strong antioxidant activity (84.56% DPPH and 96.67% ABTS at 0.55 mg/mL) and notable tyrosinase inhibition ($IC_{50} \approx 0.17$ mg/mL), outperforming conventional extracts. GC–MS analysis identified 18 bioactive compounds, predominantly triterpenoids. Overall, DES–MAE represents a sustainable approach for producing triterpenoid-rich extracts for food, cosmetic, and pharmaceutical applications.

Keywords: *Abelmoschus sagittifolius* roots, bioactive compounds, deep eutectic solvent, antioxidant activity

1. Introduction

Abelmoschus sagittifolius (Kurz) Merr., commonly known as "sâm bố chính" in Vietnam, is a traditional medicinal plant belonging to the Malvaceae family and is widely distributed across the mountainous regions of northern Vietnam [1–3]. Its roots have been extensively used in Vietnamese folk medicine for treating conditions such as chronic cough, fatigue, fever, internal heat, and menstrual disorders [2–4]. These ethnobotanical applications suggest the presence of pharmacologically active compounds in the plant. Recent phytochemical investigations have confirmed that the roots of *A. sagittifolius* are rich in bioactive triterpenoids and phenolic compounds, which exhibit diverse biological activities including antioxidant, anti-inflammatory, antiviral, and anticancer effects [5–7]. Despite this promising profile, the optimized extraction of triterpenoids from this plant using green and efficient technologies remains underexplored.

Traditionally, the extraction of triterpenoids has relied on solvent-intensive techniques such as Soxhlet or reflux extraction, which suffer from several drawbacks—long processing times, high energy consumption, and degradation of thermolabile compounds—making them incompatible with modern green chemistry principles [7,8]. As an alternative, microwave-assisted extraction (MAE) has been developed to overcome these limitations. MAE offers rapid cell wall disruption, improved mass transfer, and reduced extraction time while maintaining compound integrity [9]. Complementing MAE, deep eutectic solvents (DESs) have emerged as environmentally friendly alternatives to conventional solvents. DESs are typically composed of a hydrogen bond acceptor (HBA) and donor (HBD), forming a eutectic mixture with unique solvating abilities [10–12]. Among them, the combination of choline chloride (ChCl) and citric acid (CA) has demonstrated high polarity, excellent hydrogen-bonding capacity, and strong biodegradability, making it particularly effective in solubilizing triterpenoids

[13,14]. Citric acid not only enhances extraction via hydrogen bonding but also facilitates mild acid hydrolysis, promoting better release of intracellular metabolites [10–12].

Recent studies have successfully applied DES–MAE systems for extracting triterpenoids from various medicinal plants, including *Gleditsia sinensis*, celery leaves, and *Paris polyphylla* [15–17]. However, comprehensive investigations using DES-MAE for triterpenoid extraction from *Abelmoschus sagittifolius* remain limited. Despite its rich phytochemical profile, *A. sagittifolius* has been underexplored in modern green extraction research, presenting both a scientific gap and an opportunity to explore its potential within the framework of green chemistry principles.

Therefore, this study aimed to develop and optimize an eco-friendly extraction method for isolating triterpenoids from *A. sagittifolius* roots, using microwave-assisted extraction (MAE) with a choline chloride–citric acid-based deep eutectic solvent (ChCl–CA DES). A Box–Behnken design coupled with response surface methodology (RSM) was employed to investigate the effects of key parameters on extraction efficiency. Furthermore, the antioxidant and tyrosinase inhibitory activities of the extracts were evaluated to assess their potential applications. This research not only introduces a sustainable extraction approach but also contributes to the development of functional ingredients for potential use in nutraceuticals, cosmetics, and pharmaceuticals.

2. Materials and methods

2.1 Materials and chemicals

Fresh roots of *Abelmoschus sagittifolius* (Kurz) Merr., one-year-old, with a total fresh weight of approximately 200 kg, were collected from Quang Tri Province, Vietnam. After collection, the roots were thoroughly washed with water, sliced, and dried in a hot-air oven (Memmert UFE 500, Germany) at 50 °C for 8 h until the moisture content was reduced to below 10%. The residual moisture content of the dried root powder was determined using a moisture analyzer (MF-50, A&D, Japan) at 105 °C until constant weight, ensuring that all analytical results could be expressed on a dry weight (DW) basis. The dried material was subsequently ground into a fine powder using a laboratory grinder (IKA MF10, Germany) and sieved through a 16-mesh screen (1.25 mm aperture), yielding an average particle size of 1.20 ± 0.05 mm. The resulting powder was stored in vacuum-sealed, silver-coated zip-lock bags (EuroLab®, Vietnam) at room temperature until further use.

Analytical-grade chemicals employed in this study included ethanol (99.8%, Merck, Germany), choline chloride (≥98%, Sigma-Aldrich, USA), citric acid (99.9%, Merck, Germany), ascorbic acid (≥99%, Sigma-Aldrich, USA), 2,2-diphenyl-1-picrylhydrazyl (DPPH, >90%, Sigma-Aldrich, USA), and 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS, >99%, Sigma-Aldrich, USA). All solvents and reagents were of analytical grade and used without further purification.

2.2 Deep eutectic solvent extraction

Deep eutectic solvents (DESs) were prepared by mixing choline chloride (ChCl, 139.62 g·mol^{-1}) and citric acid (CA, 192.12 g·mol^{-1}) at molar ratios of 1:1, 1:2, 1:3, 1:4, and 1:5. The exact masses of each component were calculated from their molecular weights and weighed accordingly. Distilled water (Milli-Q, Millipore, USA) was added at levels ranging from 10 to 60% (v/v) to adjust the physicochemical properties of the mixtures. The viscosity of the ChCl–CA–water systems was measured at 25 °C using a digital viscometer (Brookfield DV2T, USA), and results showed a marked decrease with increasing water content: 1.01 ± 0.02 Pa·s (10%), 0.90 ± 0.03 (20%), 0.71 ± 0.02 (30%), 0.42 ± 0.01 (40%), 0.22 ± 0.01 (50%), and 0.11 ± 0.00 (60%). This confirmed that water addition effectively reduced viscosity, thereby improving solvent fluidity and mass transfer efficiency [18]. All DES mixtures were gently heated in a 60 °C water bath (Memmert WNB7, Germany) until clear and homogeneous solutions were obtained [11].

For extraction, 5.0 g of finely ground *A. sagittifolius* root powder was combined with the prepared DES and subjected to microwave irradiation at 400 W for 10 min using a laboratory microwave system (Model 8212, PG Instruments, UK). The mixture was subsequently shaken at ambient temperature for 50 min on an orbital shaker (IKA KS 4000, Germany). After extraction, the samples were centrifuged at 5000 × g for 10 min using a refrigerated centrifuge (Hermle Z326K, Germany), and the supernatant was collected for triterpenoid quantification. The DES composition yielding the highest triterpenoid content (mg/g DW) was then selected for further optimization experiments.

2.3 Optimization of extraction conditions

The extraction of triterpenoids was optimized using a Box–Behnken design (BBD), considering three independent variables: microwave power (100–600 W), extraction time (10–60 min), and solvent-to-solid ratio (20–70 mL/g). Each experimental run began with an initial 10 min microwave-assisted treatment, followed by

shaking at room temperature for the designated extraction period, following previous studies [5,8,9]. After extraction, the mixtures were centrifuged at 5500 rpm for 20 min, and the supernatants were stored at 1–2 °C prior to analysis. Since DES cannot be removed by rotary evaporation, the extracts were analyzed directly and the yields were expressed relative to the dry weight of the raw material (mg/g DW). The relationship between extraction conditions and triterpenoid yield was described by the following second-order polynomial model [19]:

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k} \beta_{ii} {X_i}^2 + \sum_{i>j}^{k} \beta_{ij} X_i X_j \quad (1)$$

Where X_i and X_j denote the different independent variables. Y is the predicted response. β_0, β_j, β_{jj} and β_{ij} denote the regression coefficients corresponded to intercept, linearity, square, and interaction, respectively. All the statistical analyses were done using Design-Expert 8.0.5 (State-Ease Inc., Minneapolis, MN, USA) package.

2.4 Traditional extraction method (HE)

For comparison, triterpenoids were also extracted using a conventional Soxhlet method. Briefly, 1.0 g of *A. sagittifolius* powder was refluxed with 10 mL of 80% ethanol (Merck, Germany) at 80 °C for 2 h in a Soxhlet extractor (LabTech EV400, Italy) [20]. The ethanol extract was concentrated using a rotary evaporator (Buchi R-300, Switzerland) to remove the solvent, then stored at 1–2 °C until analysis. To ensure comparability with the DES–MAE method, the extraction yields were calculated and expressed as mg/g DW.

2.5 Determination of triterpenoid content

The triterpenoid concentration in the extracts was determined using a modified colorimetric method based on the procedure described by Cai et al. [7], with oleanolic acid employed as the standard (concentration range: 0.01–0.7 mg/mL). Specifically, 0.2 mL of the extract was mixed with 0.2 mL of 5% vanillin (prepared in acetic acid) and 1.2 mL of perchloric acid ($HClO_4$). The mixture was incubated at 70 °C for 15 minutes in a water bath, allowed to cool at room temperature for 2 minutes, and subsequently diluted to 5 mL with ethyl acetate. Absorbance was recorded at 550 nm using a UV–Vis spectrophotometer, and the triterpenoid content was expressed in milligrams of oleanolic acid equivalents per gram of dry weight (mg OA/g DW).

2.6 Determination of antioxidant and tyrosinase inhibitory activities

The antioxidant capacity of the extracts was evaluated using DPPH and ABTS radical scavenging assays, with minor modifications based on the method reported by Wei et al. [21]. For the DPPH assay, 0.2 mL of extract (at triterpenoid concentrations ranging from 0.05 to 0.55 mg/mL) was added to 1.8 mL of DPPH solution and incubated in the dark for 30 minutes. Absorbance was measured at 517 nm, and ethanol served as the blank control. In the ABTS assay, 0.2 mL of the extract was added to 1.8 mL of pre-formed $ABTS^{\bullet+}$ solution, followed by incubation in the dark for 6 minutes. The absorbance was recorded at 734 nm. The radical scavenging activity in both assays was calculated using the equation:

$$\text{Scavenging activity (\%)} = (A_{\text{Control}} - A_{\text{Sample}}) \times 100 / A_{\text{Control}} \quad (2)$$

The tyrosinase inhibitory activity of the extracts was assessed using a spectrophotometric assay with L-DOPA as the substrate. The reaction system consisted of 40 µL of extract, 80 µL of 0.1 M phosphate buffer (pH 6.8), 40 µL of mushroom tyrosinase (100 U/mL), and 40 µL of 2.5 mM L-DOPA. The mixture was incubated at 37 °C for 30 minutes in the dark. Dopachrome formation was monitored by measuring the absorbance at 475 nm. Appropriate controls without extract (negative control) and blanks without enzyme were included to correct background absorbance. Ascorbic acid was used as positive controls for antioxidant and tyrosinase assays, respectively.

All experiments were performed in triplicate (n = 3), and results are expressed as mean ± standard deviation (SD). The radical scavenging activity and tyrosinase inhibition were calculated using Eq. (2), and IC_{50} values were determined from the dose–response curves.

2.7 GC–MS Analysis of Chemical Constituents

The chemical composition of the extracts was analyzed by gas chromatography–mass spectrometry (GC–MS) according to a modified protocol of Bakar et al. [14]. Prior to injection, the samples were filtered through a 0.22 µm PTFE membrane and diluted with ethyl acetate to improve volatility. To enable the detection of low-volatile and thermally labile compounds such as triterpenoids (e.g., lupeol, oleanolic acid, friedelin), derivatization was

performed using N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) to generate trimethylsilyl (TMS) derivatives, following [22]. Briefly, dried extracts were silylated by adding 250 µL of a BSTFA/TMCS/pyridine mixture (22:13:65, v/v/v) and incubated for 2 h at 30 °C. Samples were then cooled and diluted with 750 µL of hexane prior to GC–MS analysis.

Analyses were carried out on an Agilent 7890B GC system coupled with a 5977A mass selective detector (Agilent Technologies, USA) equipped with a DB-5MS capillary column (30 m × 0.25 mm i.d., 0.25 µm film thickness). The injector temperature was set at 250 °C with a split ratio of 10:1. The oven temperature was programmed from 50 °C (2 min hold) to 260 °C at 10 °C/min, with a final hold of 5 min. Helium was used as carrier gas at a constant flow rate of 1.0 mL/min. Mass spectra were acquired in EI mode (70 eV) over the range of 40–650 m/z. Compound identification was performed by comparing the obtained spectra with the NIST 14 Mass Spectral Library, and only matches with similarity indices above 80% were considered. Due to the lack of retention indices and authentic standards, the identification of triterpenoids should be regarded as tentative rather than definitive.

2.8 Scanning electron microscopy

The surface morphology of A. *sagittifolius* powder after extraction by DES-based MAE and conventional solvent extraction was examined using a Hitachi Ion Sputter E-1010 SEM. Samples were coated with a 40 nm gold layer and observed at 5 kV accelerating voltage and 5,000× magnification.

2.9 Data analysis method

Data were statistically analyzed using ANOVA at a 95% confidence level with Design-Expert 11 software. All experiments were performed in triplicate, and results are reported as mean ± standard deviation (SD).

3. Results and discussion

3.1 Selection of the DES condition

The extraction efficiency of triterpenoids from *A. sagittifolius* roots was significantly affected by the composition of the deep eutectic solvent (DES), particularly the molar ratio of choline chloride (ChCl) to citric acid (CA) and the water content incorporated into the system (Figure 1). As shown in Figure 1A, at a fixed water content of 40% (v/v), increasing the proportion of citric acid from a 1:1 to a 1:2 ChCl:CA molar ratio markedly enhanced the triterpenoid yield, which rose from 13.35 mg/g to 28.01 mg/g dry weight. This improvement can be attributed to the increased acidity and hydrogen bond donor capacity of the DES system, which enhances cell wall disruption and facilitates the release of triterpenoid compounds during microwave-assisted extraction [23]. However, further elevation of the CA proportion to 1:3 and 1:5 resulted in a substantial decrease in extraction efficiency (down to 16.56 mg/g). This phenomenon is likely due to excessive proton activity destabilizing the structural integrity of DES, elevating its viscosity, and consequently hindering mass transfer kinetics [18].

The effect of water addition was further examined at the optimal 1:2 ChCl:CA ratio (Figure 1B). Triterpenoid yield improved steadily as water content increased from 0% to 40%, with the highest efficiency (26.42 mg/g) obtained at 40%. This increase coincided with a pronounced reduction in solvent viscosity, decreasing from 1.01 Pa·s (10% water) to 0.42 Pa·s (40% water), which likely enhanced solvent diffusivity and facilitated penetration into plant tissues. Previous studies have also suggested that moderate water content may strengthen hydrogen bonding interactions with solutes, thereby improving triterpenoid solubilization [10,20,23]. By contrast, further increasing the water fraction to 50–60% resulted in a sharp decline in yield, with the 60% formulation producing ~44% lower extraction than at 40%. This reduction is plausibly linked to dilution of the eutectic matrix and disruption of its supramolecular network, diminishing its capacity to form strong hydrogen bonds and weakening its solvation power for hydrophobic analytes [19,23].

Overall, both the ChCl:CA molar ratio and the water fraction were found to critically modulate the physicochemical properties of DES and, consequently, the extraction performance. The ChCl–CA system at a 1:2 molar ratio with 40% water was identified as the most effective formulation and was selected for further optimization. The yields reported represent estimates from the vanillin–perchloric acid assay, a widely used though not fully specific method.

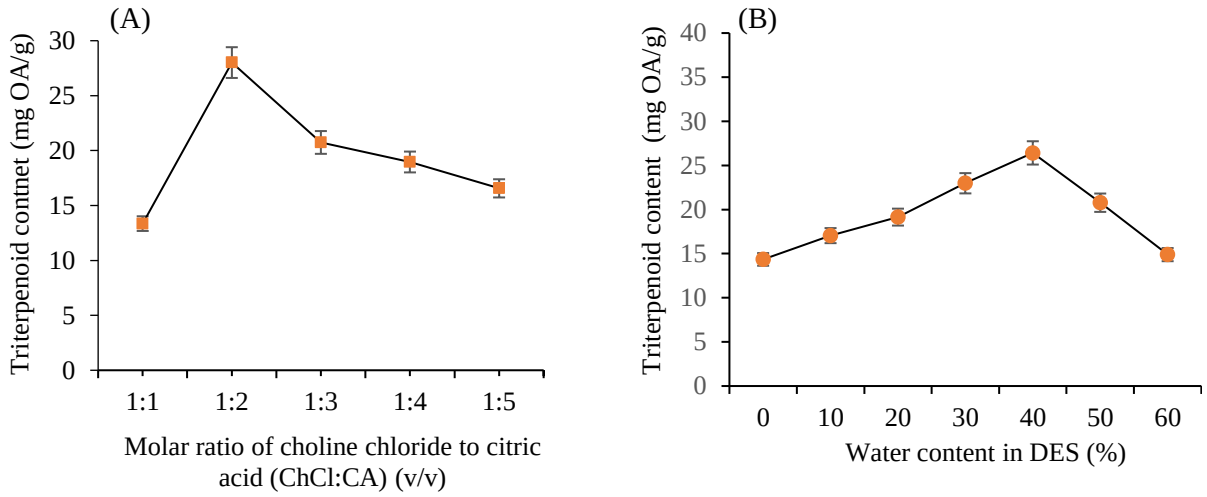

Figure 1 Effect of DES composition on triterpenoid content extracted from *A. sagittifolius* powder by microwave-assisted extraction: (A) molar ratio of choline chloride to citric acid (ChCl:CA) and (B) water content in DES.

3.2 Single factor experimental analysis

Microwave-assisted extraction (MAE) efficiency is markedly influenced by process variables such as microwave power, extraction time, and solvent-to-solid ratio [5,9]. To identify optimal conditions for triterpenoid extraction from *A. sagittifolius*, single-factor experiments were conducted to evaluate the individual effects of these parameters. When varying microwave power from 100 to 600 W while keeping the solvent-to-solid ratio and extraction time constant at 40 mL/g and 50 minutes, respectively, the yield of triterpenoids increased with increasing power, reaching a maximum of 28.54 mg/g at 300 W (Figure 2A). This increase is attributed to enhanced electromagnetic energy transfer that improves mass diffusion between the solvent and plant matrix [10,24]. However, power levels exceeding 300 W led to a significant decline in yield, likely due to thermal degradation of heat-sensitive compounds under excessive heating [9,15,25]. Similarly, extraction time showed a comparable trend (Figure 2B). When extraction was carried out at 300 W and a 40 mL/g solvent-to-solid ratio, triterpenoid content increased from 10 to 40 minutes and plateaued at 50 minutes before significantly decreasing. This suggests that 40 minutes is sufficient to reach equilibrium between solute and solvent, beyond which prolonged irradiation may result in co-extraction of impurities and degradation of target compounds [26]. Furthermore, the over exposure of triterpenoid to microwave radiation can decline the yield due to thermal degradation [24].

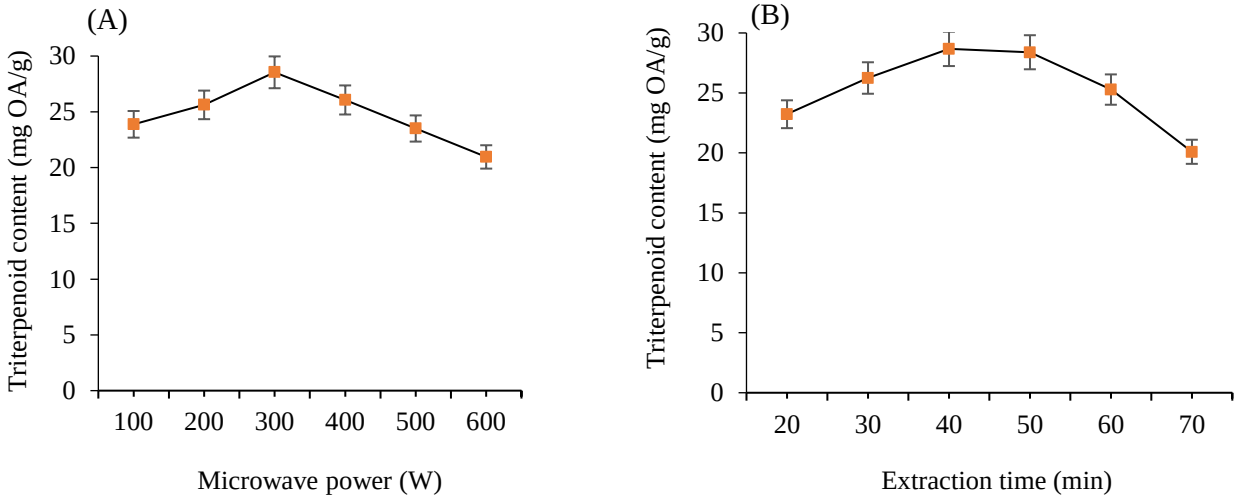

Figure 2 Effect of different: (A) microwave power (W); (B) extraction time (min); (C) solvent to liquid ratio (mL/g) on triterpenoid content.

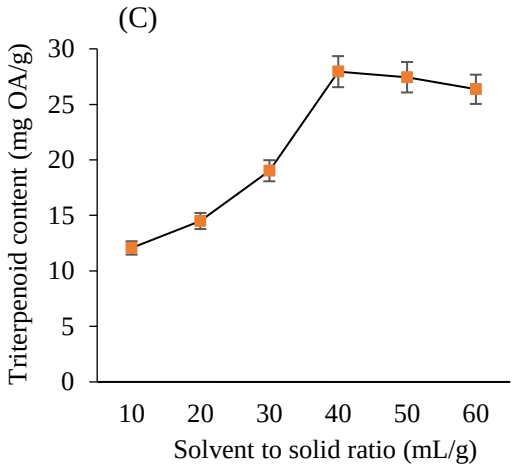

Figure 2 Effect of different: (A) microwave power (W); (B) extraction time (min); (C) solvent to liquid ratio (mL/g) on triterpenoid content.

Regarding the solvent-to-solid ratio (Figure 2C), increasing the ratio from 10 to 50 mL/g improved triterpenoid yield, with the highest yield (28.67 mg/g) observed at 40 mL/g. Enhanced solvent volume facilitates compound solubilization by lowering extraction medium viscosity and promoting better penetration into plant cells [25,27]. Nevertheless, a further increase to 60 mL/g slightly reduced yield to 26.36 mg/g, likely due to solute saturation and decreased mass transfer efficiency, while also raising processing costs and energy consumption [24]. These findings indicate that all three variables significantly affect extraction performance.

3.3 Fitting model and response surface analysis

To maximize triterpenoid yield from *A. sagittifolius* roots, microwave-assisted extraction (MAE) conditions were optimized using response surface methodology (RSM) based on a Box–Behnken design. Three independent variables were considered: microwave power (X_1, 100–600 W), extraction time (X_2, 10–60 min), and solvent-to-solid ratio (X_3, 20–70 mL/g), each evaluated at three levels. A total of 17 experimental runs were conducted, including five replicates at the central point to assess reproducibility and estimate experimental error. The observed triterpenoid contents are summarized in Table 1, with the highest yield (38.01 mg/g) recorded in Run 17, obtained under 350 W microwave power, 45 minutes of extraction, and a 45 mL/g solvent-to-solid ratio.

Table 1 Box–Behnken design of experimental conditions and observed responses for triterpenoid content of *A. sagittifolius* extracted using microwave-assisted deep eutectic extraction.

Run	Independent Variable			Triterpenoid content (Y; mg/g)		
	X_1 (W)	X_2 (min)	X_3 (mL/g)	Actual value	Predicted value	Residual
1	600 (+1)	45 (0)	70 (+1)	35.65±0.98	35.93	-0.28
2	350 (0)	45 (0)	45 (0)	37.90±1.12	37.91	-0.01
3	350 (0)	20 (-1)	70 (+1)	28.96±1.34	29.03	-0.07
4	350 (0)	45 (0)	45 (0)	37.93±0.92	37.91	0.02
5	100 (-1)	45 (0)	20 (-1)	27.92±0.78	27.62	0.3
6	100 (-1)	20 (-1)	45 (0)	28.65±1.23	28.07	0.58
7	350 (0)	45 (0)	45 (0)	37.09±1.21	37.91	-0.82
8	350 (0)	20 (-1)	20 (-1)	19.91±1.14	20.76	-0.85
9	600 (+1)	20 (-1)	45 (0)	22.16±1.09	21.75	0.41
10	350 (0)	45 (0)	45 (0)	38.67±0.98	37.91	0.76
11	350 (0)	70 (+1)	20 (-1)	28.06±1.01	27.87	0.19
12	600 (+1)	70 (+1)	45 (0)	34.65±1.14	35.23	-0.58
13	100 (-1)	45 (0)	70 (+1)	28.92±1.21	29.36	-0.44
14	350 (0)	70 (+1)	70 (+1)	35.61±1.02	34.74	0.87
15	600 (+1)	45 (0)	20 (-1)	23.04±1.09	22.55	0.49
16	100 (-1)	70 (+1)	45 (0)	27.45±1.01	27.41	0.04
17	350 (0)	45 (0)	45 (0)	38.01±1.01	37.91	0.1

Analysis of variance (ANOVA), as presented in Table 2, confirmed that the fitted quadratic model was highly significant (F-value = 107.91; $p < 0.0001$), indicating strong statistical reliability. The non-significant lack-of-fit ($F = 3.29$; $p = 0.14$) further demonstrated that the model was suitable for describing the experimental data [1–3]. Moreover, the low coefficient of variation (CV = 2.53%) suggested excellent precision, while the high coefficient of determination ($R^2 = 0.99$) and adjusted R^2 (0.98) indicated that the model could explain 98–99% of the variation in triterpenoid yield [19,24]. These statistical indices collectively confirmed the adequacy and robustness of the quadratic model for predicting extraction performance.

Among the model terms, the linear effects of X_1, X_2, and X_3, the quadratic effects of X_1^2 and X_2^2, and the interaction terms X_1X_2 and X_1X_3 were statistically significant ($p < 0.05$), as shown in Table 2. In contrast, the quadratic effect of X_3^2 was not significant and was excluded from the final regression model. This outcome implies that both microwave power and extraction time exhibited strong quadratic effects, while solvent-to-solid ratio primarily influenced the response in a linear manner. The final second-order polynomial equation predicting triterpenoid yield (Y) was expressed as:

$$Y=37.28+4.52X_1+2.03X_2+2.81X_3+4.71X_1X_2+3.88X_1X_3-8.03X_1^2-5.28X_2^2 \quad (3)$$

Table 2 Results of the ANOVA for the response surface quadratic model

Source	Sum of Squares	df	Mean Square	*F-value*	*P-value*
Model	605.70	9	67.30	107.91	< 0.0001
X_1	49.78	1	49.78	79.81	< 0.0001
X_2	26.88	1	26.88	43.11	0.0003
X_3	51.70	1	51.70	82.89	< 0.0001
X_1X_2	49.98	1	49.98	80.14	< 0.0001
X_1X_3	33.93	1	33.93	54.40	0.0002
X_2X_3	0.49	1	0.49	0.7857	0.4048*
X_1^2	85.92	1	85.92	137.76	< 0.0001
X_2^2	117.37	1	117.37	188.19	< 0.0001
X_3^2	86.49	1	86.49	138.67	< 0.0001
Lack of Fit	3.11	3	1.04	3.29	0.1399*
R^2	0.99		Adeq Precision	28.32	
Adjusted R^2	0.98		C.V. %	2.53	
Predicted R^2	0.91				

Df: degree of freedom; SS: sum of squares; MS: mean square; R^2: coefficient of determination; Adj R^2: adjusted R^2; Predicted R^2: Pred R^2; $p<0.05$ indicates statistical significance. *stands for insignificant differences ($p<0.05$).

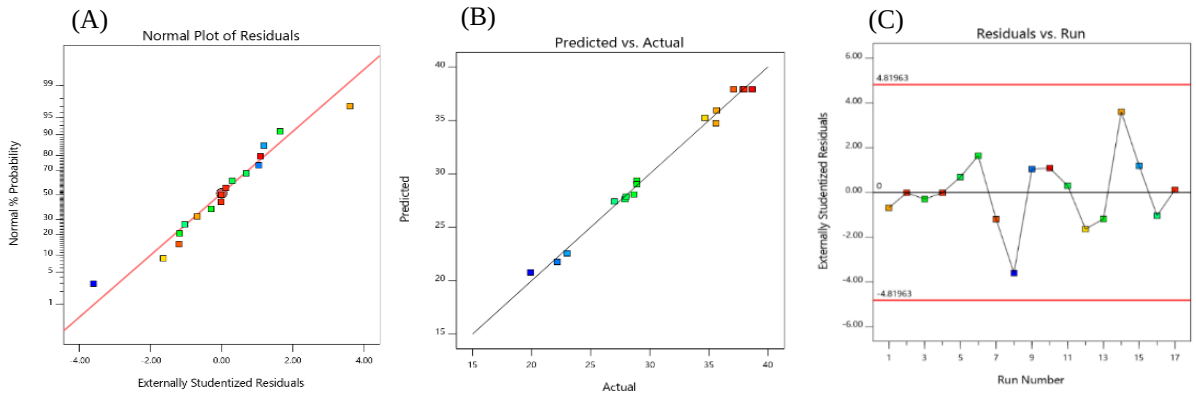

Figure 3 Analytical plots to verify the appropriateness of the model for the polysaccharide yield response: (A) Normal plot; (B) Predicted vs actual plot; (C) Residuals vs run plot

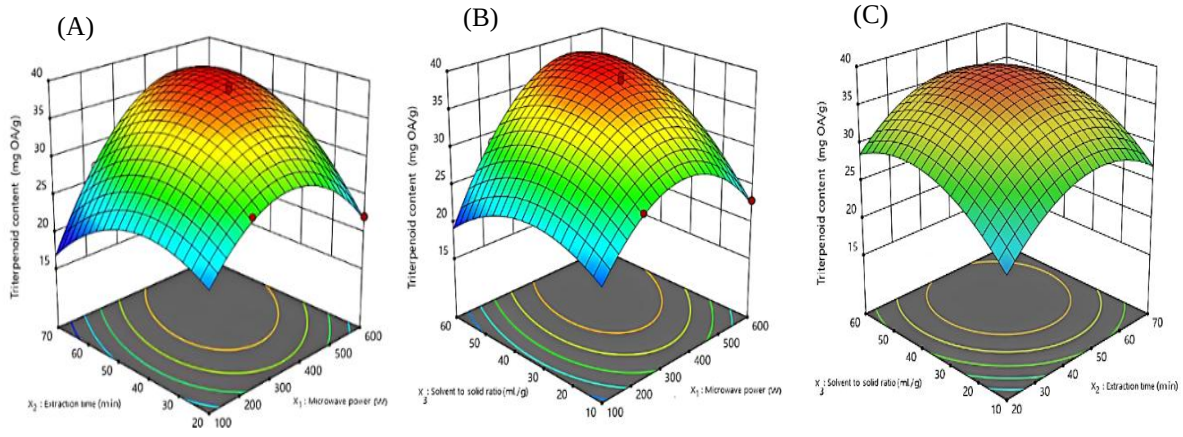

Figure 4 Response surface plots (3D) illustrating the effects of microwave-assisted extraction parameters on triterpenoid yield: (A) microwave power versus extraction time; (B) microwave power versus solvent-to-solid ratio; (C) solvent-to-solid ratio versus extraction time.

Diagnostic plots were used to validate the adequacy of the model (Figure 3). The normal probability plot of residuals confirmed that the residuals followed a normal distribution. The predicted vs. actual plot exhibited a high degree of agreement, indicating good model fit. Furthermore, the plot of standardized residuals against predicted values displayed no discernible patterns, supporting the assumptions of homoscedasticity and model validity. To visualize interactions among variables, three-dimensional (3D) response surface plots were generated (Figure 4). The three-dimensional response surface plots (Figure 4A–C) offer valuable insights into the interactive effects of the extraction parameters on triterpenoid yield.

Specifically, Figure 4A illustrates the combined influence of microwave power and extraction time at a constant solvent-to-solid ratio of 35 mL/g. Triterpenoid content was found to increase synergistically with both factors, reaching a maximum in the range of 300–350 W and 40–50 minutes. This enhancement is likely due to the improved dielectric heating at moderate microwave intensities, which accelerates the internal energy transfer, disrupts cellular structures, and promotes the migration of intracellular triterpenoids into the solvent medium [9,24]. However, beyond these optimal conditions, a progressive decline in yield was observed, which can be attributed to the thermal degradation of thermolabile compounds and the formation of undesirable radicals under prolonged or excessive microwave irradiation. These findings are consistent with previous reports by Hao et al. [27], who demonstrated similar thermal sensitivity during the extraction of bioactives from *Lactuca indica*.

Similarly, the interaction between microwave power and solvent-to-solid ratio (Figure 4B), with extraction time maintained at 45 minutes, revealed a bell-shaped trend in triterpenoid yield. The extraction efficiency improved with both increasing power and solvent volume, peaking at approximately 350–400 W and 35–40 mL/g. At these levels, the reduced viscosity and improved solute accessibility in the medium facilitate efficient mass transfer and diffusion of triterpenoids. However, further increases in the solvent-to-solid ratio (>60 mL/g) led to a noticeable decline in yield. This phenomenon may be attributed to a dilution effect that reduces the driving force for solute diffusion, as well as potential co-extraction of interfering substances that compromise purity and extraction selectivity. These results align with the principles of concentration gradient-driven mass transfer and have been previously documented in MAE studies involving other plant matrices [9,24].

The relationship between extraction time and solvent-to-solid ratio at a constant microwave power of 350 W is illustrated in Figure 4C. As extraction time increased, the triterpenoid yield rose steadily, attaining a maximum around 45 minutes. Prolonged durations beyond this point resulted in a modest decline in yield, likely due to the thermal instability of the extracted triterpenoids and potential over-extraction of undesired components. Notably, the effect of solvent volume was more pronounced at extended extraction times, suggesting that solvent availability becomes increasingly important as the extraction progresses. At shorter time intervals, the influence of solvent volume appeared minimal, implying that rapid heating and initial matrix disruption play a more dominant role during the early stages of MAE. These results reinforce previous observations [24] and underscore the importance of balancing extraction time with solvent accessibility to avoid excessive degradation or reduced selectivity. Taken together, the response surface plots emphasize the necessity of carefully optimizing all three variables—microwave power, extraction time, and solvent-to-solid ratio—in a coordinated manner. While moderate levels of each parameter synergistically enhance extraction efficiency by promoting cellular disruption, reducing solvent viscosity, and improving solute diffusion, their excessive levels may lead to counterproductive effects such as thermal decomposition, solvent dilution, or loss of specificity.

3.4 Optimum extraction conditions

Design-Expert software was employed to optimize the extraction parameters for triterpenoid recovery from *A. sagittifolius*. The optimal conditions predicted by the model were a microwave power of 365.20 W, an extraction time of 45.50 min, and a solvent-to-solid ratio of 39.71 mL/g, resulting in a predicted triterpenoid yield of 39.50 mg/g. For practical implementation, these parameters were slightly adjusted to 365 W, 46 min, and 40 mL/g. Under these experimental conditions, the triterpenoid yield reached 39.80 mg/g, which was in close agreement with the predicted value (39.50 mg/g), confirming the reliability of the optimization model. The triterpenoid yield obtained using DES-based MAE (39.80 mg/g) was significantly higher than that obtained using ethanol-based MAE (32.69 mg/g) and Soxhlet extraction (SE) with ethanol (28.14 mg/g). The differences in extraction efficiency can be attributed to the varying degrees of structural disruption of *A. sagittifolius* cells induced by each extraction method. SEM images (Fig. 5) provide clear evidence of these structural changes. The untreated raw material exhibited an intact and compact cellular structure with smooth surfaces (Fig. 5A). After Soxhlet extraction, the cell structure showed moderate surface damage and partial deformation, although much of the cellular framework remained relatively preserved (Fig. 5B). Similarly, ethanol-based MAE caused noticeable structural loosening and the formation of some cracks and cavities, indicating partial disruption of the cell wall (Fig. 5C). In contrast, samples treated with DES-based MAE under optimal conditions exhibited the most severe structural disruption, characterized by highly fragmented cell walls, numerous pores, and extensive cracks (Fig. 5D). This pronounced damage is likely due to the combined effects of microwave heating, rapid internal pressure generation, and the strong penetration ability of DES into the plant matrix, which collectively enhance cell wall rupture and facilitate the release of intracellular triterpenoids. These observations are consistent with the findings reported by Deng et al. [15], who demonstrated that DES-assisted extraction significantly increased cell wall porosity and promoted the release of triterpenoid saponins from *Gleditsia sinensis*. Therefore, DES-based MAE not only improves extraction efficiency but also effectively disrupts plant microstructures, highlighting its potential as an efficient and environmentally friendly technique for extracting bioactive compounds from *A. sagittifolius*.

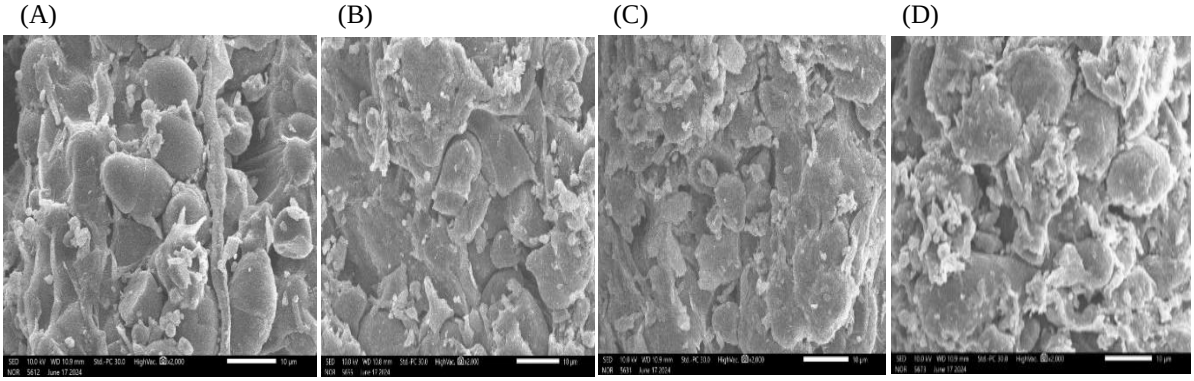

Figure 5 SEM images of *A. sagittifolius* cells under different extraction methods: (A) raw material; (B) Soxhlet extraction (SE); (C) ethanol-based microwave-assisted extraction (MAE); and (D) DES-based MAE under optimal conditions (365 W, 40 mL/g, 46 min).

3.5 In vitro antioxidant activity

The antioxidant activity of triterpenoid-rich extracts from *A. sagittifolius* was evaluated using DPPH and ABTS radical scavenging assays. Among the extraction methods examined, the microwave-assisted extraction (MAE) using a choline chloride–citric acid-based deep eutectic solvent (DES) showed the highest antioxidant activity, with ABTS and DPPH scavenging efficiencies of 96.67% and 84.56%, respectively, at a triterpenoid concentration of 0.55 mg/mL. These values were lower than those of the reference antioxidant ascorbic acid (98.15% for ABTS and 94.14% for DPPH), reflecting the inherently stronger radical scavenging capacity of the pure standard.

By contrast, ethanol-based MAE and conventional Soxhlet extraction produced extracts with lower antioxidant activities, exhibiting ABTS/DPPH scavenging efficiencies of 92.15%/81.03% and 89.10%/74.23%, respectively. The improved antioxidant performance of the DES–MAE extract is attributed to the synergistic effects of microwave-induced cell wall disruption and the favorable solvation characteristics of the DES. In particular, the extensive hydrogen-bonding network of the choline chloride–citric acid DES enhances triterpenoid solubility and extraction efficiency, thereby contributing to the higher antioxidant activity of the extract [9,15].

A similar trend was observed in the evaluation of tyrosinase inhibitory activity. The DES-based MAE extract exhibited the most potent enzyme inhibition, with an IC_{50} value of approximately 0.17 mg/mL, outperforming both ethanol-MAE (IC_{50} ~0.22 mg/mL) and Soxhlet-derived extracts (IC_{50} ~0.27 mg/mL). Notably, the IC_{50} of the DES-MAE extract was slightly lower than that of ascorbic acid (~0.15 mg/mL), but the result still showed a well-established tyrosinase inhibitor. It could be due to the ascorbic acid is a compound with strong antiradical activity. The lower IC_{50} value observed for the DES-MAE extract reflects its higher inhibitory potency, which is likely attributable to the increased concentration and improved bioavailability of triterpenoids in the extract. These findings are consistent with previous studies that have demonstrated a strong positive correlation between triterpenoid content and tyrosinase inhibitory activity [27].

Collectively, these results underscore the effectiveness of the choline chloride–citric acid DES in combination with microwave-assisted extraction as a green and efficient strategy for obtaining triterpenoid-enriched extracts from *A. sagittifolius*. The significantly enhanced antioxidant and tyrosinase inhibitory properties of the DES-MAE extract highlight its potential for application in the development of functional foods, cosmetics, and pharmaceutical products. Based on these findings, the extract obtained under optimized DES-MAE conditions was selected for further bioactivity and phytochemical characterization.

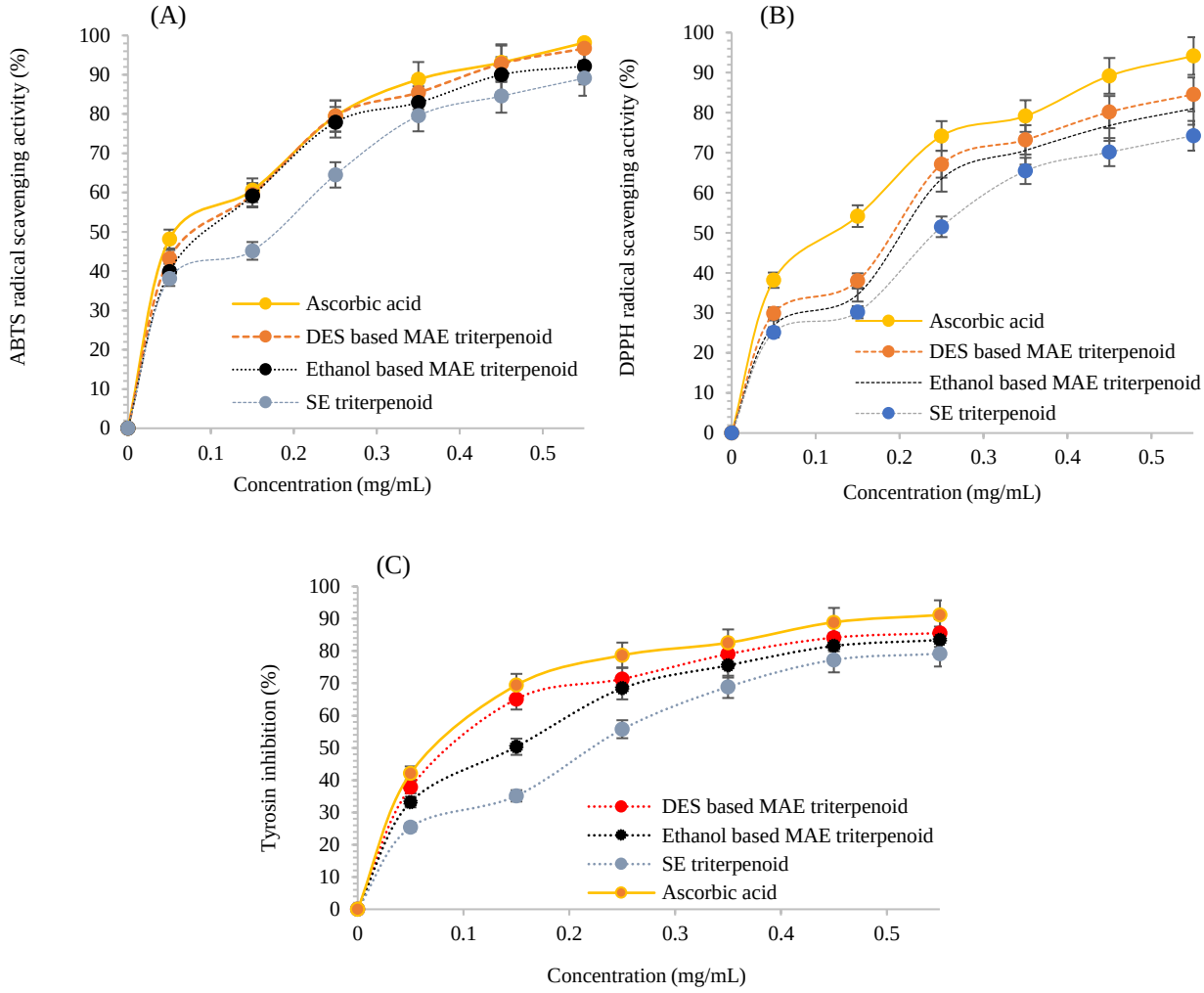

Figure 6. ABTS (A) and DPPH radical scavenging activity (B), and tyrosinase inhibition (C) of triterpenoids extracted from *A. sagittifolius* using DES-based MAE, ethanol-based MAE, and Soxhlet extraction (SE)

3.6 GC-MS analysis of bioactive compounds

Gas chromatography–mass spectrometry (GC–MS) analysis of the *A. sagittifolius* root extract revealed a total of 18 identifiable compounds spanning diverse chemical classes, including triterpenoids, sesquiterpenes, long-chain aliphatic hydrocarbons, phenolics, and esters (Fig. 7, Table 3). While triterpenoids did not dominate in terms of compound number, they accounted for a significant proportion of the total extract composition by relative abundance. Notably, friedelin, a pentacyclic triterpenoid, was the most abundant compound (21.23%), known for

its anti-inflammatory, antioxidant, and hepatoprotective properties [28]. Other triterpenoids—such as lupeol, oleanolic acid, β-amyrin, and ursolic acid—were also detected and are widely recognized for their multifunctional bioactivities [29,30].

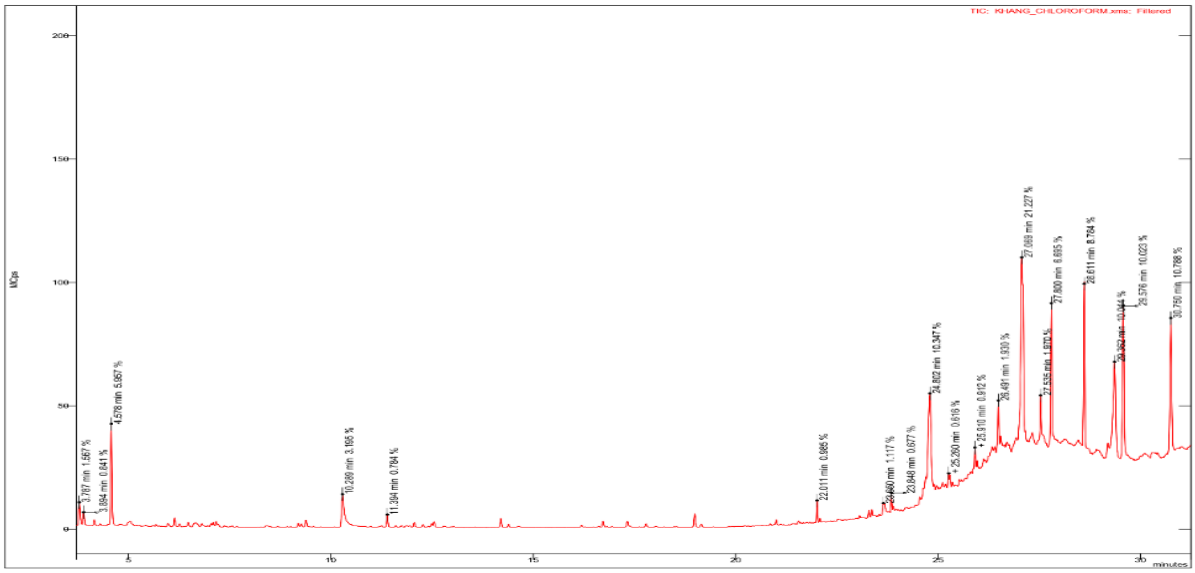

Figure 7 GC-MS results for analysis bioactive compounds from *A. sagittifolius*

Additionally, farnesane (10.02%), a sesquiterpene with structural similarities to compounds found in *Panax* species, may contribute to the extract's antimicrobial and anti-inflammatory properties. Several long-chain hydrocarbons—including (10E)-10-henicosene, 2-methylnonadecane, and 2,3-dimethylheptadecane—were also present in considerable amounts, suggesting roles in environmental stress protection. Although catechol and other phenolic compounds appeared in lower concentrations, their antioxidant potential remains relevant. The co-occurrence of bioactive triterpenoids such as lupeol, β-amyrin, and oleanolic acid with structurally similar components to *Panax* spp. supports the traditional medicinal use of *A. sagittifolius*. A small fraction of the extract (10.79%) comprised unidentified compounds, indicating the possibility of discovering novel bioactives. Overall, the GC-MS results suggest that triterpenoids, although not numerically dominant, are major contributors to the extract's bioactivity due to their high relative abundance and pharmacological relevance.

Table 3 Chemical compounds of *A. sagittifolius* extract

No.	Compound Name	RT (min)	Relative Concentration (%)	Classification Group
1	Catechol	3.787	1.567	Phenol
2	2,2-Dimethylpentanal	3.894	0.841	Aldehyde
3	Lupeol (TMS derivative)	4.578	5.957	Pentacyclic triterpenoid alcohol
4	Oleanolic acid (TMS)	10.289	3.195	Triterpenoid acid
5	β-Amyrin (TMS)	11.394	0.784	Triterpenoid
6	cis-7-Hexadecene	22.011	0.985	Alkene
7	(E)-5-Octadecene	23.650	1.117	Alkene
8	Ursolic acid (TMS)	23.848	0.677	Triterpenoid acid
9	(10E)-10-Henicosene	24.802	10.347	Alkene
10	1-Hexadecanol	25.260	0.616	Fatty alcohol
11	1-Docosene	25.910	0.912	Alkene
12	1-Hexacosene	26.491	1.930	Alkene
13	Friedelin	27.069	21.227	Triterpenoid ketone
14	Carbonic acid, eicosyl vinyl ester	27.535	1.970	Long-chain ester
15	2,3-Dimethylheptadecane	27.800	6.695	Branched alkane
16	2-Methyleicosane	28.611	8.784	Alkane
17	2-Methylnonadecane	29.362	10.044	Alkane
18	Farnesane	29.567	10.023	Sesquiterpene hydrocarbon
19	(Unidentified compound)	30.750	10.788	–

4. Conclusion

This study highlights the effectiveness of microwave-assisted extraction (MAE) with a choline chloride–citric acid deep eutectic solvent (ChCl–CA DES) for recovering bioactive compounds from *A. sagittifolius* roots. Optimal conditions (365 W, 46 min, 40 mL/g) yielded 39.80 mg/g triterpenoids, outperforming conventional methods in efficiency and antioxidant activity. GC–MS identified diverse phytochemicals, with friedelin, lupeol, oleanolic acid, and β-amyrin as major triterpenoids. The results validate the plant's traditional use and suggest its potential in functional food, nutraceutical, and pharmaceutical applications, while confirming DES–MAE as a green, efficient extraction strategy.

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6. Author contributions

Chi Hai Tran: Conceptualization, Investigation, Methodology, Data curation, Validation, Formal analysis, Writing – original draft; Hoang Nguyen Khang Le: Conceptualization, Methodology, Data curation; Thi Thu Hien Vu: Conceptualization, Investigation, Methodology, Formal analysis; Ngoc Hien Le: Conceptualization, Investigation, Formal analysis; Thanh Sang Nguyen: Formal analysis, Data curation, Validation; Van Man Phan: Conceptualization, Investigation, Data curation, Formal analysis, Writing – review & editing.

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