

Ultrasonic-assisted extraction of polyphenolic compounds from *Paederia scandens* (Lour.) Merr. Using deep eutectic solvent: optimization, identification, and comparison with traditional methods

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ABSTRACT

Ultrasonic-assisted extraction (UAE) coupled with deep eutectic solvent (DES) is a novel, efficient and green extraction method for phytochemicals. In this study, the effects of 16 DESs coupled with UAE on the extraction rate of polyphenols from *Paederia scandens* (Lour.) Merr. (*P. scandens*), an edible and medicinal herb, were investigated. DES synthesised with choline chloride and ethylene glycol at a 1:2 M ratio resulted in the highest extractability. Moreover, the effects of extraction parameters were investigated by using a two-level factorial experiment followed by response surface methodology. The optimal parameters (water content in DES of 49.2%, the actual ultrasonic power of 72.4 W, and ultrasonic time of 9.7 min) resulted in the optimal total flavonoid content (TFC) (27.04 mg CE/g DW), ferric-reducing antioxidant power (FRAP) value (373.27 $\mu\text{mol Fe(II)E/g DW}$) and 2,2'-azino-bis(3-ethylbenzthiazoline)-6-sulfonic acid radical (ABTS⁺) value (48.64 $\mu\text{mol TE/g DW}$), closely matching the experimental results. Furthermore, a comparison study demonstrated that DES-UAE afforded the higher TFC and FRAP value than traditional extraction methods. 36 individual polyphenolic compounds were identified and quantified by ultra-high-performance liquid chromatography-mass spectrometry (UHPLC-MS) in *P. scandens* extracts, and of which 30 were found in the extracts obtained by DES-UAE. Additionally, DES-UAE afforded the highest sum of individual polyphenolic compound content. These results revealed that DES-UAE enhanced the extraction efficiency for polyphenols and provided a scientific basis for further processing and utilization of *P. scandens*.

1. Introduction

Polyphenols, widely distributed in plants, are a very diverse and multifunctional group of phytochemicals, containing flavonoids, phenolic acids, anthocyanins, procyanidins, and stilbenes. Accumulating studies have shown that polyphenols have natural antioxidant

properties and play an important role in preventing food oxidation and exerting health-promoting effects [1,2]. For these reasons, polyphenols or polyphenolic extracts from herbs, fruits, and vegetables have been widely used in food, medicine, feed stuff and other fields [3,4].

Paederia scandens (Lour.) Merr. (*P. scandens*) ("Jishiteng" in Chinese), an edible herb primarily grown in southern China, Vietnam,

Abbreviations: UAE, ultrasonic-assisted extraction; DES, deep eutectic solvent; *P. scandens*, *Paederia scandens* (Lour.); Merr. ABTS⁺, 2,2'-azino-bis(3-ethylbenzthiazoline)-6-sulfonic acid radical; CE, catechin equivalent; CV, coefficient of variation; DES-UAE, ultrasonic-assisted extraction coupled with deep eutectic solvents; DW, dry weight; EtOH-UAE, ultrasonic-assisted extraction coupled with 80% ethanol; Fe(II)E, FeSO₄ equivalent; FRAP, ferric-reducing antioxidant power; MeOH-UAE, ultrasonic-assisted extraction coupled with 70% methanol; MS, mass spectra; NMR, Nuclear magnetic resonance; TE, Trolox equivalent; TFC, total flavonoid content; 3D, three-dimensional; UHPLC-MS, ultra-high-performance liquid chromatography-mass spectrometry; W-UAE, ultrasonic-assisted extraction coupled with ultrapure water.

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Japan and India, has been used as a Chinese traditional medicine for treating dyspepsia, jaundice, aches and dysentery, as well as used in many local traditional foods for centuries [5,6]. The medical injection of *P. scandens* made in China has been used for relieving pain [7]. Recent studies have reported that *P. scandens* shows anti-arthritis [6], antioxidant [8], antifungal [8], antinociceptive [9], uric-acid-lowering [10], anti-inflammatory, immunomodulatory [11], and anticonvulsant and sedative properties [12]. The plants belonging to *Paederia scandens* (Lour.) Merr. or *Paederia*, such as *Paederia scandens* (Lour.) Merr. var. *Tomentosa* and *Paederia foetida*, were reported to exert hepatoprotective effects [13], therapeutic effects on adjuvant-induced arthritis [14], antifungal, antioxidant and analgesic effects [15,7]. Some phytochemicals, including iridoid glycosides [11,16], volatile oil [16], and phenolic acids [8,16], have been identified in *P. scandens*. Moreover, flavonoids have been identified in *Paederia scandens* var. *Mairei* and *Paederia chinensis* Hance [17,18], which may be primarily responsible for their pharmacological properties. Among these phytochemicals, iridoid glycosides have been most commonly studied, while the other compounds have been explored sporadically. Due to the biological activities and health-promoting effects of polyphenols from plants, polyphenols from *P. scandens* have attracted considerable attention. Quercetin and kaempferol were identified from the fruits of *Paederia chinensis* Hance [18]. Ishikura et al. [17] isolated and identified 13 flavonoids by ^1H and ^{13}C nuclear magnetic resonance (NMR) from leaves and stems of *P. scandens* var. *Mairei*. Bordoloi et al. [8] identified 6 phenolic acids from the ethanolic extract of *P. scandens* using high-performance liquid chromatographic method with diode-array detection (HPLC-DAD) and found the potent antioxidant and antifungal activities of the ethanolic extract. Cai et al. [19] also found that leaves and stems of *P. scandens* were rich in anthocyanin, with potent antioxidant capacity. These studies also revealed that potent biological properties of *P. scandens* extracts were closely correlated with polyphenol profiles (flavonoids, phenolic acids, and anthocyanin). The extraction methods played a key role in the analysis of polyphenol profiles of the extracts [20–23]. Therefore, a high-efficiency method for extracting polyphenols from *P. scandens* is urgently needed.

DES is considered as a class of novel, efficient and green solvent, with the advantages of easy synthesis and a wide polarity range [24,25]. DES is formed with hydrogen-bond acceptors and hydrogen-bond donors via hydrogen-bond interactions [26]. Recently, DES has been used to extract bioactives, such as flavonoids and phenolic acids, from plants [20,23,24]. DES has shown higher extraction efficiency for polyphenols from plants as compared to traditional solvents [22,23]. UAE has been widely used for extracting protein, oil, and bioactives, including polyphenols, from dairy products, oilseeds, grains, vegetables and fruits [27,28]. UAE can also improve the extraction efficiency of bioactive compounds by disrupting the cell-wall structure of plants using acoustic cavitation [27,22]. DES-UAE significantly improved the extraction efficiency of polyphenols from ginger and *Moringa oleifera* L. leaves, as compared to UAE with organic solvents [22,23,29]. To the best of our knowledge, there is no study focusing on extraction of polyphenols from *P. scandens* using DES-UAE.

The present study screened the best DES to extract polyphenols from *P. scandens*, then optimized DES-UAE parameters to improve TFC and antioxidant capacity using a two-level factorial experiment, followed by response surface methodology. We also evaluated and compared the TFC, antioxidant capacity, and polyphenol profiles (identified and quantified by UHPLC-MS) of extracts obtained by DES-UAE and UAE coupled with traditional solvents.

2. Materials and methods

2.1. Materials and chemicals

The fresh aerial parts of *P. scandens* were purchased from the Dongmen Market (Haikou, Hainan Province, China) in August 2020 and

identified by Dr. Qiang Liu from the Department of Pharmacognosy, Hainan Medical University (Haikou, China). After washing with tap water, plants were dried at 50 °C for 8 h in an electric thermostat blast drying oven (DHG-9140A, Shanghai Yiheng Scientific Instrument Co. Ltd, Shanghai, China), crushed through a 60 mm mesh, packed and stored at – 20°C.

Apigenin, acacetin, diosmetin, hesperidin, rutin and quercitrin for UHPLC-MS were purchased from Qiyun Biotechnology (Guangzhou, China). Folin–Ciocalteu reagent, as well as the remaining polyphenol standards for UHPLC-MS, was purchased from Sigma Chemical Co., Ltd. (Shanghai, China). All chemicals used for DES synthesis were purchased from Aladdin Biochemical Technology Co., Ltd. (Shanghai, China). Total antioxidant capacity assay kits employing ABTS⁺ scavenging capacity method and FRAP method were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, Jiangsu, China).

2.2. Preparation of DESs

According to previously reported methods, 16 kinds of DESs were synthesized by mixing the hydrogen-bond acceptor and hydrogen-bond donor at the appropriate molar ratio with continuous stirring at 1500 rpm at 80 °C, forming a transparent and uniform liquid [24,22]. Chemicals and their molar ratios, as well as the abbreviations of the DESs, are shown in Table 1.

2.3. Determination of TFC

TFC was determined using the $\text{AlCl}_3\text{-NaNO}_2$ colorimetric method at 510 nm as described previously [30]. Briefly, the extract or a standard solution (250 μL) was mixed with deionized water (1.25 mL) and NaNO_2 solution (5% [w/w], 75 μL) sequentially. After 6 min, 10% (w/w) $\text{AlCl}_3\cdot 6\text{H}_2\text{O}$ solution (150 μL) was added, then 5 min later NaOH solution (1 M, 0.5 mL) was added followed by adjusting the total volume to 3.0 mL with deionised water. TFC was calculated by comparing the calibration curve of catechin and expressed as mg catechin equivalent per g of dry weight of plant powder (mg CE/g DW).

2.4. Antioxidant capacity

Antioxidant capacity was determined by commercial kits (FRAP and ABTS⁺ scavenging capacity assays) following the manufacturer's protocols. For FRAP assay, the extracts or FeSO_4 solutions ranging from 0.15 to 1.5 mmol (5 μL) was mixed with 180 μL of FRAP working solution and kept for 5 min at 37°C. The absorbance of the mixed solution was recorded at 593 nm. For ABTS⁺ scavenging capacity assay, the

Table 1
List of DESs.

Solvent abbreviation	Combination	Molar ratio
ChCl-MA	Choline chloride-Malic acid	1:1
ChCl-Gly	Choline chloride-Glycerol	1:2
ChCl-OA	Choline chloride-Oxalic acid	1:1
ChCl-Xyl	Choline chloride-Xylitol	1:1
Pro-Gly-1	L-Proline- Glycerol	2:5
ChCl-Lev	Choline chloride-Levulinic acid	1:2
ChCl-EG	Choline chloride-Ethylene glycol	1:2
ChCl-Glu	Choline chloride-Glucose	5:2
ChCl-TrG	Choline chloride-Triglycol	1:4
Bet-Lev	Betaine-Levulinic acid	1:2
Bet-Gly	Betaine-Glycerol	1:1
Pro-EG	L-Proline-Ethylene glycol	1:2
Pro-Lev	L-Proline-Levulinic acid	1:2
Pro-Gly-2	L-Proline-Glycerol	1:2
Pro-LA	L-Proline-Lactic acid	1:2
CA-Gly	Citric acid-Glycerol	1:2

DESs, deep eutectic solvents. DESs were chosen according to previous studies [22,23,25–27,29].

extracts or Trolox solutions ranging from 0.15 to 1.5 mmol (10 μ L) was mixed with 200 μ L of ABTS⁺ working solution and kept for 6 min at 37°C. The absorbance was recorded at 734 nm. Values were calculated by comparing standard curves of FeSO₄ and Trolox, and expressed as μ mol FeSO₄ equivalent per g of dry plant powder (μ mol Fe(II)/g DW) and μ mol Trolox equivalent per g of dry weight of plant powder (μ mol TE/g DW), respectively.

2.5. Optimization of extraction experiments

2.5.1. Screening of DESs

P. scandens powder (0.5 g) was added in the prepared DESs (5 mL, 40% water, v/v) in a centrifuge tube and then the centrifuge tube was put into a bath-type ultrasonic instrument (SB25-12DTD, Ningbo Xinzhi Biotechnology Co. Ltd) with a temperature-controlled system under an ultrasonic frequency of 40 KHz. The mixture was extracted at the actual ultrasonic power 77.0 W (the set ultrasonic power 320 W) at 40 °C for 30 min and centrifuged for 10 min at 13,000 rpm to obtain the supernatant. TFC of the supernatant was used to evaluate extraction efficiency of different DESs.

A calorimetric assay was used to measure the actual ultrasonic power [31]. Briefly, 15 L water was poured into the bath system. The ultrasonic process lasted for 30 min at the ultrasonic power set at 240 W, 300 W, 320 W or 360 W. The temperature was measured in the centrifuge tube before and after the ultrasonic process using thermometer. The temperature was collected at fifteen sites (four corners and the center at upper layer, middle layer, and bottom layer of water in the centrifuge tube from triplicate experiments. The actual ultrasonic power was calculated according to equation: the actual ultrasonic power (W) = $m \cdot C_w \cdot (dT/dt)$, where m was the mass (kg) of the water submitted to the ultrasonic processing, C_w was the specific heat of the water at constant pressure, and dT/dt was the rate of temperature rise during the process time (°C/s) [31]. The actual ultrasonic power for 240 W, 300 W, 320 W and 360 W was 57.7 W, 72.2 W, 77.0 W, and 86.6 W, respectively. In order to increase the accuracy, the actual ultrasonic power was used.

2.5.2. Two-level factorial experiment

A two-level factorial experiment was performed using Minitab v.17 (Philadelphia, PA, USA) to ascertain the effect of five factors (water content in DES [A], liquid–solid ratio [B], the actual ultrasonic power [C], ultrasonic time [D], and ultrasonic temperature [E]) and their interactive effects on TFC of *P. scandens* extracts (Table 2, Table 3), where TFC was the response. A normal plot of the standardized effects relied on position of effect points relative to the standard line, while a pareto chart of the standardized effects evaluated the significance of the primary or interactive effects according to the column magnitude in contrast to other columns [29]. The key factors with a remarkable influence on TFC of *P. scandens* were selected to conduct the response surface methodology.

2.5.3. Response surface experiment

Based on the results of two-level factorial experiment, water content in DES (A, 30%, 50% and 70%), the actual ultrasonic power (B, 57.7, 72.2 and 86.6 W), and ultrasonic time (C, 2, 10 and 18 min) were considered as the main driving factors. The impact of these three

Table 2
Factors and levels of two-level factorial experiment.

Independent variable	Units	Experimental value	
		Low (–1)	High (+1)
A: Water contents in DES	%	20	50
B: Liquid–solid ratio	mL/g	10	40
C: The actual ultrasonic power	W	57.7	86.6
D: Ultrasonic time	min	10	40
E: Ultrasonic temperature	°C	30	60

independent variables on the three related responses (TFC, FRAP value and ABTS⁺ value) were evaluated at a constant liquid–solid ratio (20 mL/g) and ultrasonic temperature (40 °C) by performing the Box–Behnken design using Design-Expert v. 8.0.5 (Table 4, Table 5, Fig. 3). The verification experiments were conducted as DES-UAE in 2.6.

2.6. Comparisons among DES-UAE and UAE coupled with traditional solvents

After mixing 0.5 g *P. scandens* powder with 10 mL of the above selected DES (choline chloride–ethylene glycol [ChCl–EG] with 49.2% of water content in it) (DES-UAE), ultrapure water (W-UAE), 80% ethanol (EtOH-UAE) or 70% methanol (MetOH-UAE), ultrasonic-assisted extraction was performed under the actual ultrasonic power 72.2 W (the set ultrasonic power 300 W) at 40 °C for 9.7 min. The supernatant was obtained by centrifugation at 13,000 rpm for 10 min. The traditional solvents used were chosen based on previous studies [27,32–35], which were efficient in polyphenolic extraction.

2.7. Identification and quantification of polyphenols by UHPLC-MS

UHPLC coupled with a Xevo triple quadrupole mass spectrometer system (Micromass Waters, Milford, MA, USA) was used for identification and quantification of polyphenols as per our previous methods, with some modifications [36]. An Acquity UHPLC BEH-C18 column (2.1 i.d. \times 100 mm, 1.7 μ m, Waters, USA) was used to separate polyphenols, with a gradient solvent system of 0.25% formic acid–water (elution A) and 0.25% formic acid–methanol (elution B) using the following parameters: 0–1 min, 5% B; 8 min, 25% B; 11 min, 60% B; 13 min, 100% B; 16 min, 100% B; and 16.1–20 min, 5% B. Polyphenols were identified using multiple reaction monitoring, and the basic structure of peaks were deduced by parent ions, fragment ions, and comparisons with published mass spectra (MS) data [37–51]. Furthermore, polyphenols were assigned by comparing the respective standards using UHPLC-MS, and their quantification was achieved by comparing the calibration curve of respective standards expressed as μ g per g of dry weight of plant powder (μ g/g DW). MS was measured between m/z 50–1000 with the following constant parameters: cone voltage, 30 V; capillary voltage, 2.0 kV; drying gas (N₂) flow, 1000 L/h; and drying gas temperature, 500 °C.

2.8. Statistical analysis

All data derived from experiments performed in triplicate were expressed as mean \pm standard deviation. Statistical analysis was performed with IBM SPSS 24.0 using one-way ANOVA, followed by Duncan's post hoc test, and p -value of < 0.05, was regarded as statistically significant.

3. Results and discussion

3.1. Selection of DES for the extraction of polyphenols from *P. Scandens*

DESs are effective for cell wall dissolution due to their high hydrogen-bond basicity, allowing efficient intermolecular interactions between the DES and cellulose strands [24,25]. Extraction efficiency is primarily related to DES polarity and viscosity, which are largely dependent on their constituents, the molar ratios of hydrogen-bond acceptors to hydrogen-bond donors and water content in DES [24,27]. DESs are commonly based on sugars, alcohols, amines, amino acids, and organic acids [26]. Several studies have shown that the compositions of DES used to efficiently extract polyphenolic compounds are based on choline chloride as the hydrogen-bond acceptors and polyalcohols, organic acids, sugar, or amides as the hydrogen-bond donors [26,27]. Therefore, in the present study, four types of hydrogen-bond acceptors (choline chloride, citric acid, betaine, and L-proline) and four types of hydrogen-bond donors (amide, acid, alcohol and glucose) were selected

Table 3
Experimental design and results of two-level factorial experiment.

Run	A: Water contents in DES (%)	B: Liquid-solid ratio (mL/g)	C: The actual ultrasonic power (W)	D: Ultrasonic time (min)	E: Ultrasonic temperature (°C)	TFC (mg CE/g DW)
1	20	40	57.7	10	60	18.52 ± 1.27
2	50	10	57.7	10	60	19.21 ± 0.39
3	50	40	57.7	40	60	19.90 ± 1.73
4	20	10	57.7	40	60	17.82 ± 1.23
5	20	40	57.7	40	30	13.99 ± 1.14
6	50	10	57.7	40	30	19.63 ± 0.75
7	20	40	86.6	40	60	14.75 ± 0.10
8	50	10	86.6	40	60	20.09 ± 0.83
9	50	40	86.6	10	60	24.90 ± 1.08
10	20	10	86.6	10	60	21.41 ± 0.79
11	20	40	86.6	10	30	21.17 ± 0.26
12	50	10	86.6	10	30	21.42 ± 0.94
13	50	40	57.7	10	30	20.08 ± 1.39
14	20	10	57.7	10	30	17.69 ± 0.60
15	50	40	86.6	40	30	19.38 ± 0.52
16	20	10	86.6	40	30	18.36 ± 0.66

Table 4
Box-Behnken design and resultant responses.

Run	A: Water content in DES (%)	B: The actual ultrasonic power (W)	C: Ultrasonic time (min)	TFC (mg CE/g DW)		FRAP (μmol Fe(II)/g DW)		ABTS ⁺ (μmol TE/g DW)	
				Actual value	Predicted value	Actual value	Predicted value	Actual value	Predicted value
1	70	72.2	18	19.42 ± 0.19	19.66	285.94 ± 9.40	290.96	47.56 ± 0.07	47.43
2	50	86.6	2	23.09 ± 0.11	23.02	339.04 ± 48.41	339.56	47.48 ± 0.04	47.40
3	50	57.7	18	22.16 ± 0.12	22.23	332.68 ± 4.96	332.16	47.36 ± 0.11	47.45
4	50	86.6	18	23.01 ± 0.12	22.77	365.85 ± 4.48	354.41	47.00 ± 0.02	46.98
5	70	57.7	10	22.97 ± 0.12	22.67	292.08 ± 6.66	287.58	48.00 ± 0.01	48.05
6	30	86.6	10	22.18 ± 0.21	22.48	353.87 ± 9.99	358.37	47.00 ± 0.11	46.95
7	50	57.7	2	22.08 ± 0.16	22.32	319.90 ± 3.35	331.34	47.83 ± 0.02	47.86
8	70	72.2	2	20.98 ± 0.33	21.04	286.06 ± 12.76	279.12	47.90 ± 0.10	47.83
9	50	72.2	10	27.59 ± 0.08	27.06	380.07 ± 18.24	372.33	48.60 ± 0.10	48.65
10	30	72.2	18	20.13 ± 0.04	20.06	335.58 ± 13.34	342.51	46.70 ± 0.16	46.77
11	30	57.7	10	21.11 ± 0.10	21.11	351.88 ± 21.03	345.46	46.97 ± 0.03	46.81
12	50	72.2	10	27.01 ± 0.08	27.06	363.64 ± 18.24	372.33	48.71 ± 0.10	48.65
13	30	72.2	2	19.26 ± 0.16	19.02	343.70 ± 34.56	338.68	47.08 ± 0.05	47.21
14	50	72.2	10	26.56 ± 0.08	27.06	373.29 ± 18.24	372.33	48.63 ± 0.10	48.65
15	70	86.6	10	22.53 ± 0.12	22.53	298.72 ± 10.26	305.14	46.82 ± 0.04	46.98

to prepare DESs. There was a significant difference among the TFC of *P. scandens* extracts obtained by the 16 DESs coupled with UAE ($p < 0.05$) (Fig. 1). The highest TFC of *P. scandens* extracts was obtained by ChCl-EG coupled with UAE (19.43 ± 0.53 mg CE/g DW), followed by ChCl-TrG coupled with UAE (17.65 ± 0.48 mg CE/g DW). Notably, CA-Gly and Bet-Gly coupled with UAE afforded the lowest TFC, which were 18.69% and 24.98% of the TFC obtained by ChCl-EG coupled with UAE. Different types of DESs were used to extract polyphenolic compounds and showed significantly different extraction efficiency [52]. Khezeli et al. [53] found that ChCl-EG (1:2) showed higher efficiency than ChCl-Gly (1:2), pure ethylene glycol and glycerol solvents in the extraction of ferulic, caffeic and cinnamic acids from seed oils. Cui et al. [54] found that 1,6-hexanediol-ChCl showed the highest efficiency in extracting

genistin, genistein and apigenin from pigeon pea roots among 18 kinds of DESs. García et al. [20] found that there was an obvious difference in polyphenolic compounds extracted by different DESs from virgin olive oil. DES polarity and its approximation to the polarity of extracts are very critical for the extraction efficiency, due to the principle of “compounds are more likely to dissolve in solvents with similar polarity” [24,27]. High viscosity of DESs results in low mass transfer and low compound diffusion [26]. For these reasons, in the present study, the highest TFC obtained by ChCl-EG coupled with UAE may be attributed to the high similarity between the polarities of ChCl-EG and polyphenols from *P. scandens*, along with suitable viscosity. Therefore, ChCl-EG was selected as the best green solvent for subsequent experiments.

Table 5
ANOVA for response surface quadratic model.

Source	df	Sum of squares		
		TFC	FRAP	ABTS ⁺
Model	9	92.49**	13527.79 **	6.35**
A- Water content	1	1.30*	6172.99**	0.81**
B- The actual ultrasonic power	1	0.77 ns	464.15 ns	0.43**
C- Ultrasonic time	1	0.06 ns	122.84 ns	0.34*
AB	1	0.57 ns	5.42 ns	0.37*
AC	1	1.47*	16.03 ns	0.00 ns
BC	1	0.01 ns	49.20 ns	0.00 ns
A ²	1	51.89***	5157.44*	2.24**
B ²	1	4.56*	432.56 ns	1.65**
C ²	1	41.70***	1809.79*	1.15**
Residual	5	0.96	667.80	0.13
R ²		0.9897	0.9530	0.9807
Adj R ²		0.97	0.8683	0.9459
Pre R ²		0.91	0.3793	0.7056
CV		1.93	3.45	0.33
Model (F-value)		53.42	11.25	28.21
Model (p-value)		0.0002	0.0079	0.0009
Lack of fit (F-value)		0.54	2.60	11.44
Lack of fit (p-value)		0.7007	0.2898	0.0815

ns, not significant ($p > 0.1$);

*, difference is significant at 0.05 level ($p < 0.05$);

** , difference is significant at 0.01 level ($p < 0.01$);

***, difference is significant at 0.001 level ($p < 0.001$).

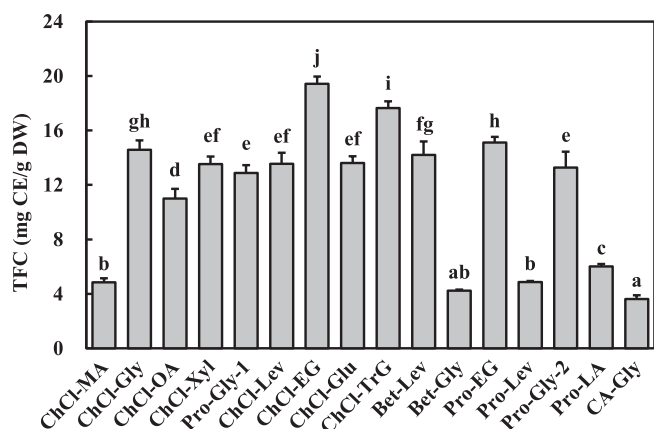


Fig. 1. Total flavonoid content (TFC) of *P. scandens* extracts obtained by 16 DESs coupled with UAE. Different letters indicate significant differences ($p < 0.05$). ChCl-MA, Choline chloride-Malic acid; ChCl-Gly; Choline chloride-Glycerol; ChCl-OA, Choline chloride-Oxalic acid; ChCl-Xyl, Choline chloride-Xylitol; Pro-Gly-1, L-Proline-Glycerol (2:5); ChCl-Lev, Choline chloride-Levulinic acid; ChCl-EG, Choline chloride-Ethylene glycol; ChCl-Glu, Choline chloride-Glucose; ChCl-TrG, Choline chloride-Triglycol; Bet-Lev, Betaine-Levulinic acid; Bet-Gly, Betaine-Glycerol; Pro-EG, L-Proline-Ethylene glycol; Pro-Lev, L-Proline-Levulinic acid; Pro-Gly-2, L-Proline-Glycerol (1:2); Pro-LA, L-Proline-Lactic acid; CA-Gly, Citric acid-Glycerol.

3.2. A two-level factorial experiment for selection of factors significantly affecting TFC

Water content in DES plays an important role in enhancing the extraction of bioactive substances from plants by altering DES polarity and lowering the viscosity [26,27]. Moreover, liquid–solid ratio, ultrasonic time, the actual ultrasonic power, and ultrasonic temperature can largely influence extraction efficiency [22,29]. In this study, the experimental model provided by a two-level factorial experiment (Table 2, Table 3) was used to generate the normal plot and pareto chart (Fig. 2) for highlighting the primary and interaction effects on TFC.

As shown in Fig. 2a, significant effects presented as single factors (water content in DES [A], the actual ultrasonic power [C], ultrasonic

time [D], and ultrasonic temperature [E]), and interactive factors (AB, AD, CD, and BD). Moreover, the magnitudes of A, C, D, E, AB, AD, CD, and BD were all larger than the significant marker line, among which the top three significant factors were A, D and C in sequence (Fig. 2b). Consequently, water content in DES, the actual ultrasonic power, and ultrasonic time were chosen for the subsequent response surface experiment.

3.3. Response surface experiment

3.3.1. Model fitting

As shown in Table 4, TFC, FRAP values, and ABTS⁺ values ranged from 19.26 – 27.59 mg CE/g DW, 285.9–380.1 μmol Fe(II)/g DW, and 46.7–48.7 μmol TE/g DW, respectively. As shown in Table 5, F-value and p-value revealed that the model was significant ($p < 0.01$), whereas the loss of fit of each model was insignificant ($p > 0.05$). For all responses, the R² was higher than 0.95 and adjusted determination coefficients (adj. R²) were close to 0.9, revealing good model accuracy and a higher correlation between experimental and predicted values, which was confirmed by the low coefficient of variation (CV). Interaction among variables was presented using three-dimensional (3D) surface plots in Fig. 3.

3.3.2. Effect of variables on TFC

As shown in Table 5, the linear effect of water content in DES (A) and secondary effect of A², B² and C² illustrated significant effects on TFC. The significant interaction between water content in DES (A) and ultrasonic time (C) on TFC was presented. The addition of water in DES varied DES polarity and viscosity [52], resulting in a change in extraction efficiency. Numerous studies found water content in DES was the main factor for DESs' high extraction efficiency for various bioactive compounds including polyphenols [22,23,26,27,29,52]. The relationship between TFC and these variables was expressed by the following equation (1):

$$Y_{TFC} = 27.06 + (0.40 \times A) + (0.31 \times B) - (0.087 \times C) - (0.38 \times AB) - (0.61 \times AC) - (0.04 \times BC) - (3.75 \times A^2) - (1.11 \times B^2) - (3.36 \times C^2) \quad (1)$$

To clarify the interaction of the three variables on the TFC, 3D response surfaces (Fig. 3A1–A3) were applied. Fig. 3B showed the significant interaction between water content in DES (A) and ultrasonic time (C) on ABTS⁺, which raised with increasing water content in DES (A) up to approximately 49%, followed by a sharp reduction. DES polarity raised with increasing water content in DES. Extraction solvents with polarity close to polyphenolic compounds showed high extractability [24,27,52]. Moreover, an increase in water content in DES reduced the viscosity of DESs, increasing mass transfer and compound diffusion [25,26]. Furthermore, ultrasonic treatment increased the extraction rate by disrupting the cell wall and facilitating solvent penetration through the plant tissue [24,27]. However, ultrasonic treatment for longer time may result in polyphenol degradation [27,28]. These results indicated high extraction efficiency of ChCl-EG coupled with UAE for polyphenols was mainly attributed to the similar polarity of ChCl-EG with polyphenols, the low viscosity of ChCl-EG that facilitated compound diffusion, and the suitable ultrasonic parameters that facilitated the solvent penetration.

3.3.3. Effect of variables on antioxidant capacity

FRAP value was significantly affected by A ($p < 0.01$), A² ($p < 0.05$) and C² ($p < 0.05$), among which water content in DES (A) showed a more significant effect on FRAP value. ABTS⁺ value was significantly affected by A, B, A², B², and C² ($p < 0.01$), followed by C and AB ($p < 0.05$). However, the interaction of AC and BC had no significant effect on ABTS⁺ value ($p > 0.05$). The antioxidant capacity (FRAP and ABTS⁺) of *P. scandens* extracts was largely affected by water content in DES, which markedly changed DES polarity. DES polarity was primarily responsible for the composition and content of polyphenols in the extracts [20–23]. Polyphenols are a large class of plant secondary metabolites with diverse

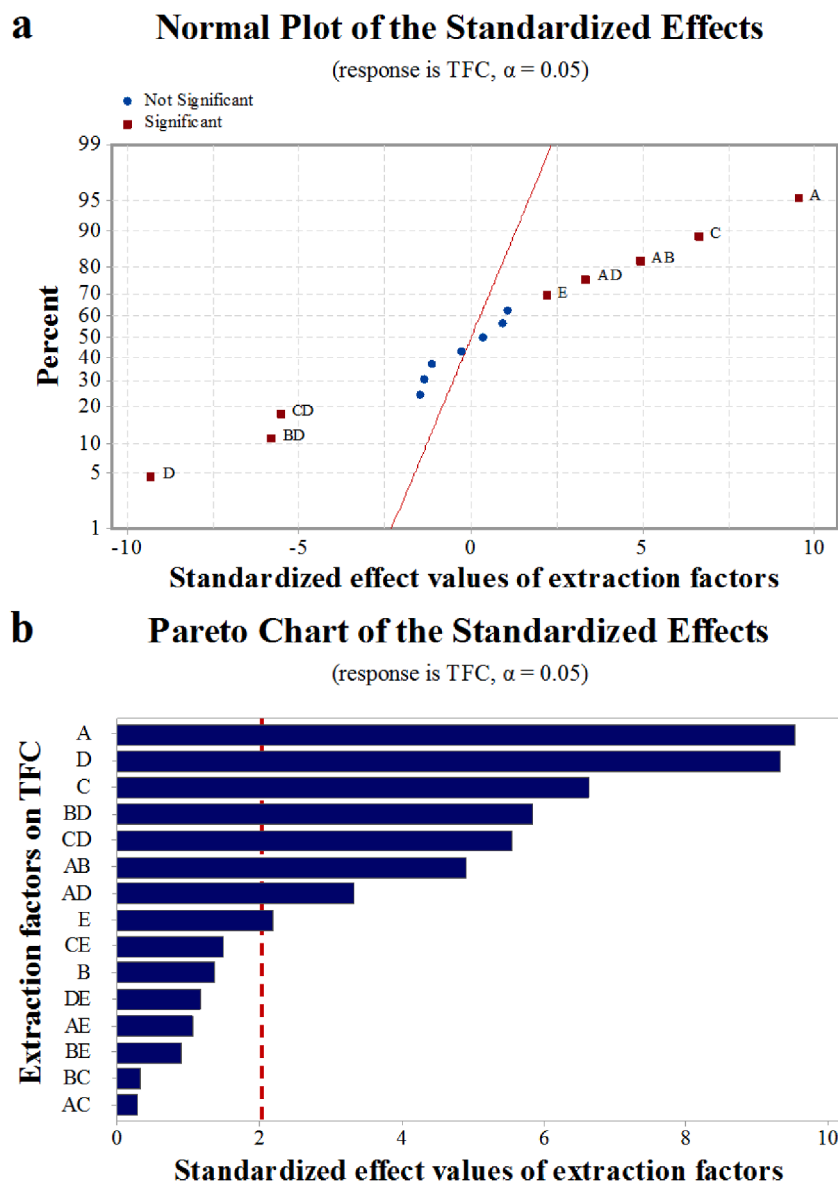


Fig. 2. The normal plot (a) and the pareto chart (b) obtained from two-level factorial experiment showing the significance of the primary and interaction effects. Factor A, water content in DES; Factor B, liquid–solid ratio; Factor C, the actual ultrasonic power; Factor D, ultrasonic time; Factor E, ultrasonic temperature.

structure and varying polarity [1]. Antioxidant capacity of extracts was closely associated with composition and content of polyphenols in the extracts [30,22,29].

Antioxidant capacity (FRAP and ABTS^+) models were demonstrated by equations (2) and (3), and the 3D surface plots (Fig. 3B1–B3, C1–C3) were established according to equations (2) and (3).

$$Y_{\text{FRAP}} = 372.33 - (27.78 \times A) + (7.62 \times B) + (3.92 \times C) + (1.16 \times AB) + (2.00 \times AC) + (3.51 \times BC) - (37.37 \times A^2) - (10.82 \times B^2) - (22.24 \times C^2) \quad (2)$$

$$Y_{\text{ABTS}^+} = 48.65 + (0.32 \times A) - (0.23 \times B) - (0.21 \times C) - (0.30 \times AB) + (9.65 \times 10^{-3} \times AC) - (3.5 \times 10^{-3} \times BC) - (0.78 \times A^2) - (0.67 \times B^2) - (0.56 \times C^2) \quad (3)$$

3.3.4. Verification of optimally predicted DES-UAE parameters

To substantiate the reliability of the response surface model design, experiments under the optimal parameters of DES-UAE predicted by the model were performed. Based on regression analysis of 3D surface plots and independent variables, the optimal extraction parameters for TFC were listed as follows: 49.2% of water content in DES, 72.4 W of the actual ultrasonic power (the set ultrasonic power 301.3 W), and 9.7 min

of ultrasonic time. In order to adjust ultrasonic power easily, the verification experiments were performed under 72.2 W of the actual ultrasonic power (the set ultrasonic power 300 W), 49.2% of water content in DES, and 9.7 min of ultrasonic time. The experimental values determined were 27.09 ± 0.48 mg CE/g DW for TFC, 335.17 ± 4.32 $\mu\text{mol Fe (II)E/g DW}$ for FRAP, and 48.91 ± 1.99 $\mu\text{mol TE/g DW}$ for ABTS^+ , which accurately matched our predicted values with a low error (Table 6).

3.4. Comparisons among DES-UAE and other methods

3.4.1. TFC and antioxidant capacity

A comparative study was conducted among DES-UAE and UAE coupled with traditional solvents to verify the high extractability of DES-UAE for polyphenols from *P. scandens*. As shown in Table 6, DES-UAE displayed significantly higher TFC and FRAP value than other methods ($p < 0.05$). The TFC and FRAP value obtained by DES-UAE were 1.51–2.02-fold and 1.37–1.79-fold of those obtained by other methods, respectively. Moreover, there was no significant difference between ABTS^+ value obtained by DES-UAE and W-UAE ($p > 0.05$), whereas DES-UAE displayed significantly higher ABTS^+ value than

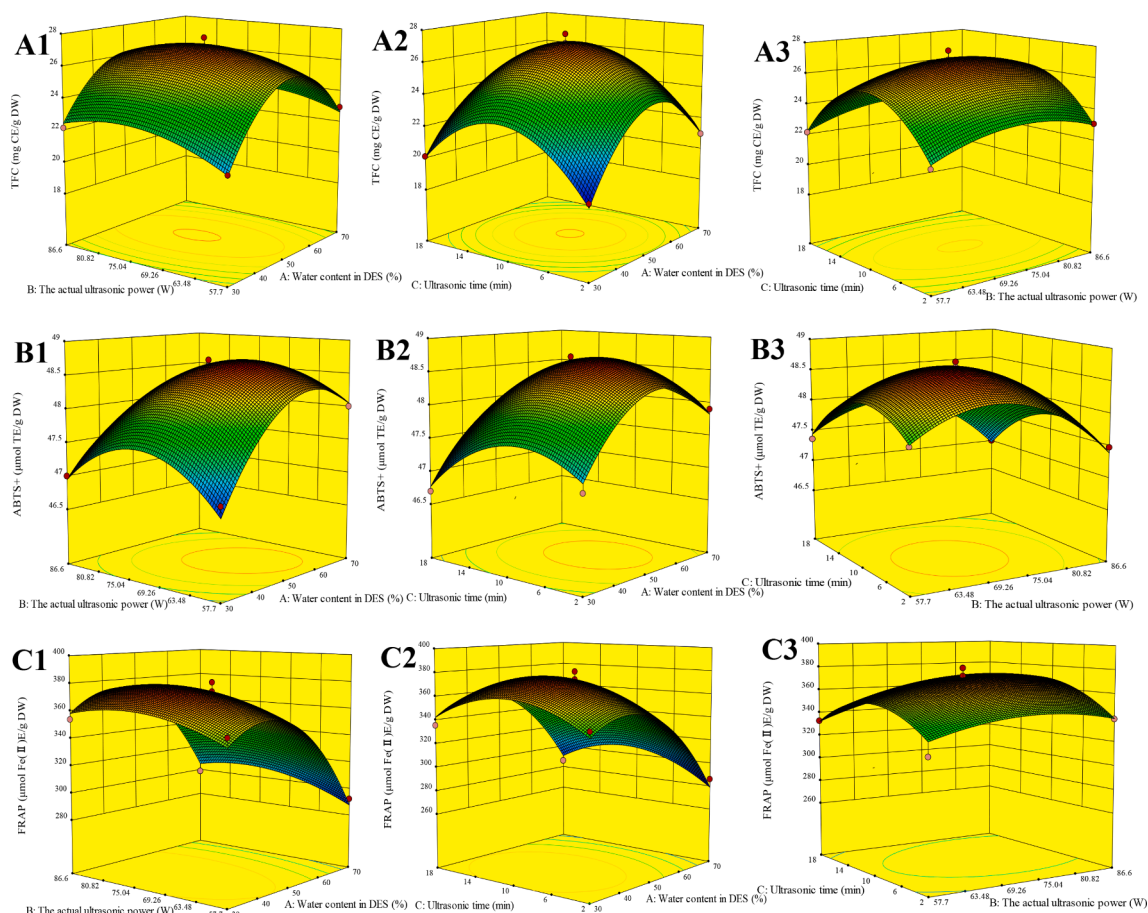


Fig. 3. 3D response surface curve showing the effects of independent variables on the TFC (A1-A3), ABTS⁺ (B1-B3) and FRAP (C1-C3). Mutual effects of water content in DES and the actual ultrasonic power on TFC (A1); Mutual effects of water content in DES and ultrasonic time on TFC (A2); Mutual effects of the actual ultrasonic power and ultrasonic time on TFC (A3); Mutual effects of water content in DES and the actual ultrasonic power on ABTS⁺ (B1); Mutual effects of water content in DES and ultrasonic time on ABTS⁺ (B2); Mutual effects of the actual ultrasonic power and ultrasonic time on ABTS⁺ (B3); Mutual effects of water content in DES and the actual ultrasonic power on FRAP (C1); Mutual effects of water content in DES and ultrasonic time on FRAP (C2); Mutual effects of the actual ultrasonic power and ultrasonic time on FRAP (C3).

Table 6
Comparative TFC and antioxidant capacities of *P. scandens* extracts obtained by DES-UAE and other methods.

Extraction methods	TFC (mg CE/g DW)	FRAP(μmol Fe (II) E/g DW)	ABTS ⁺ (μmol TE/g DW)
DES-UAE (Predicted value)	27.04	373.27	48.64
DES-UAE (Experimental value)	27.09 ± 0.48c	335.17 ± 14.32c	48.91 ± 1.99c
W-UAE	17.95 ± 0.49b	222.76 ± 11.99b	46.30 ± 3.01bc
EtOH-UAE	17.88 ± 0.41b	186.89 ± 11.41a	44.25 ± 0.16ab
MetOH-UAE	13.54 ± 0.08a	195.49 ± 2.42a	43.83 ± 0.45ab

Different letters in same column indicate significant differences ($p < 0.05$). UAE, ultrasonic-assisted extraction; DES-UAE, W-UAE, EtOH-UAE, MetOH-UAE: ultrasonic-assisted extraction coupled with deep eutectic solvent (DES-UAE), ultrapure water, 80% ethanol and 70% methanol, respectively.

other methods ($p < 0.05$). These results indicated that DES-UAE was a high efficiency method compared with UAE coupled with traditional solvents (ultrapure water, 80% ethanol, and 70% methanol), which were usually used in the previous studies and showed a high efficiency for extracting polyphenols [27,32–35,30,36]. DES-UAE reportedly improved TFC and antioxidant capacity of *Moringa oleifera* L. leave

extracts, compared with UAE coupled with traditional solvents [29,22]. DES-UAE was a novel and high efficiency method for extracting polyphenols from *P. scandens*.

3.4.2. Identification of polyphenolic compounds by UHPLC-MS

Thirty-six polyphenols comprising of 5 benzoic acids, 5 hydroxycinnamic acids, 15 flavonols, 4 flavones, 1 isoflavone, 3 flavanones, 1 anthocyanidin, 1 procyanidin, and 1 stilbene were identified in *P. scandens* extracts, all of which were further confirmed with reference standards using UHPLC-MS. Besides these, many compounds still remain unknown (Table 7).

Peaks 1, 3, 4, and 5 at m/z 169.0, 137.0, 136.96, and 196.9 with their product ions 125.0 [$M + H - CO_2$]⁺, 93.0 [$M - H - CO_2$]⁻, 108.0 [$M - H - CHO$]⁻, and 168.9 [gallic acid - H]⁻ were tentatively identified as vanillic acid, 2-hydroxybenzoic acid, protocatechualdehyde and ethyl gallate, respectively [37–39]. Vanillin (peak 2) was identified by its protonated ion [$M + H$]⁺ m/z 153.0, and product ions m/z 138.0 [$M + H - CH_3$]⁺, m/z 125.0 [$M + H - CO$]⁺, and m/z 93.0 [$M + H - CO - CH_3OH$]⁺, which was consistent with MS data from Flamini et al. [40]. Peaks 6–10, identified as *p*-coumaric acid, ferulic acid, caffeic acid, *trans*-cinnamic acid, and rosmarinic acid, showed precursor ions [$M - H$]⁻ at m/z 163.1, 193.0, 179.0, 146.95, and 358.96 and their product ions at m/z 119.0 [$M - H - CO_2$]⁻, 91.0 [$M - H - CO_2 - C_2H_4$]⁻ (peak 6), 149.0 [$M - H - CO_2$]⁻, 178.0 [$M - H - CH_3$]⁻ (peak 7), 135.0 [$M - H - CO_2$]⁻ (peak 8), 118.9 [$M - H - CO$]⁻ (peak 9), and 196.96 [dihydroxyphenyl-lactic acid - H]⁻, 161.0 [caffeic acid - H - H₂O]⁻

Table 7
Identification of polyphenolic compositions in *P. scandens* by UHPLC-MS.

Polyphenol sub-classes	Peak no.	λ_{\max} (nm)	Tentative assignment	Model	Parents ions	Fragment ions	Reference
Benzoic acid and derivatives	1	259,291	vanillic acid	+	169.0	125.0, 93.0	[37]
	2	275	vanillin	+	153.0	138.0, 125.0, 93.0	[40]
	3	260,294	2-hydroxybenzoic acid	–	137.0	93.0	[38]
	4	274,308	protocatechualdehyde	–	136.96	108.0, 81.0	[39]
	5	272	ethyl gallate	–	196.9	168.9, 124.1	[37]
Hydroxycinnamic acid and derivatives	6	270,307	<i>p</i> -coumaric acid	–	163.1	119.0, 91.0	[41,37]
	7	299,323	ferulic acid	–	193.0	178.0, 149.0, 134.0	[42,41,37]
	8	299,323	caffeic acid	–	179.0	135.0, 79.0	[42,41,37]
	9	278,306	<i>trans</i> -cinnamic acid	–	146.95	118.9, 77.0, 40.1	[38]
	10	290,328	rosmarinic acid	–	358.96	196.96, 161.0	[43]
Flavonol	11	280	catechin	–	289.07	244.9, 204.9, 137.1	[38,44]
	12	280	epicatechin	–	289.07	244.9, 204.9, 137.1	[44]
	13	270	(-)-epigallocatechin	–	304.98	179.0, 124.98	[44]
	14	270	(-)-gallocatechin	–	304.98	179.0, 124.98	[44]
	15	254,352	myricitrin	–	462.9	316.99	[45]
	16	255,347	quercetin	–	301.0	179.0, 151.0	[43,42,38]
	17	250,354	hyperoside	–	463.0	300.9, 270.9, 254.9	[38]
	18	255,355	rutin	–	609.0	301.1, 270.9, 178.7	[41]
	19	257,356	quercitrin	–	447.0	301.0, 179.0, 151.0	[42,41]
	20	257,356	isoquercitrin	–	447.0	301.0, 179.0, 151.0	[43,41]
	21	256,354	guaiaverin	–	432.9	270.9, 300.9	[46]
	22	266,348	kaempferol 3-O-rutinoside	–	592.9	254.9, 284.8	[46]
	23	264,346	astragaline	–	446.9	226.98, 254.9, 285.1	[46]
	24	254,343	isorhamnetin-3-O-glucoside	–	476.9	313.96, 242.8	[46]
Flavone	25	254,354	narcissin	+	624.9	85.1, 316.9, 478.9	[46]
	26	268,338	apigenin	+	271.0	227.0, 151.0	[42]
	27	270,335	acacetin	+	285.0	153.0	[49]
	28	267,345	diosmetin	+	301.0	153.0, 111.0, 255.0, 257.0	[50]
Isoflavone	29	252,348	cynaroside	–	446.9	107.0, 133.0, 284.8	[46]
	30	260,327	genistein	–	268.9	108.7, 132.9, 159.0	[41]
Flavanone	31	283,327	hesperidin	–	609.0	301.0	[41]
	32	270,350	isovitexin	–	430.9	280.9, 253.0	[48]
	33	270,334	cosemetin	–	430.9	268.9	[47]
Anthocyanidin	34	276,530	cyandinin	+	286.9	109.1, 137.0	[51]
Procyanidin	35	280	procyanidin B2	–	576.9	288.99, 406.9	[46]
Stilbene	36	282,308	<i>trans</i> -piceid	+	391.1	229.1, 135.0	[37]
	37	–	unknown	–	469.3	417.3, 371.2, 315.3, 217.1	
	38	–	unknown	–	747.4	471.2, 419.3, 373.4	
	39	–	unknown	–	952.9	872.3, 619.1, 277.4	
	40	–	unknown	–	944.9	625.1, 485.3, 355.1, 299.4, 281.0	

(peak 10), respectively [37,41,42,38,43]. Peaks 11 and 12 were observed as the same precursor ion $[M - H]^-$ at m/z 289.07, and the same product ions m/z 244.9 $[M - H - CO_2]^-$ and 137.1 $[M - H - 152]^-$ (typical retro Diel-Alder fragmentation) with different retention time, and were distinguished as catechin and epicatechin [38,44]. Peaks 13–14 produced the same precursor ion $[M - H]^-$ at m/z 304.98 and product ions at m/z 179.0 $[M - H - C_6H_6O_3]^-$ and 124.98 $[M - H - 152]^-$ with different retention times, and were identified as (-)-epigallocatechin and (-)-gallocatechin [44]. Gallic, chlorogenic, vanillic, ferulic, *p*-coumaric, and caffeic acids have previously been identified by HPLC-DAD from the ethanolic extract of *P. scandens* by Bordoloi et al. [8], whereas 3-hydroxy-4-methoxybenzaldehyde, 2-hydroxybenzoic acid, 2,5-dihydroxybenzoic acid methyl ester, as well as 12 iridoid glycosides, were isolated and identified from 70% ethanol extract of *P. scandens* by MS [16].

Quercetin (peak 16) with deprotonated ions $[M - H]^-$ at m/z 301.0 and retro Diel-Alder fragments of flavon-3-ols (m/z 179.0, 151.0) were identified as quercetin, which was consistent with MS data from previous studies [38,42,43]. Flavonoid glycosides containing O-glycoside usually showed a loss of glycoside as the initial breakdown [55]. Myricitrin (peak 15), was authenticated by deprotonated molecule $[M - H]^-$ ion at m/z 462.9 and product ion m/z 316.99 (aglycone myricetin) for the loss of rhamnoside (–146 Th) [45]. Peaks 17–25 were respectively identified as hyperoside, rutin, quercitrin, isoquercitrin, guaiaverin,

kaempferol 3-O-rutinoside, astragaline, isorhamnetin-3-O-glucoside, and narcissin by the loss of galactoside (–162 Th), rutinoside (–308 Th), rhamnoside (–146 Th), glucoside (–162 Th), or arabinoside (–132 Th) group [38,41,42,43,46]. Similarly, cynaroside (peak 29), hesperidin (peak 31), and cosemetin (peak 33) were distinguished by the loss of glycoside [46,41,47]. Quercetin and kaempferol were found in the fruits of *Paederia chinensis* Hance [18]. A previous study identified 13 flavonol-O-glycosides, including astragaline (kaempferol-3-O-glycoside), kaempferol-3-O-rutinoside, kaempferol-7-O-glycoside, isoquercitrin (quercetin-3-O-glycoside), kaempferol-3-O-rutinoside-7-O-glycoside, quercimeritrin (quercetin-7-O-glycoside), rutin (quercetin-3-O-rutinoside), quercetin-3-O-rutinoside-7-O-glycoside, paederinin (quercetin-3-O-rutinoside-7-O-xylosylglucoside), three unknown quercetin-O-glycosides and one unknown kaempferol-O-glycoside using 1H and ^{13}C NMR from *P. scandens* var. *Mairei*. leaves and stems [17]. Flavonol-3-O-glycoside may be the characteristic compounds of Rubiaceae, including *Paederia* plants, as the 3-O-glycoside of quercetin and kaempferol found in the present study have also previously been reported in many plants of Rubiaceae [7,56], including *P. scandens*. However, rutin and quercetin were not found in the ethanol maceration extract of *P. scandens* from India determined by HPLC-DAD [8].

Isovitexin (apigenin-6-C-glucoside) (peak 32) was characterized by the deprotonated ion $[M - H]^-$ at m/z 430.9 and product ions at m/z 280.9 corresponding to the fragment $[M - H - 150]^-$ and m/z 253.0 $[M$

– H – 150 – CO][−] as reported by Pereira et al. [48], which has a different fragmentation pathway with its isomer apigenin-7-O-glucoside (cosmetin). Apigenin (peak 26) was characterized by the protonated ion [M + H]⁺ at *m/z* 271.0 and product ions *m/z* 227.0 and 151.0 [42]. Acacetin (peak 27) and diosmetin (peak 28) were respectively observed as protonated ions [M + H]⁺ at *m/z* 285.0 and 301.0, with the same product ion at *m/z* 153.0 corresponding to the retro Diel-Alder cleavage of C-ring [49,50]. Genistein (peak 30) was characterized by the parent ion [M – H][−] at *m/z* 268.9 and product ion at *m/z* 132.9, consistent with MS data from Mattonai et al. [41]. Cyanidin (peak 34), a typical anthocyanidin, was identified by the deprotonated ion [M – H][−] at *m/z* 286.9 and product ions corresponding to fragments [M – H – 150][−] and [M – H – 150 – CO][−] [51]. Cai et al. [19] found that leaves and stems of *P. scandens* were rich in anthocyanin. Procyanidin B2 (peak 35) was identified by the deprotonated ion [M – H][−] at *m/z* 576.9 and product ion at *m/z* 288.99 corresponding to loss of the (epi)catechin entity [46]. Trans-piceid (peak 36) was identified by its loss of glucoside to form resveratrol and further assigned by comparison with retention time of *trans*-piceid standard [37].

3.4.3. Quantification of individual polyphenolic compounds by UHPLC-MS

As shown in Table 8, the number and contents of individual polyphenolic compounds obtained by DES-UAE and other methods varied considerably. There were 30 individual polyphenolic compounds in the extracts obtained by DES-UAE and MetOH-UAE, followed by EtOH-UAE (29 compounds) and W-UAE (25 compounds).

Moreover, the highest sum of individual flavonoid content and sum of individual polyphenolic compound content were obtained by DES-UAE, which were 1.16-fold and 1.13-fold higher than those obtained by W-UAE, respectively ($p < 0.05$). However, the highest sum of individual phenolic acid content was obtained by W-UAE, followed by DES-UAE, MetOH-UAE, and EtOH-UAE in sequence ($p < 0.05$).

Among phenolic acid compounds, 2-hydroxybenzoic acid, protocatechualdehyde, *p*-coumaric acid, ferulic acid, caffeic acid and *trans*-cinnamic acid were extracted by all methods. The first five were the main components of phenolic acids, and the sum of their contents ranged from 94.54% to 96.96% of the sum of individual phenolic acid content. The 2-hydroxybenzoic acid content differed among the extracts obtained by different extraction methods and ranged from 38.88 ± 0.79 to 44.26 ± 1.26 $\mu\text{g/g}$ DW. Compared with DES-UAE, W-UAE showed an insignificant 2-hydroxybenzoic acid content ($p > 0.05$), but the others showed significantly lower 2-hydroxybenzoic acid contents ($p < 0.05$). 2-hydroxybenzoic acid accounted for 66.28%–72.19% of the sum of individual phenolic acid content and 4.66%–5.52% of the sum of individual polyphenolic compound content. Bordoloi et al. [8] reported that phenolic acid compounds identified by HPLC-DAD from the ethanolic extract of *P. scandens* accounted for 100% of the sum of individual polyphenolic compound content, whereas the flavonoid compounds accounted for 0%.

Among flavonoid compounds, hyperoside, rutin, quercitrin, isoquercitrin, guaiaverin, kaempferol-3-O-rutinoside, astragaline, narcissin, apigenin, diosmetin, and cyanidin were extracted by all methods. Rutin, guaiaverin, kaempferol-3-O-rutinoside, narcissin were the main components of flavonoid compounds and the sum of their contents accounted for 88.17%–94.67% of the sum of individual flavonoid content and 79.95%–88.40% of the sum of individual polyphenolic compound content. These results revealed that rutin, guaiaverin, kaempferol-3-O-rutinoside, and narcissin were the main polyphenolic compounds of *P. scandens*. The highest rutin content was found in the extracts obtained by DES-UAE, followed by W-UAE, EtOH-UAE, and MetOH-UAE ($p < 0.05$). While the highest guaiaverin content was found in the extracts obtained by DES-UAE, EtOH-UAE, and MetOH-UAE ($p > 0.05$). There was no significant difference in kaempferol-3-O-rutinoside content among the extracts obtained by all extraction methods ($p > 0.05$). The highest narcissin content was found in the extracts obtained by DES-UAE and W-UAE ($p > 0.05$).

Table 8

Quantitation of individual polyphenolic compounds in *P. scandens* by UHPLC-MS.

Compounds	Content ($\mu\text{g/g}$ DW)			
	DES-UAE	W-UAE	EtOH-UAE	MetOH-UAE
vanillic acid	1.64 \pm 0.11a	2.01 \pm 0.13b	ND	1.80 \pm 0.10ab
vanillin	0.36 \pm 0.02a	0.42 \pm 0.02b	0.40 \pm 0.02ab	0.42 \pm 0.01b
2-hydroxybenzoic acid	42.20 \pm 1.58b	44.26 \pm 1.26b	38.88 \pm 0.79a	39.58 \pm 0.26a
protocatechualdehyde	1.92 \pm 0.18b	2.98 \pm 0.12c	1.42 \pm 0.05a	2.10 \pm 0.08b
ethyl gallate	ND	ND	0.12 \pm 0.01	ND
<i>p</i> -coumaric acid	4.78 \pm 0.19a	9.80 \pm 0.23c	5.46 \pm 0.26b	5.82 \pm 0.22b
ferulic acid	5.28 \pm 0.28b	0.86 \pm 0.04a	4.76 \pm 0.37b	7.40 \pm 0.25c
caffeic acid	2.56 \pm 0.20b	5.64 \pm 0.21c	1.70 \pm 0.10a	1.56 \pm 0.08a
<i>trans</i> -cinnamic acid	0.70 \pm 0.04ab	0.62 \pm 0.04a	0.72 \pm 0.02b	0.64 \pm 0.02a
rosmarinic acid	0.40 \pm 0.02a	ND	0.40 \pm 0.02a	0.40 \pm 0.02a
<i>Number of phenolic acids</i>	9	8	9	9
<i>Sum of individual phenolic acid content</i>	59.84 \pm 1.50b	66.58 \pm 1.45c	53.86 \pm 1.25a	59.72 \pm 0.83b
catechin	0.16 \pm 0.01b	ND	0.10 \pm 0.01a	0.16 \pm 0.01b
epicatechin	0.38 \pm 0.01a	ND	0.48 \pm 0.02b	0.50 \pm 0.02b
(-)-epigallocatechin	ND	ND	ND	0.36 \pm 0.02
(-)-gallicocatechin	0.10 \pm 0.003c	0.06 \pm 0.002a	0.08 \pm 0.002b	0.06 \pm 0.002a
myricitrin	0.70 \pm 0.03a	ND	0.88 \pm 0.04b	0.86 \pm 0.04b
quercetin	0.88 \pm 0.03b	0.06 \pm 0.003a	1.88 \pm 0.16d	1.04 \pm 0.06c
hyperoside	12.98 \pm 0.55b	10.96 \pm 0.46a	13.42 \pm 0.52b	13.38 \pm 0.39b
rutin	582.76 \pm 13.69c	472.66 \pm 9.89b	438.08 \pm 11.23a	427.68 \pm 14.68a
quercitrin	9.34 \pm 0.34b	3.62 \pm 0.16a	10.54 \pm 0.42c	10.22 \pm 0.41c
isoquercitrin	10.84 \pm 0.35b	8.90 \pm 0.35a	10.90 \pm 0.41b	11.04 \pm 0.46b
guaiaverin	56.48 \pm 3.02b	9.32 \pm 0.41a	54.62 \pm 1.56b	55.5 \pm 2.59b
kaempferol 3-O-rutinoside	108.00 \pm 6.25a	109.96 \pm 5.65a	101.82 \pm 3.65a	100.08 \pm 5.26a
astragaline	2.64 \pm 0.12a	2.48 \pm 0.13a	2.66 \pm 0.15a	2.58 \pm 0.10a
isorhamnetin-3-O-glucoside	0.22 \pm 0.01b	0.68 \pm 0.03c	ND	0.16 \pm 0.01a
narcissin	53.36 \pm 1.58b	49.46 \pm 1.99a	47.88 \pm 1.56a	49.34 \pm 1.85a
apigenin	0.34 \pm 0.02c	5.84 \pm 0.20d	0.26 \pm 0.01b	0.18 \pm 0.01a
acacetin	0.04 \pm 0.002	ND	ND	ND
diosmetin	4.40 \pm 0.14b	41.00 \pm 1.36c	4.40 \pm 0.16b	3.34 \pm 0.12a
cynaroside	0.18 \pm 0.01a	ND	0.34 \pm 0.02b	0.72 \pm 0.02c
genistein	ND	0.24 \pm 0.01	ND	ND
hesperidin	ND	0.90 \pm 0.02	ND	ND
isovitexin	ND	ND	0.06 \pm 0.002	ND
cosmetin	0.38 \pm 0.01b	ND	0.46 \pm 0.01c	0.16 \pm 0.01a
cyanidin	1.48 \pm 0.07a	11.30 \pm 0.21d	5.94 \pm 0.15c	1.92 \pm 0.10b

(continued on next page)

Table 8 (continued)

Compounds	Content ($\mu\text{g/g DW}$)			
	DES-UAE	W-UAE	EtOH-UAE	MetOH-UAE
<i>Number of flavonoids</i>	20	16	19	20
<i>Sum of individual flavonoid content</i>	845.66 \pm 24.35c	727.44 \pm 18.69b	694.80 \pm 17.62ab	679.28 \pm 23.35a
procyanidin B2	0.12 \pm 0.01a	ND	0.18 \pm 0.01b	0.24 \pm 0.01c
<i>trans-piceid</i>	ND	0.22 \pm 0.01	ND	ND
<i>Number of polyphenolic compounds</i>	30	25	29	30
<i>Sum of individual polyphenolic compound content</i>	905.62 \pm 25.85c	802.24 \pm 20.05b	748.84 \pm 18.87a	739.24 \pm 24.18a

Different letters in same row indicate significant differences ($p < 0.05$). ND, not detected; DES-UAE, W-UAE, EtOH-UAE, MetOH-UAE: ultrasonic-assisted extraction with deep eutectic solvent (ChCl-EG), ultrapure water, 80% ethanol and 70% methanol, respectively.

Typically, the polyphenol profiles depend on extraction methods, including extraction solvents, assisted extraction technology, and extraction parameters used. Traditional and eco-friendly solvents coupled with/without UAE gave significant different polyphenolic compositions of *Morinda citrifolia L.* leaves [24,27]. Su et al. [30] found that different extraction solvents and different extraction methods contributed to the variations of free and bound polyphenol profiles of litchi pulp, respectively. The present study found that polyphenol profiles of *P. scandens* among all extraction methods were greatly different, which was consistent with the previous studies [23,29,30]. The difference in polarity of extraction solvents mainly contributed to variations of polyphenol profiles, as well as TFC and antioxidant capacity of the extracts [23,27,29,30]. In the present study, with the same assisted extraction technology UAE and the identical extraction parameters, four extraction solvents (ChCl-EG, ultrapure water, 80% ethanol and 70% methanol) showed different polyphenol profiles of *P. scandens*, along with varying TFC and antioxidant activities, demonstrating the important effect of extraction solvents on extraction efficiency for polyphenols. Compared to ultrapure water, 80% ethanol and 70% methanol, ChCl-EG had a different polarity, and a strong hydrogen-bond basicity, effectively facilitating intermolecular interactions between DESs and cellulose strands of plants [25,26,27]. Among all extraction methods, DES-UAE exhibited the highest sum of individual flavonoid content and sum of individual polyphenolic compound content, as well as highest TFC and FRAP value, indicating that DES-UAE was more efficient than other methods. Moreover, UAE, considered as a green extraction technology, played a pertinent role in improving extraction rates of phytochemicals by disrupting the cell-wall structure of plants using acoustic cavitation [28,27,22]. Therefore, the ChCl-EG coupled with UAE was efficient in extracting polyphenols from *P. scandens*.

4. Conclusions

The present study revealed significant differences in the TFC and antioxidant activities among the 16 DESs coupled with UAE, and wherein choline chloride chloride and ethylene glycol at a 1:2 M ratio showed the highest extractability. DES-UAE optimization using a two-level factorial experiment followed by response surface methodology revealed the optimum parameters (water content in DES of 49.2%, the actual ultrasonic power of 72.4 W, and ultrasonic time of 9.7 min). The experimental TFC and antioxidant capacity closely matched the predicted results. Furthermore, there were significant differences in TFC, antioxidant capacity, and polyphenol profiles among DES-UAE and other extraction methods. 30 individual polyphenolic compounds were found in the extracts obtained by DES-UAE. Additionally, DES-UAE showed the highest sum of individual polyphenolic compound

content, as well as the highest TFC and FRAP value. These results revealed the high extractability of DES-UAE.

CRediT authorship contribution statement

Yuxin Liu: Methodology, Resources, Project administration, Writing – original draft. **Wang Zhe:** Investigation, Project administration. **Ruifeng Zhang:** Writing – review & editing, Funding acquisition. **Ziting Peng:** Data curation, Software. **Yuxi Wang:** Software, Supervision. **Heqi Gao:** Software, Supervision. **Zhiqiang Guo:** Validation, Writing – review & editing, Formal analysis. **Juan Xiao:** Conceptualization, Funding acquisition, Supervision, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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