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RESEARCH ARTICLE

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Qualitative and quantitative analysis of *Porana sinensis* Hemsl by UHPLC-Q-Exactive MS, TLC autographic method and DART-MS

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Abstract

Introduction: *Erycibe obtusifolia* and *E. schmidtii* are widely used in traditional Chinese medicine (TCM) to treat joint pain and rheumatoid arthritis. With the reduction of wild *E. obtusifolia* and *E. schmidtii* resources, *Porana sinensis* has been widely used as a substitute. However, few studies have been conducted on the chemical composition and quality control of *P. sinensis*.

Objective: To clarify the chemical composition and improve the quality control of *P. sinensis*.

Methodology: We developed an ultra-high performance liquid chromatography electrospray ionisation Q-Exactive Focus tandem mass spectrometry (UHPLC-ESI-Q-Exactive Focus-MS/MS) method to characterise the chemical constituents of *P. sinensis*. A strategy based on a combination of high-performance thin-layer chromatography (HPTLC) and direct analysis in real-time (DART) ion source was proposed for the identification of alkaloid components in *P. sinensis*. Thin-layer chromatography (TLC) autography for 2,2′-diphenyl-1-picrylhydrazyl free radical (DPPH˙) and TLC bioautography for xanthine oxidase were used to rapidly screen marker compounds for high-performance liquid chromatography (HPLC) determination of *P. sinensis*. Based on the selected marker compounds, a HPLC method for the quantitative determination of eight marker compounds in *P. sinensis* was developed.

Results: Eighteen compounds in *P. sinensis* were identified by UHPLC-Q-Exactive MS. Taken together with the results of TLC autography and TLC bioautography, eight compounds were chosen as marker compounds for HPLC determination of *P. sinensis*. The alkaloid components in *P. sinensis* were identified as Baogongteng A and Baogongteng C by DART-MS.

Conclusion: We systematically clarified the chemical composition of *P. sinensis* for the first time, and potentially improved its quality control. These results should promote the application of *P. sinensis* as a new resource for Caulis Erycibes.

KEYWORDS

DART-MS, *Porana sinensis*, qualitative and quantitative analysis, TLC autographic method, UHPLC-Q-Exactive MS

Zhiyong Chen and Mengmeng Wang contributed equally to this work.

1 | INTRODUCTION

The *Erycibe* genus of the Convolvulaceae family commonly grows in southern Asia, southeast Asia and Australia. Several *Erycibe* plant species are used in traditional Chinese medicine (TCM) to treat joint pain and rheumatoid arthritis.[1,2] Currently, *Erycibe* resources in the wild are unable to meet clinical needs; therefore, the caulis of *Porana sinensis* has been widely used as a substitute, as in the preparation of Fengliaoxing Fengshi Dieda wine.[3] *Porana sinensis* is a wild woody plant belonging to the family Convolvulaceae. The plant is naturally found on mountains in southeast Asia and southern Asia. Our previous study identified similarities between *P. sinensis* and *Erycibe* plant species with respect to their main active components as well as anti-nociceptive and anti-inflammatory activities.[4] According to the literature,[5] *P. sinensis* exhibited greater capacities scavenging 2,2′-diphenyl-1-picrylhydrazyl ($DPPH^{\cdot}$), (2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid ($ABTS^{+\cdot}$) and 2,2'-azobis(2-amidinopropane) dihydrochloride ($AAPH^{\cdot}$) radicals than did *E. schmidtii*. Further anti-inflammatory experiments on NO release and transcription of inflammatory factors iNOS, COX-2, IL-6 and IL-1β showed that *P. sinensis* possessed higher anti-inflammation activities than did *E. schmidtii*, with similar mechanisms. As mentioned earlier, with the reduction of the wild *Erycibe* resources, *P. sinensis* has been widely used as a substitute. Nevertheless, few studies have been conducted on the chemical composition and quality control of *P. sinensis*.

TCM is a multi-component, multi-target agent that treats complex diseases in a holistic way.[6-8] Therefore, it is essential to evaluate the chemical composition of each TCM.[9] In this study, a Q-Exactive Focus tandem mass spectrometry (MS/MS) method was used to obtain high-resolution mass spectra of the various components in *P. sinensis*. Total alkaloid contents Baogongteng A and Baogongteng C in *Erycibe* plant species are toxic substances with cholinergic functions.[10] However, alkaloids in *P. sinensis* have not been systematically explored due to the lack of detection and identification methods. In the current study, high-performance thin-layer chromatography (HPTLC)-direct analysis in real-time (DART)-mass spectrometry (MS) was employed to identify alkaloids in *P. sinensis*. DART-MS was first demonstrated and discussed in detail for coupling with HPTLC at the end of 2006.[11,12] The direct sample access at ambient conditions within a minute or even within seconds greatly contributes to the progress of HPTLC.[13,14] The proposed method provides a fast and exclusive authentication method for alkaloids in TCM.

In the Chinese Pharmacopoeia (2015), the quality standard of *P. sinensis* has not been included. Therefore, it is urgent to conduct research on quality standards for *P. sinensis*. Methods for simultaneous quantitative determination of several active compounds in a single run for *P. sinensis* have been unavailable until now. The selection of chemical markers is one of the most important features of high-performance liquid chromatography (HPLC) determination.[15] In this study, we adhere to the principle of high content and wide activity to select marker compounds. Thin-layer chromatography (TLC) autography and TLC bioautography are simple, convenient, and require no specialised equipment, accounting for their wide utilisation in screening marker compounds and active natural products.[2,16-18] Based on the simple and convenient features, we screened $DPPH^{\cdot}$ scavenging and xanthine oxidase inhibitory activities of the main components in *P. sinensis* to select marker compounds for HPLC determination. Based on the selected marker compounds, a universal method for the quantitative determination of eight marker compounds in *P. sinensis* was developed.

Briefly stated, the study systematically clarified the chemical composition of *P. sinensis*, and the proposed HPLC method can potentially improve quality control of *P. sinensis*. Combined with previous reports,[10,19] the study identifies similarities in chemical components between *P. sinensis* and *Erycibe* species; it also provides a reference for the use of *P. sinensis* as a substitute.

2 | EXPERIMENTAL

2.1 | Reagents, materials and instrumentation

Porana sinensis, *E. obtusifolia* and *E. schmidtii* samples were collected in various regions of China. The collection of samples was strictly adhered to Administrative Ordinance on Wild Medical Materials Resources (promulgated by the State Administration for Market Regulation). All the samples were authenticated by Dr Lihong Wu, Shanghai University of Traditional Chinese Medicine. Voucher specimens were deposited at Shanghai R&D Centre for Standardisation of Chinese Medicines. The origin, herbarium numbers of the samples are listed in Supporting Information Table S1. Neochlorogenic acid (MUST-12113001), cryptochlorogenic acid (MUST-12113002), 3,4-dicaffeoylquinic acid (MUST-12041114), 3,5-dicaffeoylquinic acid (MUST-12101101) and 4,5-dicaffeoylquinic acid (MUST-12081803) were purchased from Shanghai Qiming Bioengineering Institute (Shanghai, China). Scopoletin (batch number 110768-200504) and chlorogenic acid (110753-200413) were provided by the National Institute for Food and Drug Control (Beijing, China). Scopolin (purity > 98%) was isolated and purified in our laboratory and used as a reference compound after being characterised by UV and NMR spectral data. Oasis MCX (60 mg, 3 mL) mixed-mode cation-exchange solid-phase extraction columns were purchased from Waters (Milford, MA, USA). HPTLC plates used for DART-MS were purchased from Macherey-Nagel (HPTLC-Fertigplatten Nano-DURASIL-20 UV_{254}, Düren, Germany). Methanol of HPLC grade was supplied by Fisher chemical reagent company (Waltham, MA, USA). Ultrapure water was used, and all other reagents were of analytical grade.

The Agilent 1260 Infinity LC system, equipped with Agilent ChemStation B.04.03 (Agilent Technologies, Waldbronn, Germany), was used for the determination of *P. sinensis*. Q-Exactive Focus MS (Thermo Finnigan, San Jose, CA, USA) was used to analyse the chemical constituents of *P. sinensis*. The DART-SVP ion source (IonSense, Saugus, MA, USA) was coupled to a Finnigan Surveyor LCQ DECA XPplus ion trap mass spectrometer and operated by DART Control software. Automatic TLC Sampler 4, ADC_2 Automatic Developing Chamber and TLC Visualiser (Camag, Muttenz, Switzerland) were used.

2.2 | UHPLC-Q-Exactive focus-MS/MS analysis

2.2.1 | Sample preparation

All standard samples (1 μg/mL) were individually prepared in 60% methanol aqueous solution. Thus, 0.5 g powder (40 mesh) of accurately weighed *P. sinensis* (Number 2) was extracted with 50 mL of 80% methanol–water solution in a 100 mL flask for 30 min in an ultrasonic bath at room temperature. An 80% methanol–water solution was subsequently added to compensate for the loss of weight. The mixture was then filtered, and 10 mL of the final extract was collected and transferred into a 20 mL volumetric flask, which was then diluted with a 40% methanol–water solution. The resulting mixture was filtered through a 0.22-μm polytetrafluoroethylene (PTFE) syringe filter and the filtrate was transferred to an autosampler vial for analysis.

2.2.2 | Analytical conditions

Porana sinensis was analysed in the positive and negative ionisation modes. Chromatographic separations were performed on a Waters XTerra® RP18 (4.6 mm × 100 mm, 3.5 μm) column. The column temperature was maintained at 35°C, the flow rate of the mobile phase was 0.3 mL/min, and the injection volume was 2 μL. The mobile phase was composed of 0.1% formic acid aqueous solution (A) and methanol (B). Elution was conducted using a linear gradient 5–25% B within the first 12 min, 25–30% B within 12–20 min, 30–38% B within 20–28 min, 38–42% B within 28–40 min, 42–50% B within 40–45 min and 50–60% B within 45–55 min. For MS detection, the operating parameters were as follows: spray voltage, +3500 V/−3000 V; atomisation temperature, 400°C; capillary temperature, 380°C; sheath gas pressure, 60 arb; aux gas pressure, 20 arb; S-lens RF, 60 V; resolution, MS full scan 70000 full width at half maximum (FWHM); scan range, *m/z* 110–1650 for MS; *m/z* 100–1000 for MS/MS.

2.2.3 | Structure analysis procedure

Based on the high-accuracy precursor ions and product ions obtained from Q-Exactive Focus-MS/MS, the elemental compositions were calculated. Then, the most rational molecular formula was sought in various chemical databases such as ChemSpider (http://www.chemspider.com) and *m/z* cloud (https://www.mzcloud.org). Meanwhile, by searching literature sources, all components reported in the literature on *P. sinensis* and plants of the same family were summarised in a Microsoft Office Excel table to search the most rational molecular formula. When several matching compounds with the same formula were found, the fragmentation patterns and pathways of the compounds were analysed.

2.3 | Rapid identification of alkaloids in *P. sinensis* by DART-MS

2.3.1 | Sample preparation

Sample powder of 1.0 g was extracted with 50 mL of 0.1 mol/L hydrochloric acid (HCl) solution in a 100 mL flask for 30 min in an ultrasonic bath at room temperature. The filtrate of 25 mL was evaporated to dryness in a rotary evaporator at 50°C. Then the residue was dissolved in 1 mL 0.1 mol/L HCl for solid-phase extraction (SPE) purification. Oasis MCX (60 mg, 3 mL) mixed-mode cation-exchange SPE columns was first conditioned with 3 mL methanol and 3 mL water (the flow rate was adjusted to one drop in 3 s). Subsequently, a 2-mm layer of the solvent remained. The extract was then dissolved in 0.1 mol/L HCl and was transferred into the column very slowly at the same speed of dropping. The column was eluted with 3 mL of water. Subsequently, the total alkaloids were eluted from the sorbent with elution mixtures 4 mL of methanol–10% ammonia (3:1, *v/v*). The total alkaloidal fractions after SPE were evaporated to dryness in a rotary evaporator at 50°C and dissolved in 1 mL of methanol for HPTLC separation.

2.3.2 | Identification of alkaloids

Briefly, 8 μL of the total alkaloids were directly deposited (as bands) onto HPTLC plates. Then, the HPTLC plates were developed in a pre-saturated solvent tank with chloroform–methanol–formic acid (3:1:0.3, *v/v/v*) as the developing reagent and the developing distance from the application position was 8.0 cm. The plates were air-dried, sprayed with 0.2% ninhydrin in ethanol, heated under 105°C and examined under white light to localise the bands with a pencil. Photographs of the developed plates were taken with TLC Visualiser. We then cut the TLC plate with smartCUT (CAMAG) to 3 mm down the centre of these bands. The incised plates were sprayed with a solution of glycerin in methanol (1:30) and allowed to dry. We obtained all necessary information for zones of interest by holding the TLC slide in the DART mass spectrometer. All parameters were measured using DART-SVP interface software. The DART ion source was operated in positive ionisation mode according to the property of compounds. The DART settings were 200–300°C gas temperature, 200 V grid electrode voltage, and 4.0 kV discharge needle voltage. For operation of the DART source, helium gas (99.999%) was employed, whereas nitrogen gas (99.998%) was used in the standby mode. The flow rate of helium as the ionising medium was set at 3.0 L/min. The ion source was coupled to a Finnigan Surveyor LCQ DECA XPplus ion trap mass spectrometer. The mass spectrometer was carried out with the capillary temperature of 300°C and a capillary voltage of 15.0 V. The relative collision energy was set to 30.0–40.0%.

2.4 | DPPH˙ scavenging activity detected by TLC autography and xanthine oxidase inhibitory activity detected by TLC bioautography

The *P. sinensis*, *E. obtusifolia*, *E. schmidtii* samples (prepared as described earlier in section 2.2, 2 μL) and standard samples (0.1 mg/mL, 2 μL) were directly deposited (as bands) onto TLC plates. The TLC plates were developed in a pre-saturated solvent tank with ethyl acetate–methanol–formic acid (6:1:0.5) as the developing reagent. The developing distance from the application position was 8.0 cm.

The TLC autography for DPPH˙ scavenging activity was the same as described by Xiao *et al.*[16] The developed TLC plates were removed

from the chamber, and allowed to air-dry for 30 min, followed by spraying with a 2.54 mM DPPH· methanol solution. Bands with the DPPH· scavenging activity were observed as white/yellow bands on a purple background.

The TLC bioautography for xanthine oxidase inhibitory was the same as described by Ramallo *et al.*[20] The TLC plate was dipped with xanthine oxidase staining solution [agar: 0.1 mg/mL; EDTA (ethylenediaminetetraacetic acid): 1 mmol; dipotassium hydrogen phosphate/potassium dihydrogen phosphate: 50 mmol; NBT (nitro blue tetrazolium): 0.22 mg/mL; xanthine oxidase: 68 mU/mL]. After being incubated for 10 min at 38°C in an incubation chamber, the TLC plates were dipped in 3 mmol solution of xanthine, followed by a second incubation for 20 min at 38°C. Bands with xanthine oxidase inhibitory or superoxide scavenger activities were observed as white/yellow on a purple background.

2.5 | Quantitative analysis of *P. sinensis* by HPLC method

2.5.1 | Analytical conditions

All separation procedures were conducted using the Agilent Poroshell 120 EC-C_{18} column (4.6 mm × 150 mm, 2.7 μm). The mobile phase was composed of 0.1% formic acid aqueous solution (A) and methanol (B). Elution was conducted using a linear gradient 15–34% B within the first 10 min, 34% B within 10–20 min, and 34–38% B within 20–35 min. A detection wavelength of 345 nm was used. The column temperature was maintained at 25°C, the flow rate of the mobile phase was 1.0 mL/min, and the injection volume was 10 μL. Under this chromatographic analytical condition, the main compounds in *P. sinensis* could be determined without interference of other ingredients.

2.5.2 | Preparation of standard solutions

A standard stock containing eight compounds (neochlorogenic acid 180 μg/mL, scopolin 268 μg/mL, chlorogenic acid 270.4 μg/mL, cryptochlorogenic acid 168 μg/mL, scopoletin 81 μg/mL, 3,4-dicaffeoylquinic acid 188 μg/mL, 3,5-dicaffeoylquinic acid 176 μg/mL, and 4,5-dicaffeoylquinic acid 196 μg/mL) was prepared in a 60% methanol aqueous solution. Representative chromatograms for *P. sinensis* samples are presented in Figure 1.

2.5.3 | Preparation of samples

Thus, 0.5 g powder (40 mesh) of accurately weighed *P. sinensis* was extracted with 50 mL of 80% methanol–water solution in a 100 mL flask for 30 min in an ultrasonic bath at room temperature. An 80% methanol–water solution was subsequently added to compensate for the loss of weight. The mixture was then filtered, and 10 mL of the final extract was collected and transferred into a 20 mL volumetric flask, which was diluted with a 40% methanol–water solution.

2.5.4 | Methodological study

The previously prepared stock solutions were diluted 2, 4, 20, and 100 times to plot calibration curves, and 10 μL of the solution was injected into the HPLC system. The calibration curve of each analyte was constructed by plotting the peak area versus the concentration. The range of linearity, regression equation, and correlation coefficient of each compound are shown in Table S2. Inter-day assay precision was determined by analysing the known concentrations of the eight analytes in six replicates in a single day. The relative standard deviations (RSDs) of the eight compounds in this assay were 0.36%, 0.14%, 0.21%, 0.33%, 0.24%, 0.37%, 0.41%, and 0.34%. These results indicate that the proposed method provides sufficiently precise outcomes for the

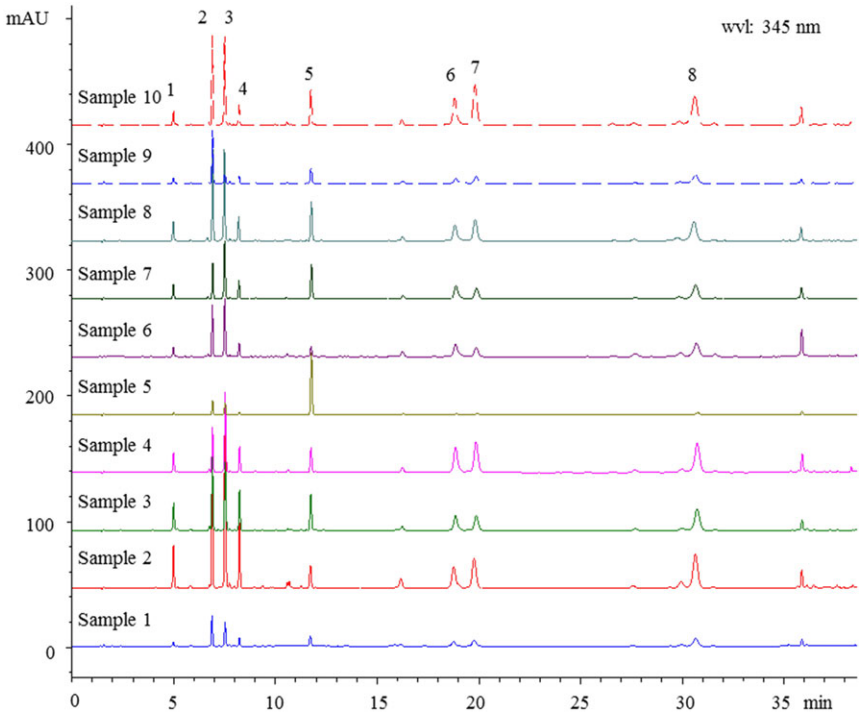

FIGURE 1 Chromatograms for simultaneous quantification of the eight active compounds in *Porana sinensis*. Neochlorogenic acid (**1**), scopolin (**2**), chlorogenic acid (**3**), cryptochlorogenic acid (**4**), scopoletin (**5**), 3,5-dicaffeoylquinic acid (**6**), 3,4-dicaffeoylquinic acid (**7**) and 4,5-dicaffeoylquinic acid (**8**) [Colour figure can be viewed at wileyonlinelibrary.com]

determination of eight active components in *P. sinensis*. For the stability test, the same sample solution was analysed for 0, 4, 8, 12, 24, and 48 h. The RSDs of the peak area (0.72%, 0.51%, 0.32%, 0.54%, 0.33%, 0.52%, 0.43% and 0.61%) showed that the sample solution was stable within 48 h. Repeatability of the analysis was determined by injecting six samples that were obtained in accordance with the sample preparation procedure. The RSDs of the peak area were 1.71%, 2.78%, 2.85%, 2.21%, 1.68%, 2.76%, 2.56% and 2.47%. The recovery test was used to assess the accuracy of the developed analytical method. Standard solutions at different concentrations (low, medium, and high) were added into the samples. The mixtures were extracted and analysed using the proposed method. Assay recovery was calculated using the following formula: Recovery (%) = (amount found – original amount)/amount added × 100%, and RSD (%) = (SD/mean) × 100%. Table S3 shows the results of the recovery test for three quantities (low, medium, and high) of the real standards.

3 | RESULTS AND DISCUSSION

3.1 | Analysis of chemical constituents of *P. sinensis* by UHPLC-Q-Exactive MS

Porana sinensis was analysed in the positive and negative ionisation modes, and the total ion chromatograms for both of these electrospray ionisation (ESI) modes are shown in Figure 2. Some of the compounds found in this study were identified based on a comparison of their retention times and high-resolution mass spectra with those of several reference standards. Thus compounds **1**, **2**, **5**, **6**, **7**, **14**, **15**, and **17** were unambiguously identified as neochlorogenic acid, scopolin, chlorogenic acid, cryptochlorogenic acid, scopoletin, 3,5-dicaffeoylquinic acid, 3,4-dicaffeoylquinic acid and 4,5-dicaffeoylquinic acid, respectively. Compounds without reference standards were identified by determining the elemental compositions of the precursor and product ions. The molecular formula and rational fragmentation patterns and pathways of these compounds were then identified based on a comparison of these data with chemical databases and the literature. A total of 12 isomers for caffeoylsinapoylquinic acid, 12 isomers for caffeoylsyringoylquinic acid, and four isomers for syringoylquinic acid are theoretically possible, and only some of them were detected in the samples. The large number of isomers contributes difficulties in identification. Data for all of these compounds are summarised in Table 1.

3.2 | Identification of alkaloid components in *P. sinensis* by DART-MS

Erycibe species are known to contain several components, including numerous tropane alkaloids. Baogongteng A is one of these tropane

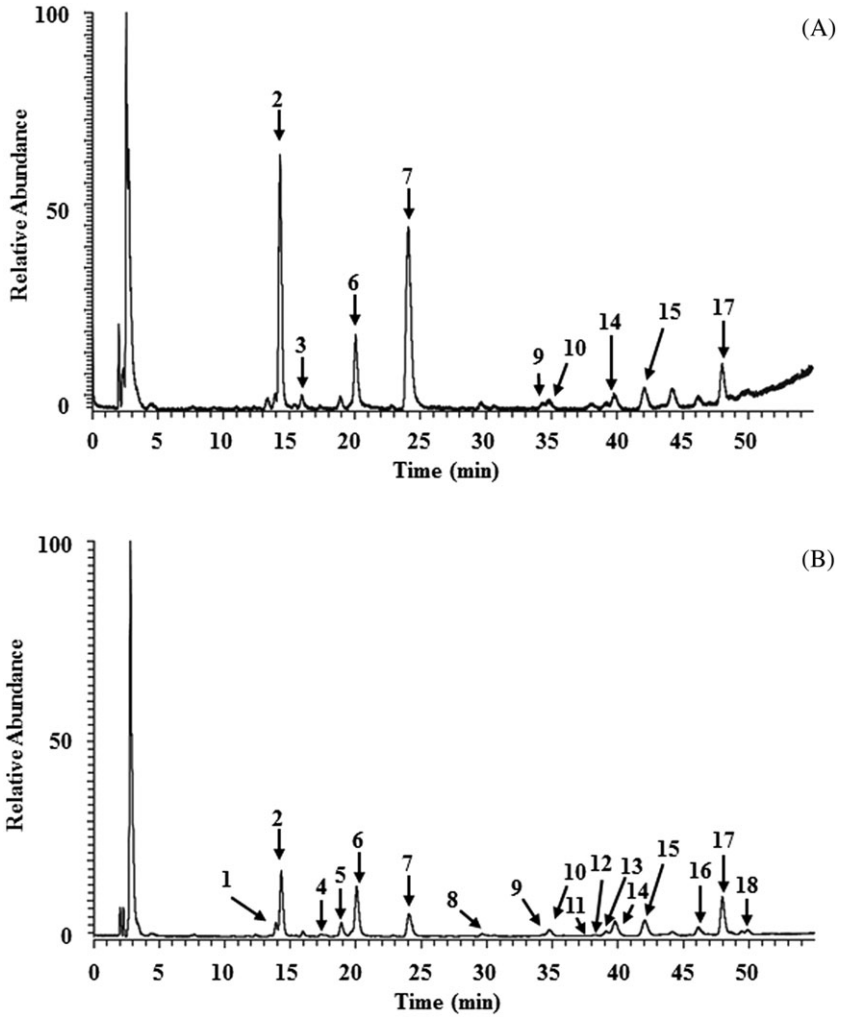

FIGURE 2 Total ion chromatogram for *Porana sinensis* in positive ion mode (A) and negative ion mode (B) using UHPLC-ESI-Q-Exactive focus-MS/MS

TABLE 1 Identification of the chemical constituents of *Porana sinensis* by UHPLC-Q-Exactive focus-MS/MS in negative and positive modes

Number	Retention time (min)	$[M + H]^+$	$[M-H]^-$	Error (ppm)	Molecular formula	Fragment ions (*m/z*)	Identification
1	13.92	—	353.0873	−0.75	$C_{16}H_{17}O_9$	191.0545, 179.0332, 161.0226, 135.0, 93.0326	Neochlorogenic acid
2	14.30	355.1016	—	−6.6	$C_{16}H_{18}O_9$	193.0495, 178.0260, 166.0712, 133.0284	Scopolin
3	15.96	509.1264	—	—	—	317.0840, 215.0313, 193.0494	—
4	17.35	—	371.0980	0.73	$C_{16}H_{20}O_{10}$	197.0440, 173.0438, 153.0539, 121.0276, 93.0327	Syringoylquinic acid
5	18.91	—	353.0872	0.49	$C_{16}H_{18}O_9$	191.0545, 179.0333, 173.0438, 135.0433, 93.0327	Chlorogenic acid
6	20.07	—	353.0875	0.79	$C_{16}H_{18}O_9$	191.0544, 179.0332, 161.0225, 127.0381, 93.0326	Cryptochlorogenic acid
7	24.03	193.0495	—	−0.04	$C_{10}H_8O_4$	178.0258, 133.0283, 122.0363	Scopoletin
8	29.67	—	711.2511	—	—	579.2085, 417.1547, 181.0490, 166.0254, 151.0018	—
9	34.25	—	312.1239	0.87	$C_{18}H_{19}NO_4$	297.0997, 190.0494, 178.0493, 148.0511, 135.0433	*N-trans*-Feruloyltyramine
10	34.77	—	533.1298	0.83	$C_{25}H_{26}O_{13}$	515.1182, 371.0973, 353.0869, 197.0441, 191.0546, 179.0334, 153.0539, 161.0226, 135.0433, 121.0277	Caffeoylsyringoylquinic acid
11	37.53	—	559.1456	0.98	$C_{27}H_{28}O_{13}$	335.0763, 223.0598, 191.0334, 176.0098, 135.0433, 104.0249	Caffeoylsinapoylquinic acid
12	38.25	—	515.1192	0.80	$C_{25}H_{24}O_{12}$	353.0869, 242.9424, 191.0545, 179.0333, 146.9593, 135.0432, 111.0432, 85.0275	Dicaffeoylquinic acid
13	39.05	—	533.1297	0.73	$C_{25}H_{26}O_{13}$	515.1182, 371.0973, 353.0869, 197.0441, 191.0546, 179.0334, 153.0539, 161.0226, 135.0433, 121.0277	Caffeoylsyringoylquinic acid
14	39.74	—	515.1190	0.60	$C_{25}H_{24}O_{12}$	353.0867, 191.0544, 173.0437, 135.0432, 111.0432, 93.0326	3,5-Dicaffeoylquinic acid
15	42.03	—	515.1193	0.90	$C_{25}H_{24}O_{12}$	353.0868, 191.0545, 179.0332, 135.0432, 85.0275	3,4-Dicaffeoylquinic acid
16	46.15	—	533.1299	0.93	$C_{25}H_{26}O_{13}$	371.0975, 197.0441, 173.0439, 153.0540, 121.0277, 93.0327	Caffeoylsyringoylquinic acid
17	47.97	—	515.1188	0.40	$C_{25}H_{24}O_{12}$	353.0872, 191.0546, 173.0439, 135.0433, 111.0432, 93.0327, 85.0276	4,5-Dicaffeoylquinic acid
18	49.91	—	559.1455	0.88	$C_{27}H_{28}O_{13}$	397.1129, 353.0868, 223.0600, 191.0545, 173.0439, 135.0433, 111.0432, 93.0326	Caffeoylsinapoylquinic acid

alkaloids that exerts myotic activities and is used to treat glaucoma.[10,21] In our previous study, total alkaloid content in *Erycibe* was shown to be the chemical basis of toxicity.[10] In the current study, a method based on TLC-DART-MS was proposed for the identification of alkaloid components in *P. sinensis*. As shown in Figure 3, the application of cation-exchange SPE and ninhydrin reagent excluded interference from other components. The *P. sinensis*, *E. obtusifolia* and *E. schmidtii* samples showed similar TLC fingerprint chromatograms. Band A exhibited quasi-molecular ions $[M + H]^-$ at *m/z* 186 in MS spectra, whereas the fragment ions of H_2O and CO were detected as neutral fragments in MS/MS spectra. On the basis of MS and MS/MS data, the compound was identified as Baogongteng A.[10,22] Band B exhibited quasi-molecular ions $[M + H]^+$ at *m/z* 144. In MS/MS analysis, peaks at *m/z* 126, 108, and 84 were primarily attributed to the loss of the neutral fragment H_2O and C_3H_6. Band B was identified as Baogongteng C.[10,22] Baogongteng A and Baogongteng C were present in all the samples and were identified as marker compounds of *P. sinensis*.

3.3 | DPPH˙ scavenging activity detected by TLC autography and xanthine oxidase inhibitory activity detected by TLC bioautography

As shown in Figure 4, both samples and reference compounds (neochlorogenic acid, chlorogenic acid, cryptochlorogenic acid, scopoletin, 3,4-dicaffeoylquinic acid, 3,5-dicaffeoylquinic acid and 4,5-dicaffeoylquinic acid) showed good DPPH˙ scavenging and xanthine oxidase inhibitory activities. The *P. sinensis*, *E. obtusifolia* and *E. schmidtii* samples showed similar active fingerprint chromatograms. In the screening process of xanthine oxidase inhibitory activity, superoxide radical production was used as the reporter for enzyme activity; therefore, compounds with radical scavenging activity would also give (false) positive results. As reported in our previous study,[2] 3,5-dicaffeoylquinic acid [IC_{50} (half maximal inhibitory concentration): 0.21 mmol/L], 3,4-dicaffeoylquinic acid (IC_{50}: 0.32 mmol/L) and 4,5-dicaffeoylquinic acid (IC_{50}: 0.26 mmol/L) showed potent xanthine oxidase inhibitory activity. The IC_{50} value of allopurinol used as the standard was 0.01 mmol/L. Scopoletin, chlorogenic acid,

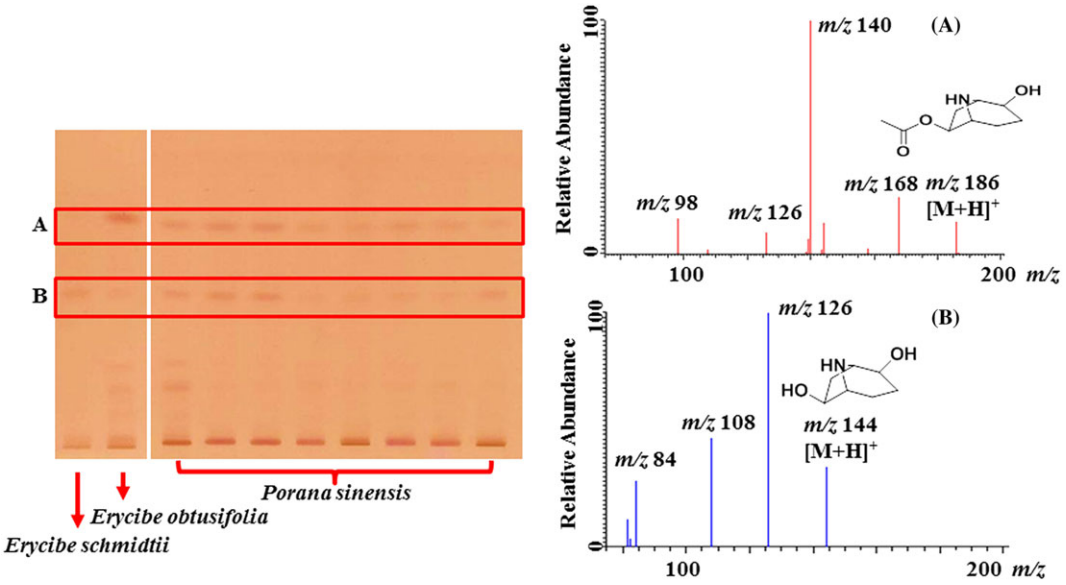

FIGURE 3 HPTLC chromatogram and product-ion spectra of main alkaloids in *Erycibe obtusifolia*, *E. schmidtii* and *Porana sinensis* [Colour figure can be viewed at wileyonlinelibrary.com]

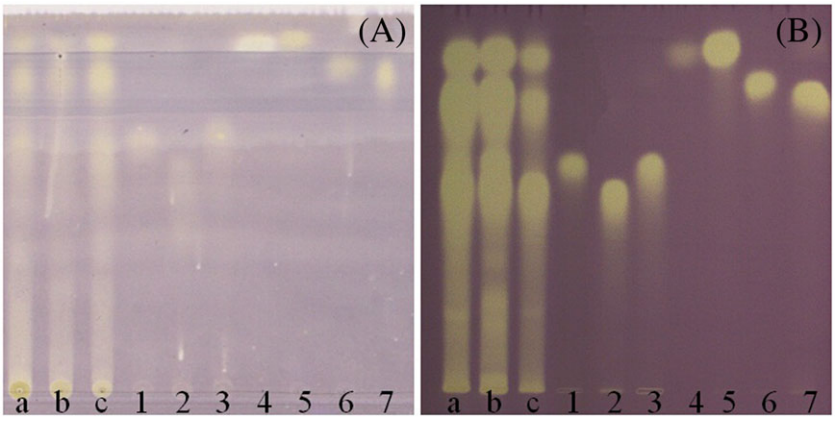

FIGURE 4 Xanthine oxidase inhibitory (A) and DPPH˙ scavenging (B) activities of *Porana sinensis* (a), *Erycibe obtusifolia* (b), *E. schmidtii* (c), 5-caffeoylquinic acid (1), chlorogenic acid (2), 4-caffeoylquinic acid (3), scopoletin (4), 3,4-dicaffeoylquinic acid (5), 3,5-dicaffeoylquinic acid (6) and 4,5-dicaffeoylquinic acid (7) [Colour figure can be viewed at wileyonlinelibrary.com]

cryptochlorogenic acid and neochlorogenic acid are superoxide radical scavengers. According to the literature,[23] scopolin is first hydrolysed into scopoletin before its conjugation by intestinal enzymes. Therefore, scopolin can also be thought of as a DPPH˙ and superoxide radical scavenger. The European Medicines Agency defines chemical markers as "chemically defined constituents which are of interest for quality control purposes".[24,25] In this study, two criteria were used to restrict the selection of marker compounds for determination: (i) through screening or reporting, the compounds must have wide range of activities; and (ii) the compounds must be contained in different batches of herbal medicine samples. Therefore, we selected eight compounds (neochlorogenic acid, scopolin, chlorogenic acid, cryptochlorogenic acid, scopoletin, 3,4-dicaffeoylquinic acid, 3,5-dicaffeoylquinic acid and 4,5-dicaffeoylquinic acid) as the marker compounds for HPLC determination of *P. sinensis*.

3.4 | Quantitative HPLC analysis of *P. sinensis*

Simultaneous quantitative determination of scopoletin, scopolin and chlorogenic acid in *P. sinensis* was performed in our previous study.[5] Methods for the simultaneous separation and quantitative determination of neochlorogenic acid, chlorogenic acid, scopolin, cryptochlorogenic acid, scolopoletin, 3,4-dicaffeoylquinic acid, 3,5-dicaffeoylquinic acid and 4,5-dicaffeoylquinic acid in a single run for *P. sinensis* are thus far unavailable. The proposed HPLC method was successfully employed for the simultaneous determination of eight active components in 10 different samples. The mean values of the three replicate injections are listed in Table 2. Compound concentration in the samples varied, and could be attributed to differences in collection time and certain storage or transportation conditions of the materials. In our previous study,[2] seven main components of the target peaks from *E. obtusifolia* were identified as neochlorogenic acid, chlorogenic acid, cryptochlorogenic acid, scopoletin, 3,4-dicaffeoylquinic acid, 3,5-dicaffeoylquinic acid and 4,5-dicaffeoylquinic acid. The results in this study identified the similarities of *P. sinensis* and *E. obtusifolia* in terms of chemical components.

In this study, we developed an ultra-high performance liquid chromatography (UHPLC)-ESI-Q-Exactive Focus-MS/MS method to characterise the chemical constituents of *P. sinensis* in the positive and negative ionisation modes. Taken together with the results of TLC autography and TLC bioautography, eight compounds were chosen as marker compounds for the HPLC determination of *P. sinensis*. *Porana sinensis* was found to contain a large amount of neochlorogenic acid, chlorogenic acid, scopolin, cryptochlorogenic acid, scopoletin, 3,4-dicaffeoylquinic acid, 3,5-dicaffeoylquinic acid and 4,5-dicaffeoylquinic acid. The proposed HPLC method can potentially improve quality control of *P. sinensis*. A strategy based on a combination of SPE and TLC-DART-MS was proposed for the identification of alkaloid components, Baogongteng A and Baogongteng C, present in all the plants and can be identified as characteristic components of *P. sinensis*. As mentioned earlier, with the reduction in wild populations of *E. obtusifolia* and *E. schmidtii*, *P. sinensis* has been widely used as a substitute. We identified similarities between *P. sinensis* and *Erycibe* species with respect to active compounds and toxic

TABLE 2 Quantification of eight active components in different samples of *Porana sinensis* (mg/g, $n = 3$)

Samples	Neochlorogenic acid	Scopolin	Chlorogenic acid	Cryptochlorogenic acid	Scopoletin	3,5-dicaffeoylquinic acid	3,4-dicaffeoylquinic acid	4,5-dicaffeoylquinic acid
1	0.205	1.37	1.06	0.351	0.282	0.810	0.848	1.29
2	2.30	2.49	4.29	2.87	0.780	3.47	4.25	5.39
3	1.45	3.25	3.11	1.80	1.01	2.30	1.82	3.54
4	1.02	1.96	3.67	1.15	0.663	3.46	3.58	4.42
5	0.127	0.656	0.589	0.167	1.70	0.682	0.741	0.931
6	0.508	2.26	2.75	0.644	0.304	2.10	1.22	2.41
7	0.807	1.58	2.82	0.865	0.988	2.23	1.49	2.56
8	1.14	4.18	4.37	1.15	1.11	2.77	2.95	3.49
9	0.289	2.34	0.567	0.343	0.457	0.804	1.01	1.50
10	0.859	4.19	4.26	1.12	1.10	3.14	5.70	5.03

components; furthermore, we provided a reference for its use as a new resource for Caulis Erycibes.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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