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Characterization of alkaloids in *Sophora flavescens* Ait. by high-performance liquid chromatography–electrospray ionization tandem mass spectrometry

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ABSTRACT

Sophora flavescens Ait., a well-known Chinese herbal medicine, is widely used in clinical practice for the treatment of viral hepatitis, cancer, gastrointestinal hemorrhage, and skin diseases. This paper is the first report on a method based on the combined use of high-performance liquid chromatography, photodiode array detection, and electrospray ionization tandem mass spectrometry for the comprehensive and systematic separation and characterization of bioactive alkaloids in *Sophora flavescens* Ait. A total of 22 constituents were identified on the basis of the extracted ion chromatograms for different $[M+H]^+$ ions of the alkaloids present in *S. flavescens* Ait. Among these, 5 constituents were unambiguously identified by comparing the experimental data on their retention times and MS^n spectra with those of the authentic compounds, and 17 other constituents were tentatively identified on the basis of their MS^n fragmentation behaviors and/or molecular weight information from literatures. Furthermore, some characteristic fragmentation pathways of the alkaloids in *S. flavescens* Ait. were detected and examined. This information may be useful for characterizing the bioactive alkaloids present in *S. flavescens* Ait. and for possible applications in formulations.

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1. Introduction

Sophora flavescens Ait. is one of the most widely used traditional Chinese medicine herbs. It has been commonly used for the treatment of viral hepatitis, cancer, and gastrointestinal hemorrhage and for skin diseases such as eczema, colpitis, and psoriasis [1].

Previous phytochemical studies on *S. flavescens* Ait. revealed that alkaloids and flavonoids are the main chemical components of this plant. So far, more than 20 alkaloids and 50 flavonoids have been isolated and identified [2,3]. The principal bioactive constituents of *S. flavescens* Ait. are major alkaloids, which were reported to exhibit sedative and inotropic effects as well as antipyretic, anti-tumor, and anti-hepatitis B virus activities [4–8].

Due to the potent pharmacological activities of the alkaloids in *S. flavescens* Ait., a simple, rapid, and efficient method for the identification of these components would greatly help in the quality control of this natural medicine and its formulations. Several methods, including thin-layer chromatography (TLC) [9], high-performance liquid chromatography (HPLC) [10,11], and capillary electrophoresis (CE) [12,13], have been developed and used in previous studies for the separation and identification of the alkaloids in *S. flavescens* Ait. However, all these reports focused on the qualitative and quantitative analysis of one or few major constituents, while the less abundant or minor compounds that are commercially unavailable have generally been neglected. One essential difference between traditional medicines and chemical drugs is that the therapeutic effects of the former are due to the synergistic contribution of several components, not just the major ones. Therefore, a more comprehensive strategy that would be applicable to most alkaloids would be valuable for characterization of *S. flavescens* Ait.

Due to its high sensitivity, rapidity, and low sample requirement, mass spectrometry (MS) is a powerful tool for the analysis of alkaloids [14,15]. Over the past few years, the use of MS coupled with HPLC (HPLC/MS) has been reported for the analysis of *S. flavescens* Ait., but most studies have generally focused only on the analysis of flavonoids in *S. flavescens* Ait. [16,17]. Zhang et al. [18] utilized HPLC/MS to obtain molecular weight information for 9 standard alkaloids, but they did not obtain the MS^n spectra of the analytes. Consequently, their characterization of the processes may be faulty because isomeric and stereoisomeric alkaloids are known to be present in *S. flavescens* Ait. Ye et al. [19] used LC–MS to characterize oxymatrine, sophoranol, sophoridine, and matrine in the serum of experimental animals treated with *S. flavescens* extracts,

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and Zhang et al. [20] used LC–MS to perform pharmacokinetic studies on matrine, oxymatrine, and oxysophocarpine in rat plasma after oral administration of the *S. flavescens* Ait. extract. In both these studies, all of the analytes were characterized by comparison with the $[M+H]^+$ ions of authentic compounds in the MS spectra, but this approach did not shed light on the MS^n spectra of alkaloids in *S. flavescens* Ait.

To our knowledge, there have been no reports on the simultaneous and comprehensive analysis of both the major and minor alkaloids in *S. flavescens* Ait. Moreover, the MS fragmentation pathways of alkaloids in *S. flavescens* Ait. have rarely been studied. In this investigation, we used high-performance liquid chromatography–photodiode array detection–electrospray ionization tandem mass spectrometry (HPLC–DAD–ESI–MS^n) to develop a simple, rapid, and valid chromatographic method for the qualitative analysis of alkaloids in *S. flavescens* Ait. The mass spectrometric data of these alkaloids was also interpreted to characterize these compounds for use in natural medicines and formulations.

2. Experimental

2.1. Chemicals and materials

Matrine, oxymatrine, sophocarpine, oxysophocarpine, and sophoridine standards were purchased from the Shanghai Tauto Biotech Corporation Limited (Shanghai, China). The purities were greater than 98% as determined by HPLC. Methanol (HPLC grade) was purchased from Merck (Darmstadt, Germany). Ammonia water and ammonium acetate (purity 99%) were purchased from Wako (Osaka, Japan). Deionized water (18 MV) was prepared by passing distilled water through a Milli-Q system (Millipore, Milford, MA, USA).

S. flavescens Ait. root was purchased from Liankang Drug Store (Beijing, China). It was authenticated by Dr. Shizhong Chen, Professor of the School of Pharmaceutical Sciences, Peking University, Beijing, China.

2.2. Sample preparation

For LC–MS analysis, the sample was ground into a fine powder (60 mesh). An 0.50 g aliquot was weighed and extracted with 50 mL of water in an ultrasonic water bath for 60 min. The solution was filtered through an 0.2 μm membrane prior to use, and a 10 μL aliquot was injected into the HPLC system for analysis.

2.3. HPLC conditions

The HPLC analyses were performed on a Shimadzu analytical HPLC instrument (Kyoto, Japan) equipped with an LC-20AD pump, CTO-20A column oven, DGU-20A3 degasser, SPD-M20A diode-array detector (DAD), and SIL-20AC auto injector. Separation was carried out on a Capcell Pak^{MG}-C18 column (5 μm, 150 mm × 4.6 mm, Shiseido) at a column temperature of 30 °C. Elution was performed with a linear gradient of A (0.02 mol/L ammonium acetate solution, adjusted with ammonia water to pH 8.0) and B (methanol) under the following conditions: 0 → 40 min, 10% B → 30% B; 40 → 50 min, 30% B → 60% B; 50 → 55 min, 60% B → 70% B; and 55 → 60 min, 70% B. The flow rate was 1 mL/min. The DAD detector was set at 205 nm, and the on-line UV spectra were recorded in the range 190–400 nm.

2.4. Mass spectrometry conditions

A hybrid ion-trap time-of-flight mass spectrometer (Shimadzu LCMS-IT-TOF, Kyoto, Japan) was connected to the Shimadzu analytical HPLC instrument via an ESI interface. The HPLC effluent was introduced into the ESI source in a post-column splitting ratio of 3:1. The curved desolvation line (CDL) temperature and block heater temperature were maintained at 200 °C. The capillary voltage, CDL voltage, and detector voltage were fixed at 4.5 kV, 10 V, and 1.7 kV, respectively. Nitrogen was used as the nebulizer gas at a flow rate of 1.5 L/min. For full-scan MS analysis, the spectra were recorded in the *m/z* range of 100–800. Data-dependent acquisition was set such that the most abundant ions in full-scan MS would trigger tandem mass spectrometry (MS^n, $n = 2$–4). In the automatic mode, all ions were first accumulated in the octopole and then rapidly pulsed into the ion trap for MS^n analyses according to the criteria settings. The collision energy for MS^n was adjusted to 50% in the LC/MS analysis, and the isolation width of precursor ions was 3.0 Th. All ions produced were finally introduced into the TOF instrument for accurate mass determination. To obtain accurate values of the masses, the entire mass range from *m/z* 100 to 800 was calibrated using the sodium trifluoroacetate cluster as the external reference. Data acquisition and processing were performed using the LC/MS solution version 3 software (Shimadzu, Kyoto, Japan), which included a formula predictor to predict chemical formulas.

3. Results and discussion

3.1. Optimization of the extraction method and chromatographic conditions

Since prolonging the reflux time would increase the content of matrine but decrease that of oxymatrine [21,22], we chose ultrasonication to extract alkaloids from *S. flavescens* Ait. In this study, the samples were extracted with chloroform–ammonia water (49.7:0.3, v/v), water, 50% methanol solution, and methanol, respectively. The obtained HPLC/MS total ion current (TIC) in the positive ion mode of these extracts indicated that when water was used as the extraction solvent, it resulted in a higher content of alkaloids and more chromatographic peaks, which is in agreement with the results of published reports [22,23]. The HPLC conditions were optimized by investigating the effects of the mobile phase and detection wavelength because these two parameters play key roles in determining the resolution and sensitivity of the method. Due to the presence of alkaloids in the herbal extract, ammonium acetate and ammonia water were added to the mobile phase to lower the polarity of these compounds and reduce the formation of broad and overlapped peaks. The optimized mobile phase consisted of 0.02 mol/L ammonium acetate solution adjusted with ammonia water to pH 8.0 as solvent A and methanol as solvent B. Using linear gradient elution, satisfactory HPLC separation results were obtained within 60 min.

A DAD was used to select the optimal wavelength for detecting the constituents in the HPLC chromatogram. In the full-scan experiment, the alkaloids showed weak absorption in the range of 190–400 nm. Fig. 1a shows the HPLC chromatogram at 205 nm.

3.2. Analysis of alkaloids in S. flavescens Ait. using HPLC–MS/MS

More than 20 alkaloids are found in *S. flavescens* Ait. Matrine, oxymatrine, sophoridine, oxysophocarpine, and sophocarpine are the major alkaloids present [2,24]. Their chemical structures are quite similar, as shown in Fig. 2. Separation in the positive and negative ion modes was attempted with these compounds, and the results indicated that although the standards did not yield a signal in the negative mode, they provided good mass spectra data in the positive ion mode. Therefore, the HPLC–MS/MS analysis was conducted in the positive ion mode.

The difficulties in analyzing the alkaloids present in *S. flavescens* Ait. can be mainly attributed to three features: (1) most of the alkaloid skeletons lack a conjugated system, due to which common UV

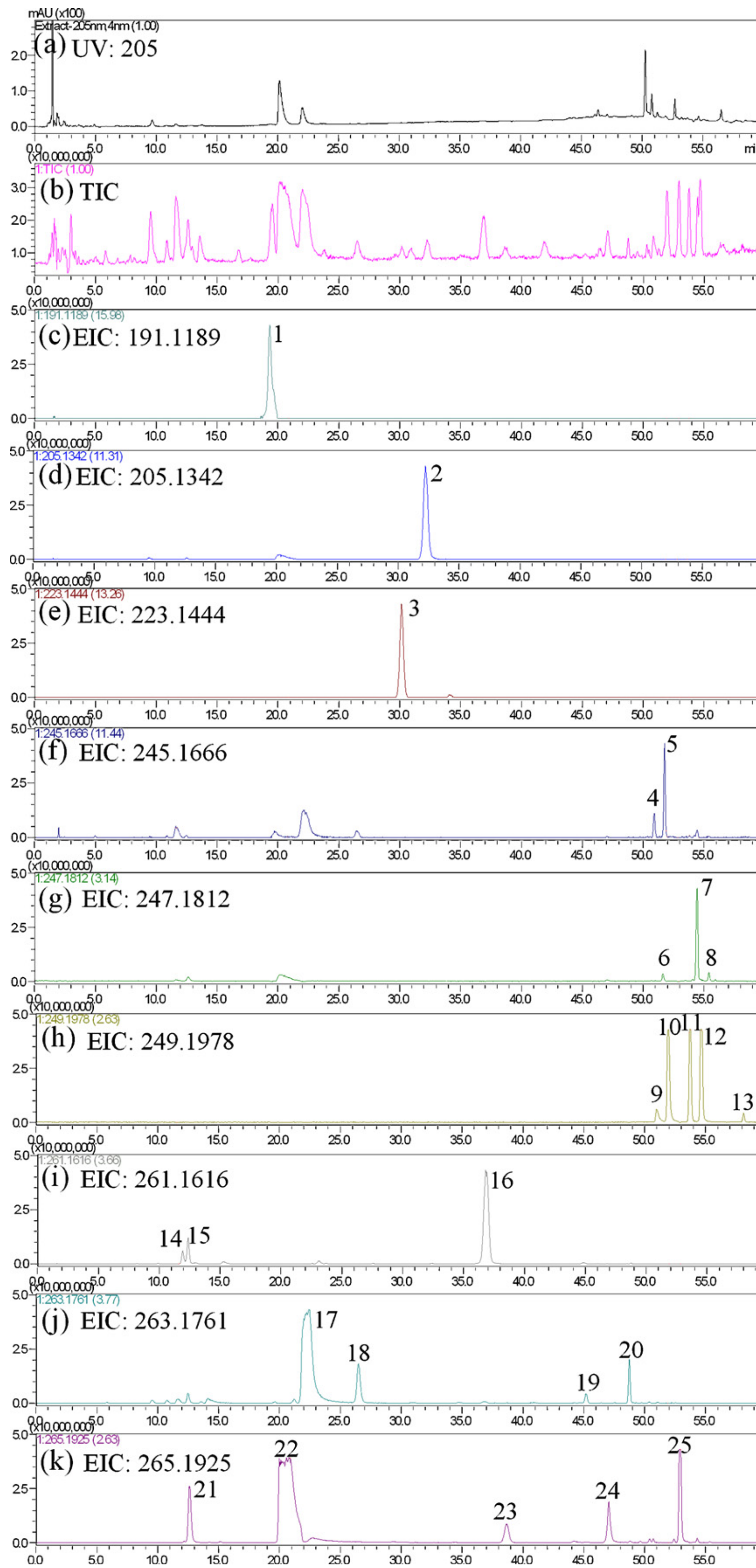


Fig. 1. Identification of alkaloids in *Sophora flavescens* Ait. by HPLC–MS/MS analysis. Top to bottom: the chromatograms represent the HPLC/UV profile recorded at 205 nm, HPLC/MS total ion current (TIC) in the positive ion mode, and extracted ion chromatograms for different $[M+H]^+$ ions with a mass window of 0.01 Da. Peak assignments are the same as those described in Table 1.

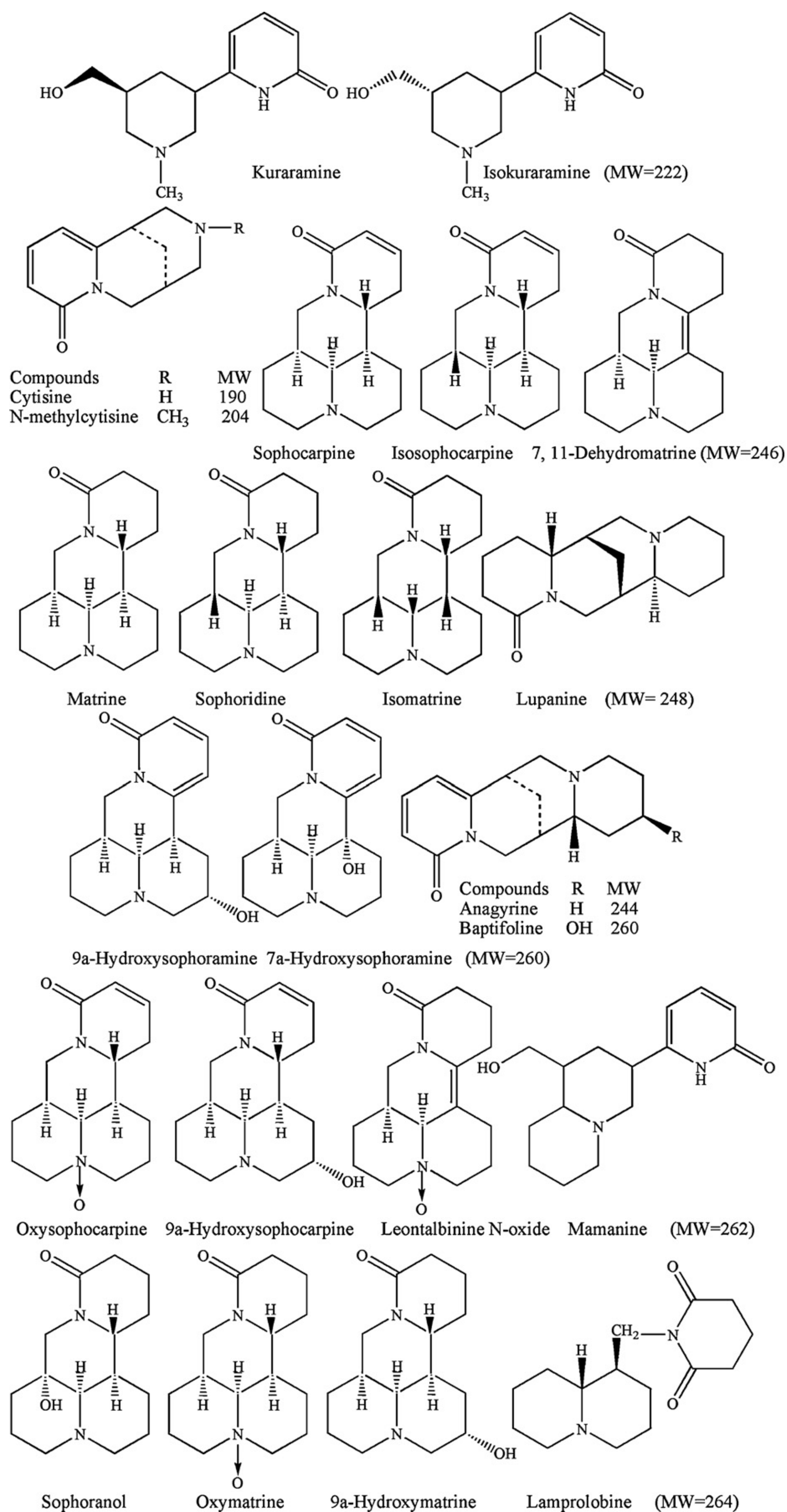


Fig. 2. The structures and molecular weights of the alkaloids identified in Radix *Sophora flavescens*.

Table 1
Characterization of the alkaloids in *Sophora flavescens* Ait. by HPLC–DAD–ESI–MS^n.

Peak no.	t_p (mm)	Experimental m/z	Predicted formula	Theoretical m/z	Error (ppm)	ESI–MS/MS m/z f% base peak)	Identification
1	19.48	191.1189	$C_{11}H_{14}N_2O$	191.1184	2.62	148(100)	Cytisine
2	32.21	205.1342	$C_{12}H_{16}N_2O$	205.1341	0.49	146(91), 108(100)	N-methy Icy ti sine
3	30.18	223.1444	$C_{12}H_{18}N_2O_2$	223.1447	−1.34	191(100), 162(78)	Kuraramine/Isokuraramine
4	50.85	245.1666	$C_{15}H_20N_2O$	245.1654	4.89	227(100), 150(39), 148(42)	Unidentified
5	51.76	245.1666	$C_{15}H_{20}N_2O$	245.1654	4.89	148(100), 98(23)	Anagyrine
6	51.56	247.1812	$C_{15}H_{22}N_2O$	247.1810	0.81	179(100), 150(90), 148(51), 136(72)	Isosophocarpine
7	54.41	247.1812	$C_{15}H_{22}N_2O$	247.1810	0.81	227(19), 179(100), 150(93), 148(40), 136(63)	Sophocarpine
8	55.39	247.1812	$C_{15}H_{22}N_2O$	247.1810	0.81	176(100), 148(13)	7,11-Dehydromatrin
9	50.98	249.1978	$C_{15}H_{24}N_2O$	249.1967	4.41	176(100), 150(15),148(10)	Isomatrine
10	52.02	249.1978	$C_{15}H_{24}N_2O$	249.1967	4.41	176(100), 150(10),148(5)	Sophoridine
11	53.74	249.1978	$C_{15}H_{24}N_2O$	249.1967	4.41	231(89), 150(100),148(53)	Unidentified
12	54.72	249.1978	$C_{15}H_{24}N_2O$	249.1967	4.41	176(100), 150(12), 148(6)	Matrine
13	58.06	249.1978	$C_{15}H_{24}N_2O$	249.1967	4.41	206(22), 166(33), 136(100)	Lupanine
14	11.89	261.1616	$C_{15}H_{20}N_2O_2$	261.1603	4.98	243(100), 150(25)	9a-Hydroxysophoramine
15	12.28	261.1616	$C_{15}H_{20}N_2O_2$	261.1603	4.98	243(100), 136(82)	7a-Hydroxysophoramine
16	36.88	261.1616	$C_{15}H_{20}N_2O_2$	261.1603	4.98	243(44), 164(24), 114(100)	Baptifoline
17	22.20	263.1761	$C_{15}H_{22}N_2O_2$	263.1760	0.38	263(88), 245(100), 150(53), 138(39)	Oxy sophocarpine
18	26.55	263.1761	$C_{15}H_{22}N_2O_2$	263.1760	0.38	245(33), 164(100)	9a-Hydroxysophocarpine
19	45.08	263.1761	$C_{15}H_{22}N_2O_2$	263.1760	0.38	245(9), 195(82), 166(100)	Leontalbinine N-oxide
20	48.69	263.1761	$C_{15}H_{22}N_2O_2$	263.1760	0.38	231(100)	Mamanine
21	12.59	265.1925	$C_{15}H_{24}N_2O_2$	265.1916	3.39	247(100), 205(42)	Sophoranol
22	20.57	265.1925	$C_{15}H_{24}N_2O_2$	265.1916	3.39	265(100), 247(26), 205(35), 148(53)	Oxymatrine
23	38.57	265.1925	$C_{15}H_{24}N_2O_2$	265.1916	3.39	166(42), 164(100)	Unidentified
24	47.12	265.1925	$C_{15}H_{24}N_2O_2$	265.1916	3.39	247(52), 150(63), 148(100), 112(20)	9a-Hydroxymatrine
25	52.92	265.1925	$C_{15}H_{24}N_2O_2$	265.1916	3.39	150(100)	Lamprolobine

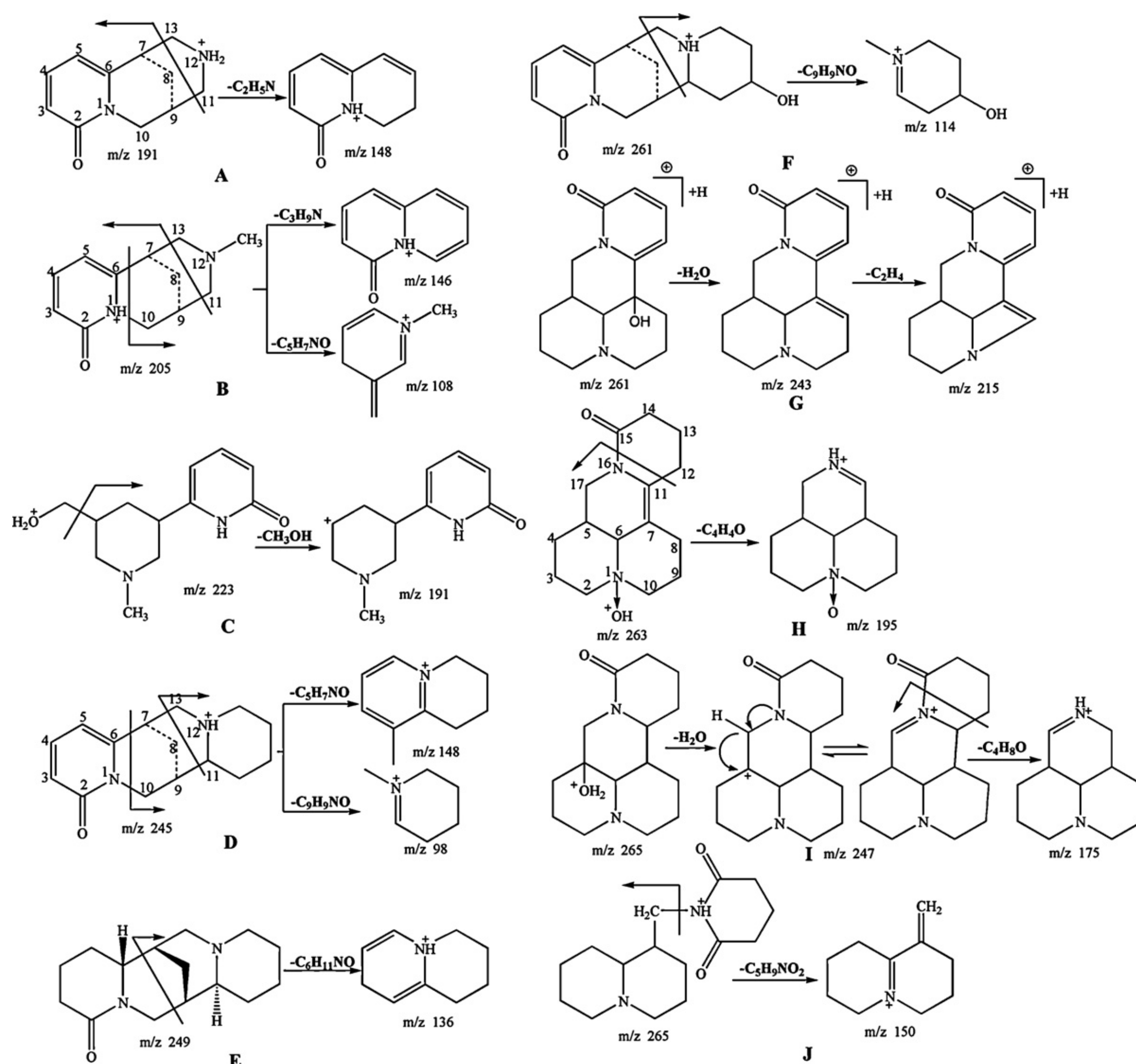
detection cannot be used; (2) isomeric and stereoisomeric alkaloids are generally present, and the identification of chromatographic peaks on the basis of retention times under the chromatographic conditions employed may be ambiguous; and (3) the levels of some minor alkaloids are extremely low, necessitating the use of a highly sensitive detector.

By using an HPLC–MS/MS method in this study, we could overcome the above-mentioned analytical problems. As shown in Fig. 1b, under the optimized chromatographic conditions described in Section 2.3, most of the analytes were well separated, with the exception of three sets of overlapping peaks at around 21 min, 52 min, and 54 min. Fortunately, MS can distinguish overlapping peaks through extracted ion chromatogram (EIC) experiments if the coeluting components have different m/z values. Fig. 1c–k clearly illustrates the separation of overlapping peaks based on the EIC of the ions at m/z 191.1189, 205.1342, 223.1444, 245.1666, 247.1812, 249.1978, 261.1616, 263.1761, and 265.1925, respectively. The identification of each chromatographic peak in the sample extract was achieved by multiple procedures. First, in the MS spectra, the constituents exhibited their protonated ions $[M+H]^+$ in the positive ion mode. This information yielded the molecular weights of the unknown components. Second, use of the accurate mass data recorded in the TOF mass spectrometer (error < 5 ppm) together with the formula predictor software offered the authentic molecular formulas of the unknown constituents. Third, the molecular formulas and MS^n fragmentation behaviors helped in elucidating the structures of the compounds. In this study, 5 peaks were identified by comparing the retention times and MS^n spectra of these compounds with those of authentic standards, and 17 peaks were tentatively identified based on their MS^n fragmentation behaviors. The results are listed in Table 1, and the structures of these compounds are shown in Fig. 2.

The EIC of the ions at m/z 191.1189, 205.1342, and 223.1444 (shown in Fig. 1c–e) indicated that H_2O extract of *S. flavescens* Ait. contained one constituent each of molecular weights 190, 204, and 222, respectively. The molecular formulas of these compounds were predicted to be $C_{11}H_{14}N_2O$, $C_{12}H_{16}N_2O$, and $C_{12}H_{18}N_2O_2$, respectively, based on the accurate mass data recorded in the TOF mass spectrometer (error < 5 ppm). The MS^2 spectrum of m/z 191 yielded a characteristic peak at m/z 148. This component was tentatively identified as cytosine based on the known constituents of *S. flavescens* Ait. [12], and the ion at m/z 148 was ascribed to the scissions of bonds 7–13 and 9–11 of cytosine (shown in Scheme 1A). The CID mass spectrum of the ion at m/z 205 contained two product ions at m/z 146 and 108, which could have arisen from the cleavage of bonds 7–13/9–11 and 6–7/1–10 of N-methylcytisine [18], respectively (shown in Scheme 1B). A base peak at m/z 191 was observed in the MS^2 spectrum of m/z 223 due to the loss of CH_3OH from $[M+H]^+$, which was in agreement with the structure of kuraramine/isokuraramine [2]. Its proposed fragmentation is depicted in Scheme 1C.

The EIC of the ion at m/z 245.1666 exhibited two peaks at t_R values of 50.85 min and 51.76 min (shown in Fig. 1f; the peaks at an approximate t_R of 23 min are product ion peaks), and the accurate mass data suggested that these two components of *S. flavescens* Ait. had the same molecular formula as $C_{15}H_{20}N_2O$. The MS^2 spectrum of m/z 245 at t_R = 50.85 min displayed ions at m/z 227, 150, and 148. This compound could not be characterized on the basis of its MS^n spectra or other reported data. The ion with m/z 245 at t_R = 51.76 min exhibited two product ions at m/z 148 and 98. This compound was tentatively identified as anagyrine [2,18]. The cleavage of bonds 7–13/9–11 and 6–7/1–10 of protonated anagyrine resulted in the ions at m/z 148 and 98, respectively, and the fragmentation pathway is shown in Scheme 1D.

The EIC of the ion at m/z 247.1812 exhibited three peaks at t_R values of 51.56 min, 54.41 min, and 55.39 min, respectively (shown in Fig. 1g). All these components had a molecular formula of $C_{15}H_{20}N_2O$. The peak at t_R = 54.41 min was unambiguously identified as sophocarpine after comparison of its retention time and MS^n spectra with those of the standard. The CID mass spectrum of sophocarpine $[M+H]^+$ yielded product ions at m/z 227, 179, 150, 148, and 136. Among these, the ion at m/z 179 was the base one, which was generated by the loss of C_4H_4O from the D ring of $[M+H]^+$ by the retro Diels–Alder (RDA) cleavage. The peak at t_R = 51.56 min was low, and its MS^2 spectrum also exhibited ions at m/z 179, 150, 148, and 136, similar to sophocarpine but with low signal intensity ion at m/z 227. This compound was tentatively identified as isosophocarpine [2]. The MS^2 spectrum of m/z 245 at t_R = 55.39 min



Scheme 1. Characteristic fragmentation pathways of cytosine (A), N-methylcytisine (B), kuraramine/isokuraramine (C), anagyrine (D), lupanine (E), baptifoline (F), 7α-hydroxysophocarpine (G), leontalbinine N-oxide (H), sophoranol (I), and lamprolobine (J) in the positive ion mode.

yielded ions at m/z 176 and 148. This compound was tentatively identified as 7,11-dehydromatrine based on the known alkaloids in *S. flavescens* Ait [2,25]. The molecular formula was deduced as $C_{15}H_{20}N_2O$.

The EIC of the ion at m/z 249.1978 exhibited five peaks at t_R values of 50.98 min, 52.02 min, 53.74 min, 54.72 min, and 58.06 min, respectively (shown in Fig. 1h). These components all had a molecular formula of $C_{15}H_{24}N_2O$. The peaks at t_R values of 52.02 min and 54.72 min were unambiguously identified as sophoridine and matrine after comparison with the standard. Sophoridine and matrine are a pair of stereoisomers that exhibit the same MS^n spectra. The MS^2 spectra of the $[M+H]^+$ ions at m/z 249 both produced ions at m/z 176, 150, and 148. It is noteworthy that the MS^n spectra of the peak at t_R = 50.98 min was the same as those of sophoridine and matrine, indicating that this compound is a stereoisomer of sophoridine and matrine. It was tentatively named isomatrine [18,25]. The MS^2 spectrum of the ion at m/z 249 with t_R = 53.74 min displayed ions at m/z 231, 150, and 148. This compound could not be characterized on the basis of its MS^n spectra or other reported data. The MS^2 spectrum of the ion at m/z 249 and t_R = 58.06 min exhibited a predominant product ion at m/z 136, which was identified as lupanine [2,24]. Its characteristic fragmentation pathway is shown in Scheme 1E.

The EIC of the ion at m/z 261.1616 exhibited three peaks at t_R = 11.89 min, 12.28 min, and 36.88 min (shown in Fig. 1i). All these components have the molecular formula $C_{15}H_{20}N_2O_2$. The peak at t_R = 36.88 min displayed product ions at m/z 243, 164, and 114 in the MS^2 spectrum. This component was identified as baptifoline [2], and the base peak at m/z 114 was formed by the cleavage of bonds 7–13 and 9–11 (shown in Scheme 1F). The MS^2 spectrum of the peak at t_R = 11.89 min showed product ions at m/z 243 and 150 whereas that of the peak at t_R = 12.28 min exhibited ions at m/z 243 and 136. The ions with m/z 243 were both formed by the loss of H_2O from the parent ions, indicating that these two compounds are hydroxylalkaloids. These were identified as 7α-hydroxysophoramine and 9α-hydroxysophoramine, respectively, based on the known alkaloids in *S. flavescens* Ait. [2,25], and their molecular formula was deduced to be $C_{15}H_{20}N_2O_2$. The MS^3 spectrum of the ion with m/z 243 and t_R = 12.28 min exhibited a characteristic ion at m/z 215, corresponding to the loss of C_2H_4 from $[M+H-H_2O]^+$ with m/z 243 (the fragment of 28.0324 was identified as C_2H_4, not CO_2, based on the exact mass). This component was characterized as 7α-hydroxysophoramine, and its fragmentation pathway is shown in Scheme 1G. Thus, the peak with t_R = 11.89 min was further tentatively identified as 9α-hydroxysophoramine.

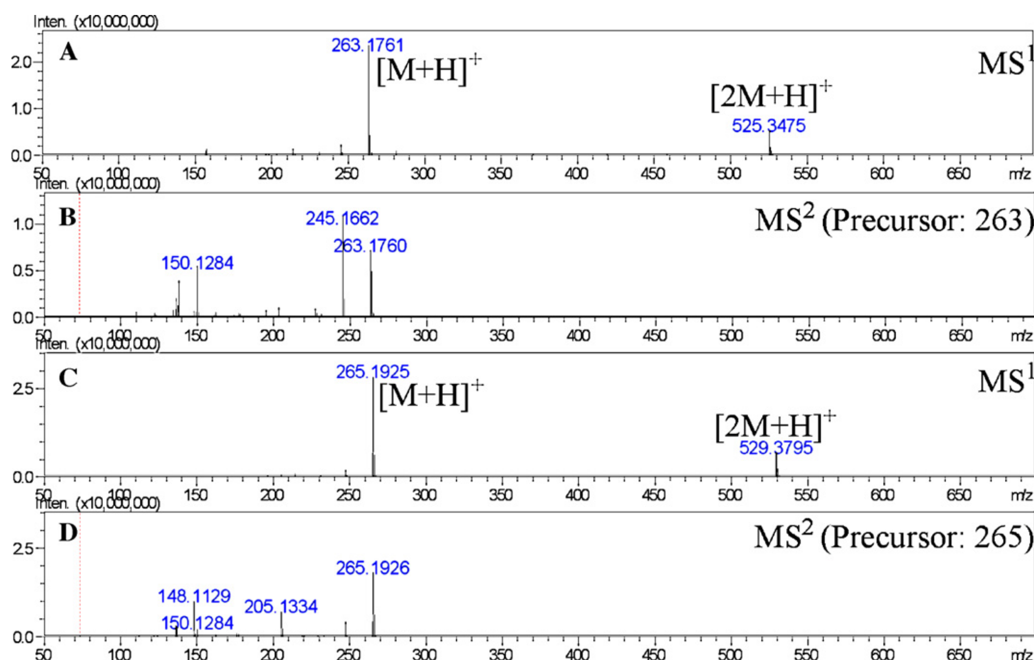


Fig. 3. The MS^n spectra of alkaloids in the positive ion mode: (A) the MS spectrum of oxysophocarpine; (B) the MS^2 spectrum of oxysophocarpine; (C) the MS spectrum of oxymatrine; (D) the MS^2 spectrum of oxymatrine.

The EIC of the ion at m/z 263.1761 exhibited four peaks at t_R = 22.20 min, 26.55 min, 45.08 min, and 48.69 min (shown in Fig. 1j). All these components had the molecular formula $C_{15}H_{22}N_2O_2$. The peak at t_R = 22.20 min was unambiguously identified as oxysophocarpine after comparison with the standard. It is interesting to note that oxysophocarpine had an $[M+H]^+$ ion at m/z 263 and a $[2M+H]^+$ ion at m/z 525 in the MS spectrum (shown in Fig. 3A), indicating that oxysophocarpine was easily polymerized to the dipolymer in the gas phase. Moreover, it is also noteworthy that the unfragmented parent ion at m/z 263 in the MS^2 spectrum still had a high signal intensity (shown in Fig. 3B). In addition to the ion at m/z 263, the MS^2 spectrum also exhibited ions at m/z 245, 150, and 138. The characteristic ion at m/z 195 in the MS^2 spectrum of the peak at t_R = 45.08 min was used to identify this compound as leontalbinine N-oxide [26]. Due to the presence of the double bond between C-7 and C-11, protonated leontalbinine N-oxide was easily dissociated at bonds 11–12 and 15–16, fragmenting C_4H_4O from the D ring to give rise to the ion at m/z 195 in the MS^2 spectrum (shown in Scheme 1H). The peak at t_R = 48.69 min exhibited a predominant ion at m/z 231 in the MS^2 spectrum due to the loss of CH_3OH, indicating that it was similar to kuraramine/isokuraramine. Therefore, this component was characterized as mamanine [2]. The MS^2 spectrum of the peak at t_R = 26.55 min displayed ions at m/z 245 and 164 and was tentatively identified as 9α-hydroxysophocarpine [25].

The EIC of the ion at m/z 265.1925 exhibited five peaks at t_R = 12.59 min, 20.57 min, 38.57 min, 47.12 min, and 52.92 min, respectively (shown in Fig. 1k). All these components had a molecular formula of $C_{15}H_{24}N_2O_2$. The peak at t_R = 20.57 min was unambiguously identified as oxymatrine by comparison with the standard. It is noteworthy that oxymatrine also exhibited a $[2M+H]^+$ ion in the MS spectrum and showed a high-signal intensity unfragmented parent ion $[M+H]^+$ in the MS^2 spectrum, similar to oxysophocarpine (shown in Fig. 3C and D). These fragmentation pathways could be used to characterize oxy-alkaloids in *S. flavescens* Ait. The peaks at t_R = 12.59 min and 47.12 min both showed an $[M+H-H_2O]^+$ ion with m/z 247 without a high-signal intensity unfragmented parent ion $[M+H]^+$ of m/z 263 in the MS^2 spectrum, suggesting that these were hydroxyl-alkaloids but not oxy-alkaloids. As the MS^3 spectrum of the ion with m/z 247 and t_R = 12.59 min exhibited an ion at m/z 175, this compound was identified as sophoranol [18,19]. Its fragmentation pathway is shown in scheme II. The peak at t_R = 47.12 min was further characterized as 9α-hydroxymatrine based on the known alkaloids in *S. flavescens* Ait [24]. The peak at t_R = 52.92 min exhibited one ion at m/z 150 in the MS^2 spectrum, which was identified as lamprolobine [27] (the fragmentation is shown in Scheme 1J). The MS^2 spectrum of the peak at t_R = 38.57 min showed ions at m/z 166 and 164, and it could not be characterized on the basis of its MS^n spectra or the reported alkaloids in *S. flavescens* Ait.

4. Conclusions

An HPLC–DAD–ESI–MS^n method was developed to chemically analyze the alkaloids present in *S. flavescens* Ait. In total, 22 alkaloids were identified or tentatively characterized based on their retention times and MS fragmentation behaviors. Among these, leontalbinine N-oxide and lamprolobine were characterized in *S. flavescens* Ait. for the first time.

Some significant characteristic fragmentation behaviors of these alkaloids in the positive ion mode were also observed. First, oxysophocarpine and oxymatrine exhibited a $[2M+H]^+$ ion in the MS spectrum and a high-signal intensity unfragmented parent ion $[M+H]^+$ in the MS^2 spectrum. These fragmentation pathways could be used to characterize oxy-alkaloids in *S. flavescens* Ait. Second, the characteristic ions at m/z 148, 146 and 108, 148 and 98, 136, 114, 195, and 150 in the MS^2 spectrum are of great importance for characterizing cytosine, N-methylcytisine, anagyrine, lupanine, baptifoline, leontalbinine N-oxide, and lamprolobine, respectively. Third, the dissociation of CH_3OH from $[M+H]^+$ dominated the fragmentation processes of kuraramine/isokuraramine and mamanine. All of the acquired mass data were useful for detecting and identifying these alkaloids in complex samples.

In comparison with conventional UV detection, MS provides a more sensitive and specific method for the qualification of alkaloids in *S. flavescens* Ait. This study provides chemical support for chromatographic fingerprint technology and should help to improve the quality control standard of *S. flavescens* Ait.

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