Published in final edited form as:

Phytochem Anal. 2014 ; 25(6): 485–494. doi:10.1002/pca.2517.

UPLC Separation and QToF–MS Identification of Major Alkaloids in Plumula Nelumbinis

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Abstract

Introduction—As an essential medicine and tea source in many countries, Plumula Nelumbinis potentially exerts its major biological activities through its alkaloids. However, its activities are not fully understood due to the lack of studies on its chemical components.

Objective—To establish an Ultra Performance Liquid Chromatography–Diode-Array Detector (UPLC–DAD) method, combined with an Electrospray Ionization–Quadrupole Time-of-flight Mass Spectrometry (ESI–QToF MS), for the separation and identification of Plumula Nelumbinis alkaloids.

Methods—The eluant from an UPLC separation of an ethanol extract of Plumula Nelumbinis was directly infused into an ESI–QToF MS system. Both positive and negative ion modes of ESI with low and high Collision Energy (CE) were used to obtain sufficient MS information.

Results—21 alkaloids were tentatively identified based on their chromatographic characteristics, UV spectra, exact mass, MS fragments, and literature reports. They consist of 6 *bis*-1-benzyltetrahydroisoquinoline, 11 benzyltetrahydroisoquinoline (containing 2 glycoalkaloids and 2 quaternary ammoniums), 2 aporphine, one proaporphine, and one indole alkaloids. Eleven were identified in Plumula Nelumbinis for the first time and 7 were firstly reported in *Nelumbo nucifera* Gaertn. Five compounds, namely norcoclaurine-4'-*O*-glucoside, norcoclaurine-6-*O*-glucoside, isolotusine, 6-demethyl-4'-methyl-*N*-methylcoclaurine and *N*-norisoliensinine, were characterized and proposed as new compounds.

Conclusion—The established UPLC–DAD–ESI–QToF–MS method is efficient for systematic identification of the alkaloids in Plumula Nelumbinis extract.

Keywords

Alkaloids; Plumula Nelumbinis; Ultra Performance Liquid Chromatography; QToF–MS

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Introduction

Plumula Nelumbinis (called “*Lian Zi Xin*” in Chinese), the dried embryo of the seed of *Nelumbo nucifera* Gaertn., is a widely used traditional Chinese medicine and green tea in most Asian countries. Previous studies on its activities suggested its usage for treatment of Alzheimer’s disease (Lin *et al.*, 2013), hypertension (Hu *et al.*, 1990; Yang *et al.*, 1999), arrhythmia (Qian 2002), platelet aggregation (Yu and Hu 1996), and stomach cancer (Shi *et al.*, 2003). Alkaloids (Chen *et al.*, 2007; Lin *et al.*, 2013) including liensinine, isoliensinine, and neferine, are demonstrated as main bioactive compounds responsible for these activities. These isolated alkaloids exhibit various activities such as butyrylcholinesterase (BChE) inhibition (Lin *et al.*, 2013), anti-human immunodeficiency virus (anti-HIV) (Kashiwada *et al.*, 2005), and anti-oxidation (Jia *et al.*, 1994) activities.

Chen and co-authors (Chen *et al.*, 2007) has previously reported pronuciferine, lotusine, nuciferine, liensinine, isoliensinine and neferine using high-performance liquid chromatography coupled with electrospray ionization time-of-flight mass spectrometry (HPLC–ESI–Tof–MS). Similarly, (Lin *et al.*, 2013) reported norisoliensinine, 6-hydroxy-norisoliensinine, liensinine, isoliensinine and neferine using HPLC–ESI–IT–Tof–MS (IT: Ion-trap) and liensinine, isoliensinine and neferine were quantified using HPLC–ESI–MS/MS (Li *et al.*, 2008).

In the present study, an Ultra Performance Liquid Chromatography with Quadrupole Time-of-flight Mass Spectrometry (UPLC–QToF MS) was applied to systematically identify major alkaloids in Plumula Nelumbinis.

EXPERIMENTAL

Materials and Chemicals

A Plumula Nelumbinis sample was purchased from Beijing Tong-Ren-Tang Group Co., Ltd. (Beijing, China), and identified by Dr. Ailiang Chen (Institute of Quality Standards and Testing Technology for Agro-products, Chinese Academy of Agricultural Sciences, Beijing, China) as the embryo of the seed of *Nelumbo nucifera* Gaertn. Analytical grade ethanol, HPLC-grade acetonitrile and water were purchased from Thermo Fischer Scientific (Fremont, CA, USA); ammonium acetate, formic acid and other reagents were purchased from Sigma (St. Louis, MO, USA).

Sample preparation

The sample was extracted as previously described (Lin *et al.*, 2013). Briefly, Plumula Nelumbinis was crushed into fine powder (approx. 2 g) which was weighed accurately and extracted with 90% ethanol (50 mL) in an ultrasonic water bath for 30 min. This extraction procedure was repeated three times. Solvent from the combined extracts was removed under reduced pressure and the powder residue dissolved in methanol (50 mL). The methanol solution was filtered through a 0.45 µm membrane prior to use and an aliquot (1 µL) was injected into the UPLC system.

UPLC conditions

The UPLC analyses were performed on a Waters ACQUITY I-Class UPLC system (Waters, Milford, USA) consisting of a binary pump, a column manager, an autosampler, a degasser, and a diode-array detector (DAD). An Agilent Zorbax Eclipse Plus C18 Column (2.1 × 50 mm, 1.8 μm) (Agilent Technology, Santa Clara, CA) maintained at 35 °C was used with a mobile phase consisting of a linear gradient of A (0.02 M acetic ammonium in water) and B (acetonitrile) under the following conditions: 0 → 1 → 3.5 → 7 → 7.5 → 8.5 → 9.5 → 10 min, 5% → 10% → 15% → 20% → 25% → 70% → 85% → 100%B. The flow rate was 0.3 mL/min and the DAD was operated in the range of 200–400 nm but 282 nm was used (Chen *et al.*, 2007; Lin *et al.*, 2013) for displaying chromatograms.

MS conditions

The UPLC was coupled to a Xevo™ G2-S QToF Mass Spectrometer (Waters, Milford, USA), a quadrupole hybrid with orthogonal acceleration time-of-flight tandem mass spectrometer. The scan range was set at 50 to 1200 Da in both positive and negative modes. The capillary and cone voltages were set at 3.0 kV and 30 V, respectively. The desolvation gas was maintained at 800 L/h at a temperature of 500 °C. The cone gas was set at 100 L/h with a source temperature of 150 °C. The data acquisition rate was set to 0.3 s, with a 20 s interval. All data were collected in centroid mode, the lockspray frequency was set at 20 s. Leucine-enkephalin solution (100 ng/mL) was utilized as the lock mass compound with a flow rate of 5 μL/min (*m/z* 556.2771 and 554.2615 for positive and negative modes, respectively). The MS data were collected with full scan mode for both positive and negative ion modes with low (6 V) and high (ramp from 20 V to 40 V) collision energy (CE) data channels to get the intact precursor ions (MS) and the fragment ions (MS/MS). All the data acquisition and analyses were controlled by Waters MassLynx v4.1 software.

RESULTS AND DISCUSSION

Using extracted ion chromatograms (EIC) displaying the protonated molecular ion ((M + H)⁺, positive ion mode) or the deprotonated molecular ion ((M – H)[–], negative ion mode) for anticipated or potential alkaloids allowed tentative identification of 21 alkaloids (Fig. 1). Both the positive and negative ion modes were found to be efficient for the identification of alkaloids in *Plumula Nelumbinis*.

The UV Chromatogram was shown in Figure 2A and MS data produced by low CE were shown in Figure 2B and 2C. As an accurate tool, QToF MS can largely overcome the separation problem associated with certain overlapping peaks in UPLC. As shown in Figure 2D, all peaks can be distinguished well based on the extracted ion chromatogram (EIC) of the ions for 21 alkaloids. As shown in Figure 1, the chemical structures of these compounds are quite similar. Among them, lotusine, liensinine, isoliensinine and neferine are the major alkaloids in the present study, consistent with existing literature reports (Chen *et al.*, 2007; Li *et al.*, 2008). MS and MS/MS data in both positive and negative modes were listed in Table 1, each characteristic fragment was predicted to yield the molecular formula within 5 ppm. These 21 components were classified into five groups based on their structures: bis-1-benzyltetrahydroisoquinolines, benzyltetrahydroisoquinolines, aporphines, proaporphines

and indole alkaloids. Among them, eleven alkaloids were identified in Plumula Nelumbinis for the first time, seven were first reported in the plant *Nelumbo nucifera* GAERTN., and five compounds (namely norcoclaurine-4'-*O*-glucoside, norcoclaurine-6-*O*-glucoside, isolotusine, 6-demethyl-4'-methyl-*N*-methylcoclaurine and *N*-norisoliensinine) were proposed as new compounds according to their MS spectra.

Identification of bisbenzyltetrahydroisoquinoline alkaloids for peaks 14–16 and 18–20

For peaks **18**, **19** and **20** (Figure 2 D10–D11) in positive ion mode, the molecular formula of ions at m/z 611.3116 and 625.3278 were predicted as $C_{37}H_{43}N_2O_6$ ($[M+H]^+$) and $C_{38}H_{45}N_2O_6$ ($[M+H]^+$). The UV spectra of these peaks showed maximal absorption wavelengths at 229 and 281 nm, 227 and 283 nm, and 224 and 282 nm, respectively. In MS/MS, peak **18** showed ions at m/z 489.2389 ($C_{29}H_{33}N_2O_5$), 312.1235 ($C_{18}H_{18}NO_4$) and 206.1179 ($C_{12}H_{16}NO_2$). These data were consistent with **18** being liensinine (Chen *et al.*, 2007; Li *et al.*, 2008). As an isomer of liensinine, two ions at m/z 475.2234 ($C_{28}H_{31}N_2O_5$, $[489-CH_2]^+$) and 192.1025 ($C_{11}H_{14}NO_2$, $[206-CH_2]^+$) of compound **19** indicated a different substitution position, and was tentatively identified as isoliensinine (Chen *et al.*, 2007; Li *et al.*, 2008; Lin *et al.*, 2013). **20** showed one more methyl group than **18** and **19**; and its two major ions (m/z 489 and 206) are the same as that of liensinine. Thus **20** was tentatively identified as neferine based on its MS behaviors (Chen *et al.*, 2007; Li *et al.*, 2008; Lin *et al.*, 2013). Data in Table 1 were also consistent with their identifications.

The EIC of m/z 597.2969 ($C_{36}H_{41}N_2O_6$, $[M+H]^+$) in positive ion mode exhibited three peaks (peak **14**, **15** and **16** shown in Figure 2 D8). This ion indicates one less methyl group than isoliensinine. They exhibited a major ion (m/z 192.1024, $C_{11}H_{14}NO_2$), the same one of isoliensinine, in MS/MS. This indicated possible demethylation of R_3 (Figure 1). In addition, detailed fragments (Table 1) strongly suggested their similarity with isoliensinine. Compound **14** and **15** were thus tentatively identified as norisoliensinine and 6-hydroxynorisoliensinine (Lin *et al.*, 2013), and **16** was tentatively proposed to have a secondary amine due to its latter retention time and was given the trial name *N*-norisoliensinine. The proposed fragmentation pathway of **16** was depicted in Figure 3.

Identification of benzyltetrahydroisoquinoline alkaloids for peaks 4–8 and 10–13

The EIC of m/z 314.1751 ($C_{19}H_{24}NO_3$, $[M+H]^+$) of **5**, **6**, **7** and **8** in Figure 2 D4 indicated they were substituted benzyltetrahydroisoquinoline alkaloids (Li *et al.*, 2008; Segrero-Nieves 1986). In positive MS/MS, both of **5** (Figure 4A) and **6** yielded same fragments m/z 269.1181 ($C_{17}H_{17}O_3$, cleavages of C_1-N_2 and N_2-C_3), 237.0917 ($C_{16}H_{13}O_2$, additional loss of CH_3O- at C_6), 209.0969 ($C_{15}H_{13}O$) and 175.0761 ($C_{11}H_{11}O_2$), while peaks **7** (Figure 4B) and **8** showed an extra ion (m/z 283.1333, $C_{18}H_{19}O_3$) indicating a neutral fragment loss of CH_3NH_2- from a tertiary amine. This is the same behavior of **16** (from m/z 597 to 556 Figure 3). The loss of $CH_3(CH_3)NH-$ from 314 for **5** and **6** indicated a quaternary ammonium group which was consistent with their greater tailing effect and shorter retention times. Therefore **5** and **6** were tentatively identified as lotusine (Li *et al.*, 2008) and isolotusine, and **7** and **8** were tentatively identified as 4'-methyl-*N*-methylcoclaurine and arnepavine as known constituents in Plumula Nelumbinis (Segrero-Nieves 1986). The

fragmentation pathways of lotusine (**5**) and 4'-methyl-*N*-methylococlaurine (**7**) are shown in Figure 4A and Figure 4B.

Compounds **10**, **11** and **12** in Figure 2 D6 ($[M+H]^+$, $C_{18}H_{22}NO_3$) have one less methyl group than **7** (or **5**, **6**, **8**). In (+)MS/MS, their spectra (Figure 4C of peak **12**) are almost the same, very similar to **7** (Figure 4B) except absences of m/z 314 and 283. The loss of $CH_3NH_2^-$ from m/z 300 suggested they were all *N*-methyl tertiary amines. Since **10** and **11** are close to each other in UPLC, the second CH_3^- was tentatively assigned at C_7 for **10** (*N*-methylisococlaurine) or C_6 for **11** (*N*-methylococlaurine) (Kunitomo *et al.*, 1973; Mukherjee *et al.*, 2009). Methylation of C_{13} OH group was possibly assigned for **12** and named 6-demethyl-4-identifie*N*-methylococlaurine as a new compound after comparing with **7**.

The ion at m/z 286.1444 ($C_{17}H_{20}NO_3$, **13**) in Figure 2 D7 indicated one less methyl group than **10** (**11** or **12**). The (+)MS/MS data of this compound (Table 1) was similar with other identified benzyltetrahydroisoquinoline compounds, suggesting their structure similarities. The loss of NH_3^- (m/z 269) suggested **13** a secondary amine, and it was tentatively identified as coclaurine as a common component in *Nelumbo nucifera* (Kashiwada *et al.*, 2005). The fragments of **13** were further explained in Figure 4 E. For peak **4** (EIC of m/z 272.1281, $C_{16}H_{18}NO_3$) in Figure 2 D3, it indicated one less methyl group than coclaurine (**13**), and was tentatively identified as norcoclaurine by reported MS information (Kashiwada *et al.*, 2005; Koshiyama *et al.*, 1970).

Identification of glycoalkaloids for peaks 1 and 2

The EIC of m/z 434.1809 ($C_{22}H_{27}NO_8$) gave two polar compounds (Figure 2 D1). The ion at m/z 272.1288 ($C_{16}H_{18}NO_3$) in MS/MS (Figure 4 D) indicated a loss of glucose ($C_6H_{10}O_5$) moiety (Agnihotri *et al.*, 2008; Kashiwada *et al.*, 2005; Mukherjee *et al.*, 2009). The ions at m/z 255.1021 ($C_{16}H_{15}O_3$, $[272-NH_3]^+$) and 161.0602 ($C_{10}H_9O_2$, $[272-NH_3-C_6H_5O]^+$) indicated the aglycone of the two glycosides was norcoclaurine. The glycosylated compounds were tentatively assigned as norcoclaurine-4'-*O*-glucoside (**1**) and norcoclaurine-6-*O*-glucoside (**2**), respectively.

Identification of aporphine alkaloids for peaks 9 and 21

Peak **21** (m/z 296.1645, $C_{19}H_{22}NO_2$) (Figure 2 D1) displayed m/z 265.1231 ($C_{18}H_{17}O_2$, $[M+H-CH_3NH_2]^+$), 250.0996 ($C_{17}H_{14}O_2$), 235.0765 ($C_{16}H_{11}O_2$), 219.0807 ($C_{16}H_{11}O$), 191.0860 ($C_{15}H_{11}$), 179.0855 ($C_{14}H_{11}$) in their MS/MS, suggesting the presence of *N*-methyl group. It was tentatively identified as nuciferine (Agnihotri *et al.*, 2008; Luo *et al.*, 2005). Peak **9** (m/z 298.1438, $C_{18}H_{20}NO_3$) in Figure 2 D5 indicated one more OH group and one less methyl group than nuciferine, it also showed similar UV spectrum indicating its aporphine structure. MS/MS spectrum have shown successive losses of two methyl groups (m/z 283 and 268 in Table 1) and one H_2O (m/z 254), indicating two methoxy and one hydroxyl groups. This compound was tentatively identified as nornuciferidine reported as a natural alkaloid (Rasamizafy *et al.*, 1987) shown in Figure 4 F.

Identification of proaporphine alkaloid for peaks 17 and indole alkaloid for peak 3

Peak **17** (m/z 312.1606, $C_{19}H_{22}NO_3$) in Figure 2 D9 exhibited ions at m/z 283.1330 ($C_{18}H_{19}O_3$, $[M+H-CH_3N]^+$), 269.1175 ($C_{17}H_{17}O_3$), 177.0905 ($C_{11}H_{13}O_2$) (Table 1) in (+)MS/MS. It was tentatively identified as pronuciferine after comparing its UV and MS spectra (Kunitomo *et al.*, 1973; Li *et al.*, 2008; Mukherjee *et al.*, 2009). For peak **3** (Figure 2 D2), the precursor m/z 160.0763 ($C_{10}H_{10}NO$, $[M+H]^+$) yielded m/z 146.0606 (C_9H_8NO), 130.0655 (C_9H_8N) and 115.0545 (C_9H_7), indicating the losses of one methoxy and one carbonyl groups. Based on its UV spectrum (UV_{max} at 220 and 273 nm), compound **3** and it was tentatively identified as a known compound 2-methyl-3-formylindole.

In summary, the acquired UPLC/DAD/QToF-MS data are powerful for rapid identification of various alkaloids in *Plumula Nelumbinis*. This study may provide the helpful chemical basis to explain the pharmacological and diary efficacies of *Plumula Nelumbinis*, and facilitate further drug development and quality control of this material.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was partially supported by an NIH instrument grant 1S10OD010678-01 to WL and funds from the Department of Pharmaceutical Sciences, College of Pharmacy, the University of Tennessee Health Science Center.

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Figure 1.
Structures of 21 identified compounds

Figure 2.

The chromatogram at 282 nm (A), TIC in positive (B) and negative (C) ion modes at low CE, and EICs (D1–D12) of the components in Plumula Nelumbinis sample.

Figure 3.
The proposed fragmentation pathway of *N*-norisoliensinine (compound **16**) in positive ion mode.

Figure 4. The MS/MS spectra (high CE) and possible fragmentation schemes of lotusine (A, compound **5**), 4'-methyl-*N*-methylcoclaurine (B, compound **7**), 6-demethyl-4'-methyl-*N*-methylcoclaurine (C, compound **12**), norcoclaurine-6-*O*-glucoside (D, compound **2**), coclaurine (E, compound **13**) and nuciferidine (B, compound **9**) in positive ion mode.

Characterization of constituents in *P. nelumbinis* by UPLC-QToF-MS and MS/MS analysis in positive and negative ion modes. All source spectra are shown in Supplementary Materials.

Table 1

No.	Identification	t_R^d (min)	(+/-) experimental MS (error in ppm)	(+/-) predicted formula	(+) experimental MS ^E (formula)	(-) experimental MS ^E (formula)
1 <i>a,b,c</i>	Norococlaurine-4'- <i>O</i> -glucoside	0.49	434.1814 (-0.2)/432.1660 (0.5)	C ₂₂ H ₂₈ N ₂ O ₈ /C ₂₂ H ₂₆ N ₂ O ₈	272.1285(C ₁₆ H ₁₈ NO ₃), 255.1021(C ₁₆ H ₁₅ O ₃), 161.0602(C ₁₀ H ₉ O ₂), 107.0496(C ₇ H ₇ O)	270.1131(C ₁₆ H ₁₆ NO ₃), 239.0704(C ₁₅ H ₁₁ O ₃), 162.0555(C ₉ H ₈ NO ₂)
	Norococlaurine-6- <i>O</i> -glucoside	1.34	434.1817 (0.5)/432.1663 (1.2)	C ₂₂ H ₂₈ N ₂ O ₈ /C ₂₂ H ₂₆ N ₂ O ₈	272.1288(C ₁₆ H ₁₈ NO ₃), 255.1023(C ₁₆ H ₁₅ O ₃), 161.0602(C ₁₀ H ₉ O ₂), 145.0650(C ₁₀ H ₉ O), 123.0447(C ₇ H ₇ O ₂), 107.0497(C ₇ H ₇ O)	270.1136(C ₁₆ H ₁₆ NO ₃), 162.0555(C ₉ H ₈ NO ₂), 137.0603(C ₈ H ₉ O ₂)
3 <i>a,b</i>	2-methyl-3-fornylindole	1.05	160.0763 (0.6)/-	C ₁₀ H ₁₀ NO/-	146.0606(C ₉ H ₈ NO), 130.0655(C ₉ H ₈ N), 115.0545(C ₉ H ₇)	-
4 <i>b</i>	Norococlaurine	2.02	272.1290(1.1)/270.1128(0.3)	C ₁₆ H ₁₈ N ₂ O ₃ /C ₁₆ H ₁₆ NO ₃	255.1023(C ₁₆ H ₁₅ O ₃), 161.0606(C ₁₀ H ₉ O ₂), 143.0496(C ₁₀ H ₇ O), 123.0450(C ₇ H ₇ O ₂), 115.0551(C ₉ H ₇), 107.0500(C ₇ H ₇ O)	162.0556(C ₉ H ₈ NO ₂) 135.0441(C ₈ H ₇ O ₂)
5	Lotusine	2.26	314.1761 (1.6)/312.1602 (0.6)	C ₁₉ H ₂₄ N ₂ O ₃ /C ₁₉ H ₂₂ N ₂ O ₃	269.1181(C ₁₇ H ₁₇ O ₃), 237.0917(C ₁₆ H ₁₅ O ₃), 209.0969(C ₁₅ H ₁₃ O), 175.0761(C ₁₁ H ₁₁ O ₂), 143.0499(C ₁₀ H ₇ O), 121.0656(C ₈ H ₆ O), 107.0500(C ₇ H ₇ O)	239.0703(C ₁₅ H ₁₁ O ₃), 221.0602(C ₁₅ H ₉ O ₂), 209.0599(C ₁₄ H ₉ O), 193.0652(C ₁₄ H ₉ O ₂), 162.0554(C ₉ H ₈ NO ₂)
6 <i>a,b,c</i>	Isolotusine	2.64	314.1757 (0.3)/312.1599 (-0.3)	C ₁₉ H ₂₄ N ₂ O ₃ /C ₁₉ H ₂₂ N ₂ O ₃	269.1179(C ₁₇ H ₁₇ O ₃), 255.1021(C ₁₆ H ₁₅ O ₃), 237.0924(C ₁₆ H ₁₃ O ₂), 209.0969(C ₁₅ H ₁₃ O), 161.1605(C ₁₀ H ₉ O ₂), 143.0500(C ₁₀ H ₇ O), 107.0501(C ₇ H ₇ O)	239.0706(C ₁₅ H ₁₁ O ₃), 221.0603(C ₁₅ H ₉ O ₂), 209.0600(C ₁₄ H ₉ O), 193.0652(C ₁₄ H ₉ O), 145.0289(C ₉ H ₅ O ₂)
7	4'-methyl- <i>N</i> -methylococlaurine	4.18	314.1759 (1.0)/312.1597 (-1.0)	C ₁₉ H ₂₄ N ₂ O ₃ /C ₁₉ H ₂₂ N ₂ O ₃	283.1333(C ₁₈ H ₁₉ O ₃), 269.1179(C ₁₇ H ₁₇ O ₃), 237.0918(C ₁₆ H ₁₃ O ₂), 209.0969(C ₁₅ H ₁₃ O), 190.0873(C ₁₁ H ₁₂ NO ₂), 175.0760(C ₁₁ H ₁₁ O ₂), 143.0499(C ₁₀ H ₇ O), 121.0655(C ₈ H ₆ O), 107.0501(C ₇ H ₇ O)	239.0710(C ₁₅ H ₁₁ O ₃), 221.0599(C ₁₅ H ₉ O ₂), 147.0452(C ₉ H ₇ O ₂)

No.	Identification	R_f^D (min)	(+/-) experimental MS (error in ppm)	(+/-) predicted formula	(+) experimental MS ^E (formula)	(-) experimental MS ^E (formula)
8	Arnepavine	5.50	314.1758 (0.6)/312.1594 (-1.9)	C ₁₉ H ₂₄ NO ₃ /C ₁₉ H ₂₂ NO ₃	283.1331(C ₁₈ H ₁₉ O ₃), 237.0911(C ₁₆ H ₁₃ O ₂), 206.1177(C ₁₂ H ₁₆ NO ₂), 190.0880(C ₁₁ H ₁₂ NO ₂), 151.0753(C ₉ H ₉ O ₂), 145.0650(C ₁₀ H ₉ O), 121.0649(C ₈ H ₉ O), 107.0493(C ₇ H ₇ O)	–
9a,b	Normuciferidine	2.53	298.1447 (1.3)/296.1284 (-1.0)	C ₁₈ H ₂₀ NO ₃ /C ₁₈ H ₁₈ NO ₃	283.1213(C ₁₇ H ₁₇ NO ₃), 268.0977(C ₁₆ H ₁₄ NO ₃), 254.1188(C ₁₆ H ₁₆ NO ₂), 240.1027(C ₁₅ H ₁₄ NO ₂), 226.1236(C ₁₅ H ₁₆ NO), 190.0869(C ₁₁ H ₁₂ NO ₂), 176.0715(C ₁₀ H ₁₀ NO ₂), 148.0766(C ₉ H ₁₀ NO), 107.0500(C ₇ H ₇ O)	239.0706(C ₁₅ H ₁₁ O ₃), 221.0603(C ₁₅ H ₉ O ₂), 209.0603(C ₁₄ H ₉ O ₂), 193.0652(C ₁₄ H ₉ O), 145.0289(C ₉ H ₅ O ₂)
10 ^b	N-methylisococlaurine	3.53	300.1603 (1.0)/298.1441 (-0.7)	C ₁₈ H ₂₂ NO ₃ /C ₁₈ H ₂₀ NO ₃	269.1180(C ₁₇ H ₁₇ O ₃), 237.0919(C ₁₆ H ₁₃ O ₂), 209.0971(C ₁₅ H ₁₃ O), 175.0757(C ₁₁ H ₁₁ O ₂), 145.0653(C ₁₀ H ₉ O), 107.0497(C ₇ H ₇ O)	133.0289(C ₈ H ₅ O ₂)
11 ^b	N-methylcoclaurine	3.84	300.1605 (1.7)/298.1441 (-0.7)	C ₁₈ H ₂₂ NO ₃ /C ₁₈ H ₂₀ NO ₃	269.1177(C ₁₇ H ₁₇ O ₃), 237.0912(C ₁₆ H ₁₃ O ₂), 209.0964(C ₁₅ H ₁₃ O), 143.0496(C ₁₀ H ₇ O), 107.0496(C ₇ H ₇ O)	223.0762(C ₁₅ H ₁₁ O ₂)
12a,b,c	6-demethyl-4'-methyl-N-methylcoclaurine	5.96	300.1599 (-0.3)/298.1440 (-1.0)	C ₁₈ H ₂₂ NO ₃ /C ₁₈ H ₂₀ NO ₃	269.1177(C ₁₇ H ₁₇ O ₃), 237.0914(C ₁₆ H ₁₃ O ₂), 209.0965(C ₁₅ H ₁₃ O), 192.1018(C ₁₁ H ₁₄ NO ₂), 175.0756(C ₁₁ H ₁₁ O ₂), 143.0494(C ₁₀ H ₇ O), 107.0496(C ₇ H ₇ O)	–
13 ^b	Coclaurine	5.62	286.1444(0.3)/284.1285 (-0.7)	C ₁₇ H ₂₀ NO ₃ /C ₁₇ H ₁₈ NO ₃	269.1178(C ₁₇ H ₁₇ O ₃), 237.0910(C ₁₆ H ₁₃ O ₂), 209.0961(C ₁₅ H ₁₃ O), 143.0492(C ₁₀ H ₇ O), 115.0544(C ₉ H ₇), 107.0496(C ₇ H ₇ O)	176.0708(C ₁₀ H ₁₀ NO ₂)
14	Norisoliensinine	7.06	597.2966 (0.2)/595.2803 (-0.8)	C ₃₆ H ₄₁ N ₂ O ₆ /C ₃₆ H ₃₉ N ₂ O ₆	566.2549(C ₃₅ H ₃₆ NO ₆), 554.2543(C ₃₄ H ₃₆ NO ₆), 489.2400(C ₂₉ H ₃₃ N ₂ O ₅), 475.2241(C ₂₈ H ₃₁ N ₂ O ₅), 446.1977(C ₂₇ H ₂₈ NO ₅), 296.1298(C ₁₈ H ₁₈ NO ₃), 206.1184(C ₁₂ H ₁₆ NO ₂), 192.1030(C ₁₁ H ₁₄ NO ₂), 175.0765(C ₁₁ H ₁₁ O ₂), 107.0499(C ₇ H ₇ O)	282.1125(C ₁₇ H ₁₆ NO ₂)

No.	Identification	t_R^d (min)	(+/-) experimental MS (error in ppm)	(+/-) predicted formula	(+) experimental MS ^b (formula)	(-) experimental MS ^b (formula)
15	6-hydroxynorisoliensinine	8.08	597.2970 (0.8)/595.2813 (0.8)	C ₃₆ H ₄₁ N ₂ O ₆ /C ₃₆ H ₃₉ N ₂ O ₆	566.2535(C ₃₃ H ₃₆ NO ₆), 554.2540(C ₃₃ H ₃₆ NO ₆), 489.2370(C ₂₉ H ₃₃ N ₂ O ₅), 475.2228(C ₂₈ H ₃₁ N ₂ O ₅), 432.1815(C ₂₅ H ₂₆ NO ₅), 312.1243(C ₁₈ H ₁₈ NO ₄), 296.1271(C ₁₈ H ₁₈ NO ₃), 206.1177(C ₁₂ H ₁₆ NO ₂), 192.1021(C ₁₁ H ₁₄ NO ₂), 107.0499(C ₇ H ₇ O)	282.1127(C ₁₇ H ₁₉ NO ₃), 268.0969(C ₁₆ H ₁₇ NO ₃)
16 ^{a,b,c}	N-norisoliensinine	8.42	597.2969 (0.7)/595.2813 (0.8)	C ₃₆ H ₄₁ N ₂ O ₆ /C ₃₆ H ₃₉ N ₂ O ₆	566.2534(C ₃₃ H ₃₆ NO ₆), 554.2534(C ₃₃ H ₃₆ NO ₆), 436.2114(C ₂₅ H ₂₆ NO ₅), 418.2005(C ₂₅ H ₂₈ NO ₄), 314.1381(C ₁₈ H ₂₀ NO ₄), 206.1172(C ₁₂ H ₁₆ NO ₂), 192.1017(C ₁₁ H ₁₄ NO ₂), 176.1066(C ₁₁ H ₁₄ NO), 121.0646(C ₈ H ₉ O), 107.0499(C ₇ H ₇ O)	581.2669(C ₃₃ H ₃₇ N ₂ O ₆), 548.2445(C ₃₃ H ₃₄ N ₂ O ₅), 473.2079(C ₂₈ H ₂₉ N ₂ O ₅), 190.0880(C ₁₁ H ₁₂ NO ₂), 176.0715(C ₁₀ H ₁₀ NO ₂)
17	Pronuciferine	8.28	312.1606 (1.9)/-	C ₁₉ H ₂₂ NO ₃ /-	283.1330(C ₁₈ H ₁₉ O ₃), 269.1175(C ₁₇ H ₁₇ O ₃), 177.0905(C ₁₁ H ₁₃ O ₂)	-
18	Liensinine	8.47	611.3129 (1.3)/609.2966(0.2)	C ₃₇ H ₄₃ N ₂ O ₆ /C ₃₇ H ₄₁ N ₂ O ₆	580.2709(C ₃₃ H ₃₈ NO ₆), 568.2702(C ₃₃ H ₃₈ NO ₆), 503.2549(C ₃₀ H ₃₅ N ₂ O ₅), 489.2389(C ₂₉ H ₃₃ N ₂ O ₅), 446.1977(C ₂₅ H ₂₈ NO ₅), 312.1235(C ₁₈ H ₁₈ NO ₄), 206.1179(C ₁₂ H ₁₆ NO ₂), 192.1018(C ₁₁ H ₁₄ NO ₂), 174.0911(C ₁₁ H ₁₂ NO), 162.0912(C ₁₀ H ₁₂ NO), 107.0492(C ₇ H ₇ O)	595.2799(C ₃₃ H ₃₉ N ₂ O ₆), 577.2704(C ₃₃ H ₃₇ N ₂ O ₅), 487.2221(C ₂₉ H ₃₁ N ₂ O ₅), 473.2070(C ₂₈ H ₂₉ N ₂ O ₅), 386.1748(C ₂₅ H ₂₄ NO ₅), 297.1366(C ₁₉ H ₁₈ N ₂ Na), 282.1129(C ₁₇ H ₁₆ NO ₃)
19	Isoliensinine	8.60	611.3123 (0.3)/609.2970 (0.8)	C ₃₇ H ₄₃ N ₂ O ₆ /C ₃₇ H ₄₁ N ₂ O ₆	580.2710(C ₃₃ H ₃₈ NO ₆), 568.2699(C ₃₃ H ₃₈ NO ₆), 503.2549(C ₃₀ H ₃₅ N ₂ O ₅), 489.2383(C ₂₉ H ₃₃ N ₂ O ₅), 475.2234(C ₂₅ H ₃₁ N ₂ O ₅), 432.1811(C ₂₅ H ₂₆ NO ₅), 206.1180(C ₁₂ H ₁₆ NO ₂), 192.1025(C ₁₁ H ₁₄ NO ₂), 176.1071(C ₁₁ H ₁₄ NO), 121.0650(C ₈ H ₉ O)	577.2701(C ₃₃ H ₃₇ N ₂ O ₅), 534.2271(C ₃₃ H ₃₂ NO ₅), 487.2234(C ₂₉ H ₃₁ N ₂ O ₅), 473.2079(C ₂₈ H ₂₉ N ₂ O ₅), 441.1808(C ₂₇ H ₂₅ N ₂ O ₄), 386.1748(C ₂₅ H ₂₄ NO ₅), 326.1392(C ₁₉ H ₂₀ NO ₄), 312.1602(C ₁₉ H ₂₂ NO ₃), 296.1284(C ₁₈ H ₁₉ NO ₃), 190.0868(C ₁₁ H ₁₂ NO ₂), 176.0711(C ₁₀ H ₁₀ NO ₂), 121.0291(C ₇ H ₅ O ₂)

No.	Identification	R^d_t (min)	(+/-) experimental MS (error in ppm)	(+/-) predicted formula	(+) experimental MS ^b (formula)	(-) experimental MS ^b (formula)
20	Neferine	8.90	625.3280 (0.3)/623.3117(-0.6)	C ₃₈ H ₄₅ N ₂ O ₆ /C ₃₈ H ₄₃ N ₂ O ₆	611.3129(C ₃₇ H ₄₃ N ₂ O ₆), 594.2869(C ₃₇ H ₄₀ NO ₆), 582.2859(C ₃₇ H ₄₀ NO ₆), 503.2543(C ₃₀ H ₃₅ N ₂ O ₅), 489.2391(C ₂₉ H ₃₃ N ₂ O ₅), 446.1963(C ₂₇ H ₂₈ NO ₅), 328.1549(C ₁₉ H ₂₂ NO ₄), 206.1182(C ₁₂ H ₁₆ NO ₂), 192.1023(C ₁₁ H ₁₄ NO ₂), 176.1071(C ₁₁ H ₁₄ NO), 135.0806(C ₉ H ₁₁ O), 121.0651(C ₈ H ₆ O)	591.2861(C ₃₇ H ₃₉ N ₂ O ₅), 548.2444(C ₃₅ H ₃₄ NO ₅), 518.1972(C ₃₅ H ₃₈ N ₂ O ₅), 487.2234(C ₂₉ H ₃₄ N ₂ O ₅), 455.1969(C ₂₈ H ₃₂ N ₂ O ₄), 440.1858(C ₂₈ H ₃₀ NO ₄), 402.1708(C ₂₅ H ₂₄ NO ₄), 386.1756(C ₂₅ H ₂₄ NO ₃), 312.1602(C ₁₉ H ₂₂ NO ₃), 296.1287(C ₁₈ H ₁₈ NO ₃), 264.1026(C ₁₇ H ₁₄ NO ₂), 176.0712(C ₁₀ H ₁₀ NO ₂), 121.0290(C ₇ H ₅ O ₂)
21	Nuciferine	9.20	296.1654 (1.0)/-	C ₁₉ H ₂₂ NO ₂ /-	265.1231(C ₁₈ H ₁₇ O ₂), 250.0996(C ₁₇ H ₁₄ O ₂), 235.0765(C ₁₆ H ₁₁ O ₂), 219.0807(C ₁₆ H ₁₁ O), 191.0860(C ₁₅ H ₁₁), 179.0855(C ₁₄ H ₁₁)	-

^a firstly identified in *Nelumbo nucifera* Gaertn.

^b firstly identified in *Plumula Nelumbinis*;

^c proposed as a new compound;

^d retention time.