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ORIGINAL ARTICLE

UFLC-PDA-MS/MS Profiling of Seven *Uncaria* Species Integrated with Melatonin/5-Hydroxytryptamine Receptors Agonistic Assay

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Abstract

Uncariae Ramulus Cum Uncis (Gou-Teng), the dried hook-bearing stems of several *Uncaria* plants (Rubiaceae), is a well-known herbal medicine in China. The clinical application of Gou-Teng is bewildered for the morphological and chemical similarity between different species. In order to discern their chemical and biological difference, an ultra-fast liquid chromatography equipped with ion trap time-of-flight mass spectrometry (UFLC-IT/TOF-MS) combining with melatonin (MT_1 and MT_2) and 5-hydroxytryptamine ($5\text{-}HT_{1A}$ and $5\text{-}HT_{2C}$) receptors agonistic assay in vitro was conducted on seven *Uncaria* species. As a result, 57 compounds including 35 indole alkaloids, ten flavonoids, five triterpenoids, five chlorogenic analogues, and two other compounds were characterized based on their MS/MS patterns and UV absorptions. Specifically, cadambine-type and corynanthein-type alkaloids were exclusively present in *U. rhynchophylla* and *U. scandens*, whereas corynoxine-type alkaloids were commonly detected in all the seven *Uncaria* plants. Three *Uncaria* species, *U. rhynchophylla*, *U. macrophylla*, and *U. yunnanensis* showed obviously agnostic activity on four neurotransmitter receptors (MT_1, MT_2, $5\text{-}HT_{1A}$, and $5\text{-}HT_{2C}$). This first-time UFLCMS-IT-TOF analyses integrated with biological assay on seven *Uncaria* plants will provide scientific viewpoints for the clinical application of Gou-Teng.

We dedicate this paper to Prof. Sun Han-Dong on the occasion of his 80th birthday.

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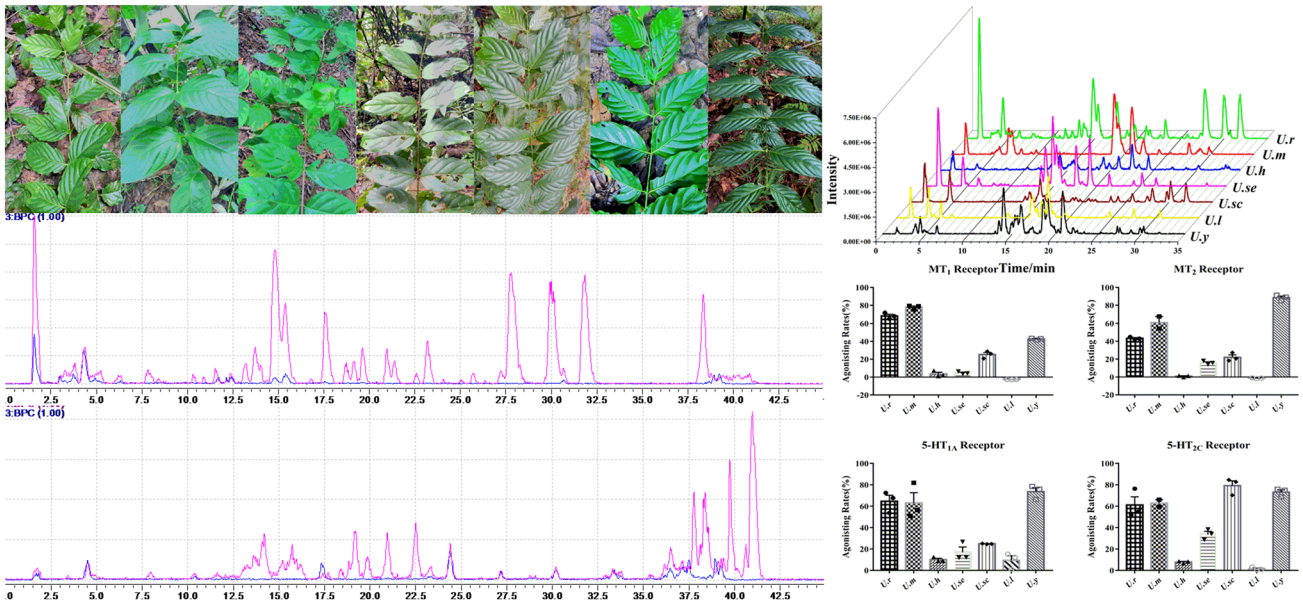
Graphic Abstract



Keywords Uncariae Ramulus Cum Uncis · *Uncaria* plants · LCMS-IT-TOF analyses · Melatonin and 5-hydroxytryptamine receptors

1 Introduction

Uncariae Ramulus Cum Uncis (Gou-Teng), the dried hook-bearing stems of *Uncaria* plants (Rubiaceae), is a well-known traditional Chinese medicine (TCM), which has long been used for the treatment of hypertension, fever, headache, dizziness, stroke, and bilious disorders in China [1–4]. In addition to monotherapies, Gou-Teng is also prescribed in many formulae, such as Diao-Teng San (Cho-Deung-San in Korean and Choto-san in Japanese) and Yi-Gan San (Yokukansan in Japanese) [2]. Indole alkaloids as the characteristic constituents of *Uncaria* plants are responsible for the hypotensive effects, *e.g.* rhynchophylline and hirsutine showing antihypertensive and antiarrhythmic effects [5, 6]. According to the latest Chinese Pharmacopoeia (2015 edition), five *Uncaria* plants, namely *Uncaria rhynchophylla* (*U. r*), *Uncaria macrophylla* (*U. m*), *Uncaria sinensis* (*U. si*), *Uncaria hirsuta* (*U. h*), and *Uncaria sessilifructus* (*U. se*), are documented as the official resource of Gou-Teng [7]. Furthermore, several *Uncaria* plants, *e.g. Uncaria scandens* (*U. sc*), *Uncaria laevigata* (*U. l*), and *Uncaria yunnanensis* (*U. y*), are also used as the substitutes of Gou-Teng in prescriptions [8, 9]. Although recent studies have manifested the antidepressant-like effects of *U. rhynchophylla* and *U. lanosa*, and locomotor decreasing effects of *U. rhynchophylla*, *U. macrophylla*, and *U. sinensis* [10–12], few reports can discern the difference regarding the chemical profiles and biological activities between different *Uncaria* species. Thus, the clinical application of Gou-Teng is bewildered for the morphological and chemical similarity between different *Uncaria* plants. Different from the cardiovascular effect, the psychiatric property and active constituents of Gou-Teng are still disputed. Melatonin (MT) and 5-hydroxytryptamine (5-HT) receptors are two types of neurotransmitter receptors closely related to mental diseases [13–16], and thus are used to evaluate the psychiatric effects of different *Uncaria* plants. The present study applied an ultra-fast liquid chromatography equipped with ion trap time-of-flight mass spectrometry (UFLC-IT/TOF-MS) and combined with melatonin and 5-hydroxytryptamine receptors agonistic assay to discern seven *Uncaria* species regarding their chemical profiles and psychiatric properties.

2 Results and Discussions

2.1 LCMS-PDA Analyses

Seven *Uncaria* plants were analyzed by UFLC-PDA-MS/MS to provide their respective base peak chromatograms (BPCs) in both positive and negative modes (Fig. 1). In total, 57 compounds including 35 indole alkaloids, ten flavonoids, five triterpenoids, five chlorogenic acids, and two

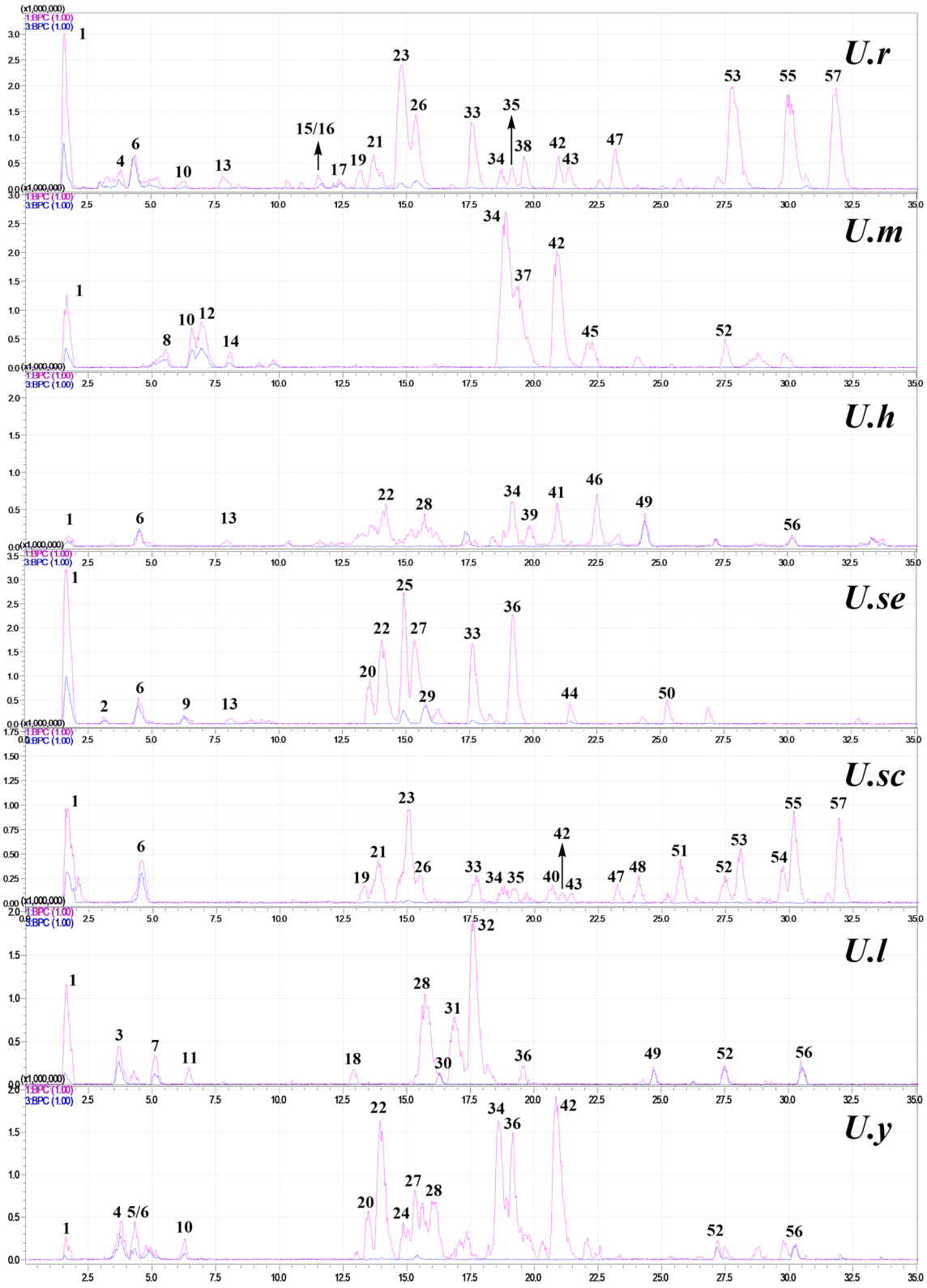


Fig. 1 Base peak chromatograms (BPCs) of seven *Uncaria* plants in positive (1 BPC) and negative (3 BPC) modes

other compounds were characterized according to their UV absorptions, MS/MS fragmentations, retention time, and comparing with the reported compounds (Table 1).

2.1.1 Indole Alkaloids

Indole alkaloids are the characteristic constituents in *Uncaria* plants with high response in positive mode MS. In this investigation, a number of 35 indole alkaloids were described and divided into six subclasses including cadambine-type (**19**, **21**, **23**, **26**, **47**), vinsosamide-type (**15**), D-*seco*-type (**18**, **25**, **33**, **38**, **44**, **50**), corynoxine-type (**11**, **20**, **22**, **24**, **27**, **28**, **31**, **32**, **34**, **35**, **36**, **37**, **42**), corynantheine-type (**40**, **43**, **45**, **48**, **51**, **53**, **55**, **57**), and ajmalicine-type (**39**, **54**). In accordance with the previous investigation [17], D-*seco* alkaloids commonly generated the characteristic fragmentation ions ascribed to the loss of 17 Da (NH_3) in the MS^2 experiment; the indole and oxindole alkaloids could be differentiated from their respective maximal UV absorptions around 280 nm (indole) or 240 nm (oxindole); the numbers and types of glycosyl moieties were determined by the mass defects between the parent and fragment ions.

2.1.1.1 Cadambine-Type Alkaloids Peak **21** was identified as cadambine from the $[M+H]^+$ ion at *m/z* 545.2129 with the diagnostic MS^2 ions at *m/z* 383.1612 ($C_{21}H_{22}N_2O_5$) and 351.1245 ($C_{20}H_{18}N_2O_4$), corresponding to the sequential loss of glycosyl and MeOH moieties [18]. Peak **19** showed the loss of 17 Da from 565 to 548, and the loss of 162 Da from 548 to 386, which was characteristic for the hydrated derivative of cadambine [18]. Peaks **23**, **26**, and **47** possessed the same molecular formula of $C_{27}H_{34}N_2O_{10}$ with two more hydrogens than **21**. In the MS^2 spectra, the identical fragmentation at *m/z* 385 ($C_{21}H_{24}N_2O_5$) and 367 ($C_{21}H_{22}N_2O_4$) suggested closely related structures. In accordance with the previous reports, 3α-dihydrocadambine, 3β-dihydrocadambine, and 3β-isodihydrocadambine were reasonably suggested [19].

2.1.1.2 Vincosamide-Type Alkaloids Peak **15** showing a molecular formula of $C_{38}H_{50}N_2O_{19}$ was deduced from the $[M+H]^+$ ion at *m/z* 839.3054. In the positive MS^2 experiment, the sequential losses of three glycosyl moieties ($C_6H_{10}O_5$, 162 Da) suggested the presence of three glucosyl in the structure. Finally, this compound was isolated under the guidance of LCMS analysis, and identified to be 2′-*O*-[β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl]-11-hydroxyvincosamide based on rigid 1D and 2D NMR spectroscopic data [20].

2.1.1.3 D-seco Indole Alkaloids D-*seco* indole alkaloids can be well recognized from the diagnostic MS^2 ions attributed to the neutral loss of 17 Da (NH_3) from the precursor ions. Peaks **33** and **38** were assigned with the same molecular formula of $C_{27}H_{34}N_2O_9$ from the $[M+H]^+$ ion at *m/z* 531. Their similar MS^2 fragmentations at *m/z* 514 ($C_{27}H_{31}NO_9$) and 352 ($C_{21}H_{21}NO_4$) indicated a pair of isomers, which were generated from the cleavage of 3-*epi*-strictosidine and strictosidine [21]. Peak **18** with a molecular weight of 516 was deduced to be the demethylated derivative of **38**, owing to a CH_2 (14 Da) less in the molecular formula. The MS^2 fragmentation ion at *m/z* 338.1568 implied the successive loss of 17 Da (NH_3) and 162 Da ($C_6H_{10}O_5$), by which this compound was assigned as strictosidinic acid [22]. The molecular formula of **25** was determined as $C_{28}H_{30}N_2O_{11}$ by the protonated ion ($[M+H]^+$) at *m/z* at 571.1896 and deprotonated ion ($[M-H]^-$) at *m/z* 569.1780. In the MS^2 experiment, the sequential losses of 162 Da ($C_6H_{10}O_5$), 18 Da (H_2O), and 14 Da (CH_2) was consistent with the presence of glucosyl, hydroxyl, and methoxyl groups. From the above analyses, peak **25** was tentatively assigned as desoxycordifoline that had been isolated from *Chimarrhis turbinate* [23]. Peaks **44** and **50** shared the molecular weight of *m/z* 930 and 902, respectively, corresponding to the chemical composition of $C_{44}H_{54}N_2O_{20}$ and $C_{44}H_{58}N_2O_{18}$. The sequential losses of two 162 Da ($C_6H_{10}O_5$) indicated the presence of two glucosyls. Taking its UV absorption at 219 nm into consideration, peak **44** was tentatively deduced to be neonaucleoside C [24]. Similarly, peak **50** was attributed to be bahienoside B from the fragments at *m/z* 341.1434 ($C_{19}H_{20}N_2O_4$) and 323.1406 ($C_{19}H_{18}N_2O_3$), by retrieving the compounds isolated from the same genus [25].

2.1.1.4 Corynoxine-Type Alkaloids The spirocyclic corynoxine-type alkaloids account for the largest number of indole alkaloids within *Uncaria* genus. Generally, this type of alkaloids can be well recognized by their UV maximum absorption at about 240 nm [17]. Peaks **34**, **37**, and **42** were isomers with the equal molecular formula of $C_{22}H_{28}N_2O_4$, which were determined by the $[M+H]^+$ ion at *m/z* 385. The MS^2 fragments at *m/z* 353 and 321 were attributed to the consecutive losses of methoxyl groups. The ion at *m/z* 267 indicated the loss of the C_5-side chain. By comparing their relative retention time on octadecylsilyl (ODS) column, they were deduced as isorhynchophylline, corynoxine, and rhynchophylline [26]. Peaks **27** and **31** occupied the same molecular weight of 384, corresponding to the molecular formula of $C_{21}H_{24}N_2O_5$. Their MS^2 fragments at *m/z* 367, 351, and 335 accounting for the lost H_2O and two additional oxygen atoms indicated an oxygenated derivative of rhynchophyllic acid. Likewise, peaks **24** and **35** were deduced as dehydro-derivatives of rhychophylline, and peak **11** was proposed as the demethylated derivative of rhychophylline [27].

Table 1 Characterization of peaks in seven *Uncaria* plants by UFLC-DAD-MS/MS analyses

No.	t_R (min)	MW	MF	DBE	MS	MS/MS	λ_{max} (nm)	Name
1	1.59	342	$C_{12}H_{22}O_{11}$	2	Pos: 381.0792 ($[M+K]^+$, − 0.2 mDa) Neg: 387.1170 ($[M+HCOO]^-$, +2.6 mDa)	Pos: − Neg: 387 → 341.1091 ($C_{12}H_{22}O_{11}$)	201	Sucrose
2	3.06	376	$C_{16}H_{24}O_{10}$	5	Pos: 399.1258 ($[M+Na]^+$, − 0.4 mDa) Neg: 375.1301 ($[M-H]^-$,+0.4 mDa)	Pos: 399 → 377.1439($C_{16}H_{24}O_{10}$), 215.0678 ($C_{13}H_{10}O_3$) Neg: −	234	Loganic acid
3	3.71	354	$C_{16}H_{18}O_9$	8	Pos: 355.1017 ($[M+H]^+$, − 0.7 mDa) Neg: 353.0877 ($[M-H]^-$, − 0.1 mDa)	Pos: 355 → 163.0406 ($C_9H_6O_3$), 145.0335 ($C_9H_4O_2$) Neg: 353 → 191.0565 ($C_7H_{12}O_6$)	221 243 325	Neochlorogenic acid
4	3.80	578	$C_{30}H_{26}O_{12}$	18	Pos: 579.1465 ($[M+H]^+$, − 3.2 mDa) Neg: 577.1327 ($[M-H]^-$, − 2.5 mDa)	Pos: 579 → 409.0915 ($C_{22}H_{16}O_8$), 301.0701 ($C_{16}H_{12}O_6$), 287.0553 ($C_{15}H_{10}O_6$), 259.0128, 247.0453 Neg: 577 → 425.0872 ($C_{22}H_{18}O_9$), 407.0766 ($C_{22}H_{16}O_8$), 285.0352, 245.0817	279	Procyanidin B1
5	4.31	290	$C_{15}H_{14}O_6$	9	Pos: 291.0841 ($[M+H]^+$, − 2.2 mDa) Neg: 289.0712 ($[M-H]^-$, − 0.6 mDa)	Pos: 291 → 273.0741 ($C_{15}H_{12}O_5$), 139.0423 ($C_7H_6O_3$), 123.0342 ($C_7H_6O_2$) Neg: −	280	Catechin
6	4.58	354	$C_{16}H_{18}O_9$	8	Pos: 355.1016 ($[M+H]^+$, − 0.8 mDa) Neg: 353.0873 ($[M-H]^-$, − 0.5 mDa)	Pos: 355 → 163.0407 ($C_9H_6O_3$), 145.0254($C_9H_4O_2$) Neg: 353 → 191.0569 ($C_7H_{12}O_6$)	218 234 325	Chlorogenic acid
7	5.10	354	$C_{16}H_{18}O_9$	8	Pos: 355.1023 ($[M+H]^+$, − 0.1 mDa) Neg: 353.0887 ($[M-H]^-$,+0.9 mDa)	Pos: 355 → 163.0401 ($C_9H_6O_3$) Neg: 353 → 191.0565 ($C_7H_{12}O_6$)	218 234 325	Cryptochlorogenic acid
8	5.57	578	$C_{30}H_{26}O_{12}$	18	Pos: 579.1480 ($[M+H]^+$, − 1.7 mDa) Neg: 577.1330 ($[M-H]^-$, − 2.2 mDa)	Pos: 579 → 427.1024 ($C_{22}H_{18}O_9$), 409.0924 ($C_{22}H_{16}O_8$), 301.0766 ($C_{16}H_{12}O_6$), 287.0693 ($C_{15}H_{14}O_6$) Neg: 577 → 425.0911 ($C_{22}H_{18}O_9$), 407.0742 ($C_{22}H_{16}O_8$)	280	Procyanidin B2
9	6.22	354	$C_{16}H_{18}O_9$	8	Pos: 355.1013 ($[M+H]^+$, − 1.1 mDa) Neg: 353.0870 ($[M-H]^-$, − 0.8 mDa)	Pos: 355 → 163.0420 ($C_9H_6O_3$) Neg: 353 → 191.0573 ($C_7H_{12}O_6$)	218 234 325	Isochlorogenic acid
10	6.28	290	$C_{15}H_{14}O_6$	9	Pos: 291.0841 ($[M+H]^+$, − 2.2 mDa) Neg: 289.0704 ($[M-H]^-$, − 1.4 mDa)	Pos: 291 → 139.0411 ($C_7H_6O_3$), 123.0342 ($C_7H_6O_2$) Neg: −	280	Epicatechin
11	6.43	370	$C_{21}H_{26}N_2O_4$	10	Pos: 371.1973 ($[M+H]^+$, +0.8 mDa) Neg: −	Pos:371 → 353.1889 ($C_{21}H_{24}N_2O_3$), 267.1463 ($C_{17}H_{18}N_2O$), 229.1376 ($C_{14}H_{16}N_2O$) Neg: −	241	Corynoxinic acid
12	7.00	562	$C_{30}H_{26}O_{11}$	18	Pos: 563.1514 ($[M+H]^+$, − 3.4 mDa) Neg: 561.1392 ($[M-H]^-$, − 1.0 mDa)	Pos: 563 → 411.1049 ($C_{22}H_{18}O_8$), 393.0997 ($C_{22}H_{16}O_7$), 291.0856 ($C_{15}H_{14}O_6$), 273.0778 ($C_{15}H_{12}O_5$) Neg: 561 → 407.0755 ($C_{22}H_{16}O_8$), 289.0693 ($C_{15}H_{14}O_6$), 187.0425	275	Fisetinidol-(4α → 8)- epicatechin
13	7.87	468	$C_{21}H_{24}O_{12}$	10	Pos: 469.1323 ($[M+H]^+$, − 1.8 mDa) Neg: −	Pos: 469 → 317.0994 ($C_{16}H_{12}O_7$) Neg: −	278	Gallocatechol *C*-glucoside
14	8.09	562	$C_{30}H_{26}O_{11}$	18	Pos: 563.1526 ($[M+H]^+$, − 2.2 mDa) Neg: 561.1400 ($[M-H]^-$, − 0.2 mDa)	Pos: 563 → 411.1014 ($C_{22}H_{18}O_8$), 393.0943 ($C_{22}H_{16}O_7$), 291.0822 ($C_{15}H_{14}O_6$), 287.0646 ($C_{15}H_{10}O_6$), 267.0542, 231.0657 Neg: 561 → 407.0783 ($C_{22}H_{16}O_8$), 289.0707 ($C_{15}H_{14}O_6$)	277	Fisetinidol-(4β → 8)- epicatechin

Table 1 (continued)

No.	t_R (min)	MW	MF	DBE	MS	MS/MS	λ_{max} (nm)	Name
15	11.59	838	$C_{38}H_{50}N_2O_{19}$	15	Pos: 839.3054 ($[M+H]^+$, − 2.7 mDa) Neg: 883.3029($[M+HCOO]^-$, +3.9 mDa)	Pos:839 → 677.2546 ($C_{32}H_{40}N_2O_{14}$), 515.1975 ($C_{26}H_{30}N_2O_9$), 353.1502 ($C_{20}H_{20}N_2O_4$), 283.1141 ($C_{16}H_{14}N_2O_3$) Neg: 883 → 837.2849 ($C_{38}H_{50}N_2O_{19}$), 675.2278 ($C_{32}H_{40}N_2O_{14}$), 495.1688($C_{26}H_{28}N_2O_8$), 281.0865 ($C_{16}H_{14}N_2O_3$)	283	Vincosamide 11,6′-di-*O*-*β*-D- glucopyranoside
16	11.66	610	$C_{27}H_{30}O_{16}$	13	Pos: 611.1585 ($[M+H]^+$, − 2.2 mDa) Neg: 609.1454 ($[M-H]^-$, − 0.7 mDa)	Pos:611 → 303.0473 ($C_{15}H_{10}O_7$) Neg: 609 → 301.0321 ($C_{15}H_{10}O_7$), 255.0311 ($C_{14}H_8O_5$)	253 348	Rutin
17	12.40	464	$C_{21}H_{20}O_{12}$	12	Pos: 465.1019 ($[M+H]^+$, − 0.9 mDa) Neg: 463.0871 ($[M-H]^-$, − 1.1 mDa)	Pos: 465 → 303.0497 ($C_{15}H_{10}O_7$) Neg: 463 → 301.0300 ($C_{15}H_{10}O_7$), 271.0146 ($C_{14}H_8O_6$)	255 354	Hyperoside
18	12.92	516	$C_{26}H_{32}N_2O_9$	12	Pos:517.2206 ($[M+H]^+$, + 2.5 mDa) Neg: –	Pos: 517 → 338.1546 ($C_{20}H_{19}NO_4$), 276.1250 ($C_{19}H_{17}NO$) Neg: –	203 280	Strictosidinic acid
19	13.24	564	$C_{27}H_{36}N_2O_{11}$	11	Pos: 565.2385 ($[M+H]^+$, − 0.7 mDa) Neg: –	Pos: 565 → 548.2101 ($C_{27}H_{33}NO_{11}$), 386.1677 ($C_{21}H_{23}NO_6$), 354.1487 ($C_{20}H_{19}NO_5$) Neg: –	220 279	Hydrated cadambine
20	13.55	368	$C_{21}H_{24}N_2O_4$	11	Pos: 369.1801 ($[M+H]^+$, − 0.8 mDa) Neg: –	Pos:369 → 337.1568 ($C_{20}H_{20}N_2O_3$), 267.1447 ($C_{17}H_{18}N_2O$), 241.1439 ($C_{15}H_{16}N_2O$), 213,1067 ($C_{13}H_{12}N_2O$), 160.0747 ($C_{10}H_9NO$) Neg: –	205 240	Cisocorynoxeine
21	13.86	544	$C_{27}H_{32}N_2O_{10}$	13	Pos: 545.2108 ($[M+H]^+$, − 2.2 mDa) Neg: –	Pos: 545 → 383.1612 ($C_{21}H_{22}N_2O_5$), 351.1245 ($C_{20}H_{18}N_2O_4$), 263.1091 ($C_{16}H_{10}N_2O_2$), 227.1193($C_{14}H_{14}N_2O$) Neg: –	280	Cadambine
22	14.08	368	$C_{21}H_{24}N_2O_4$	11	Pos: 369.1800 ($[M+H]^+$, − 0.9 mDa) Neg: –	Pos:369 → 337.1590 ($C_{20}H_{20}N_2O_3$), 291.1455 ($C_{19}H_{18}N_2O$), 265.1246 ($C_{17}H_{16}N_2O$), 213,0997 ($C_{13}H_{12}N_2O$), 160.0682 ($C_{10}H_9NO$) Neg: –	206 241	18,19-Dehydrocorynoxinic acid
23	14.81	546	$C_{27}H_{34}N_2O_{10}$	12	Pos: 547.2269 ($[M+H]^+$, − 1.7 mDa) Neg: 591.2205 ($[M+HCOO]^-$, + 1.0 mDa)	Pos:547 → 385.1801 ($C_{21}H_{24}N_2O_5$), 367.1688 ($C_{21}H_{22}N_2O_4$), 349.1577 ($C_{21}H_{20}N_2O_3$), 335.1372 ($C_{20}H_{18}N_2O_3$), 317.1258 ($C_{20}H_{16}N_2O_2$) Neg: 591 → 545.2097 ($C_{27}H_{34}N_2O_{10}$)	220 278	3*α*-Dihydrocadambine
24	14.86	382	$C_{22}H_{26}N_2O_4$	11	Pos: 383.1955 ($[M+H]^+$, − 1.0 mDa) Neg: –	Pos:383 → 351.1743 ($C_{21}H_{22}N_2O_3$), 241.1262 ($C_{15}H_{16}N_2O$) Neg: –	205 244	Isocorynoxeine
25	14.90	570	$C_{28}H_{30}N_2O_{11}$	15	Pos:571.1896 ($[M+H]^+$, − 2.6 mDa) Neg: 569.1780 ($[M-H]^-$, + 0.3 mDa)	Pos: 571 → 409.1426 ($C_{22}H_{20}N_2O_6$), 391.1250 ($C_{22}H_{18}N_2O_5$), 377.1120 ($C_{21}H_{16}N_2O_5$), 359.1064 ($C_{21}H_{14}N_2O_4$), 341.0952 ($C_{21}H_{12}N_2O_3$), 313.0973 ($C_{20}H_{12}N_2O_2$) Neg: 569 → 389.1187 ($C_{22}H_{18}N_2O_5$)	216 238 275 374	Deoxycordifoline

Table 1 (continued)

No.	t_R (min)	MW	MF	DBE	MS	MS/MS	λ_{max} (nm)	Name
26	15.33	546	$C_{27}H_{34}N_2O_{10}$	12	Pos: 547.2252 ($[M+H]^+$, − 3.4 mDa) Neg: 591.2193 ($[M+HCOO]^-$, − 0.2 mDa)	Pos:547 → 385.1762 ($C_{21}H_{24}N_2O_5$), 367.1697 ($C_{21}H_{22}N_2O_4$), 353.1595 ($C_{20}H_{20}N_2O_4$), 335.1441 ($C_{20}H_{18}N_2O_3$) Neg: –	218 280	3β-Dihydrocadambine
27	15.38	384	$C_{21}H_{24}N_2O_5$	11	Pos: 385.1739 ($[M+H]^+$, − 1.9 mDa) Neg: 429.1682 ($[M+HCOO]^-$, + 1.5 mDa)	Pos:385 → 367.1698 ($C_{21}H_{22}N_2O_4$), 351.1696 ($C_{21}H_{22}N_2O_3$), 335.1338 ($C_{20}H_{18}N_2O_3$), 267.1399 ($C_{17}H_{18}N_2O$), 239.1202 ($C_{15}H_{14}N_2O$) Neg: –	206 240	Oxocorynoxinic acid
28	15.69	368	$C_{21}H_{24}N_2O_4$	11	Pos: 369.1811 ($[M+H]^+$, + 0.2 mDa) Neg: –	Pos:369 → 337.1588 ($C_{20}H_{20}N_2O_3$), 309.1647 ($C_{19}H_{20}N_2O_2$), 291.1455 ($C_{19}H_{18}N_2O$), 265.1246 ($C_{17}H_{16}N_2O$), 160.0810 ($C_{10}H_9NO$) Neg: –	204 240	18,19-Dehydrocorynoxinic acid B
29	15.74	448	$C_{21}H_{20}O_{11}$	12	Pos: 449.1068 ($[M+H]^+$, − 1.0 mDa) Neg: 447.0939 ($[M-H]^-$, + 0.6 mDa)	Pos: 449 → 303.0510 ($C_{15}H_{10}O_7$) Neg: 447 → 301.0358 ($C_{15}H_{10}O_7$), 271.0288 ($C_{14}H_8O_6$)	204 255 348	Quercetin 3-rhamnoside
30	16.28	516	$C_{25}H_{24}O_{12}$	14	Pos: 517.1337 ($[M+H]^+$, − 0.4 mDa) Neg: 515.1187 ($[M-H]^-$, − 0.8 mDa)	Pos: – Neg: 515 → 353.0882 ($C_{16}H_{18}O_9$), 173.0401 ($C_7H_{10}O_5$)	247 326	3,5-Dicaffeoylquinic acid
31	16.89	384	$C_{21}H_{24}N_2O_5$	11	Pos: 385.1756 ($[M+H]^+$, − 0.2 mDa) Neg: 429.1686 ($[M+HCOO]^-$, + 1.9 mDa)	Pos:385 → 367.1671 ($C_{21}H_{22}N_2O_4$), 351.1721 ($C_{21}H_{22}N_2O_3$), 223.1210 ($C_{15}H_{14}N_2$) Neg: –	249	Oxorhynchophyllic acid
32	17.59	368	$C_{21}H_{24}N_2O_4$	11	Pos: 369.1817 ($[M+H]^+$, + 0.8 mDa) Neg: –	Pos: 369 → 337.1650 ($C_{20}H_{20}N_2O_3$), 309.1566 ($C_{19}H_{20}N_2O_2$), 241.1312 ($C_{15}H_{16}N_2O$), 187.0844 ($C_{11}H_{10}N_2O$), 160.0773 ($C_{10}H_9NO$) Neg: –	246	Demethylcorynoxeine
33	17.63	530	$C_{27}H_{34}N_2O_9$	12	Pos: 531.2308 ($[M+H]^+$, − 2.9 mDa) Neg: 575.2225 ($[M+HCOO]^-$, − 2.1 mDa)	Pos:531 → 514.2026 ($C_{27}H_{31}NO_9$), 352.1577 ($C_{21}H_{21}NO_4$), 334.1493 ($C_{21}H_{19}NO_3$) Neg: –	220 279	3-Epistrictosidine
34	18.62	384	$C_{22}H_{28}N_2O_4$	10	Pos: 385.2112 ($[M+H]^+$, − 1.0 mDa) Neg: –	Pos: 385 → 353.1852 ($C_{21}H_{24}N_2O_3$), 321.1618 ($C_{20}H_{20}N_2O_2$), 267.1494 ($C_{17}H_{18}N_2O$), 241.1331 ($C_{15}H_{16}N_2O$), 187.0798 ($C_{11}H_{10}N_2O$) Neg: –	206 243	Isorhynchophylline
35	19.16	382	$C_{22}H_{26}N_2O_4$	11	Pos: 383.1959 ($[M+H]^+$, − 0.6 mDa) Neg: –	Pos:383 → 351.1671 ($C_{21}H_{22}N_2O_3$), 319.1480 ($C_{20}H_{18}N_2O_2$), 267.1530 ($C_{17}H_{18}N_2O$), 215.1098 ($C_{13}H_{14}N_2O$), 160.0682 ($C_{10}H_9NO$) Neg: –	202 240	Corynoxeine
36	19.21	368	$C_{21}H_{24}N_2O_4$	11	Pos: 369.1783 ($[M+H]^+$, − 2.6 mDa) Neg: –	Pos:369 → 337.1539 ($C_{20}H_{20}N_2O_3$), 293.1347 ($C_{18}H_{16}N_2O_2$), 267.1553 ($C_{17}H_{18}N_2O$), 239.1232 ($C_{15}H_{14}N_2O$), 160.0760 ($C_{10}H_9NO$) Neg: –	207 242	Demethylisocorynoxeine

Table 1 (continued)

No.	t_R (min)	MW	MF	DBE	MS	MS/MS	λ_{max} (nm)	Name
37	19.32	384	$C_{22}H_{28}N_2O_4$	10	Pos: 385.2133 ($[M+H]^+$, +1.1 mDa) Neg: –	Pos:385 → 353.1875 ($C_{21}H_{24}N_2O_3$), 321.1619 ($C_{20}H_{20}N_2O_2$), 265.1339 ($C_{17}H_{16}N_2O$), 241.1334 ($C_{15}H_{16}N_2O$), 187.0697 ($C_{11}H_{10}N_2O$) Neg: –	210 243	Corynoxine
38	19.62	530	$C_{27}H_{34}N_2O_9$	12	Pos: 531.2311 ($[M+H]^+$, − 2.6 mDa) Neg: 575.2231 ($[M+HCOO]^-$, − 1.5 mDa)	Pos:531 → 514.2082 ($C_{27}H_{31}NO_9$), 352.1586 ($C_{21}H_{21}NO_4$), 334.1556 ($C_{21}H_{19}NO_3$) Neg: –	219 280	Strictosidine
39	19.89	352	$C_{21}H_{24}N_2O_3$	11	Pos: 353.1854 ($[M+H]^+$, − 0.6 mDa) Neg: –	Pos:353 → 321.1647 ($C_{20}H_{20}N_2O_2$), 222.1198 ($C_{12}H_{15}NO_3$), 210.1126 ($C_{11}H_{15}NO_3$), 144.0798 ($C_{10}H_9N$) Neg: –	219 280	Ajmalicine
40	20.63	354	$C_{21}H_{26}N_2O_3$	10	Pos: 355.1994 ($[M+H]^+$, − 2.2 mDa) Neg: 353.1879 ($[M-H]^-$, +0.8 mDa)	Pos: 354 → 224.1340 ($C_{12}H_{17}NO_3$), 212.1241 ($C_{11}H_{17}NO_3$), 144.0792 ($C_{10}H_9N$) Neg: –	220 281	Sitsirikine
41	20.96	580	$C_{31}H_{48}O_{10}$	8	Pos: 581.3333 ($[M+H]^+$, +1.3 mDa) Neg:579.3123 ($[M-H]^-$, − 1.2 mDa)	Pos: 581 → 389.2065 ($C_{26}H_{28}O_3$) Neg: –	202	Demythyl atropuroside C
42	20.96	384	$C_{22}H_{28}N_2O_4$	10	Pos: 385.2101 ($[M+H]^+$, − 2.1 mDa) Neg: –	Pos: 385 → 353.1847 ($C_{21}H_{24}N_2O_3$), 321.1589 ($C_{20}H_{20}N_2O_2$), 267.1539 ($C_{17}H_{18}N_2O$), 265.1373 ($C_{17}H_{16}N_2O$), 160.0632 ($C_{10}H_9NO$) Neg: –	210 242	Rhynchophylline
43	21.37	352	$C_{21}H_{24}N_2O_3$	11	Pos: 353.1829 ($[M+H]^+$, − 3.1 mDa) Neg: 351.1726 ($[M-H]^-$, +1.2 mDa)	Pos: 353 → 304.1399 ($C_{20}H_{17}NO_2$), 222.1162 ($C_{12}H_{15}NO_3$), 210.1111 ($C_{11}H_{15}NO_3$), 144.0861 ($C_{10}H_9N$) Neg: –	219 278	Geissoschizine
44	21.48	930	$C_{44}H_{54}N_2O_{20}$	19	Pos: 931.3357 ($[M+H]^+$, +1.4 mDa) Neg:929.3174 ($[M-H]^-$, − 2.3 mDa)	Pos: 931 → 769.2802 ($C_{38}H_{44}N_2O_{15}$), 719.2172($C_{37}H_{38}N_2O_{13}$), 607.2281 ($C_{32}H_{34}N_2O_{10}$), 557.1858 Neg: 929 → 749.2512 ($C_{38}H_{42}N_2O_{14}$), 517.1466 ($C_{24}H_{26}N_2O_{11}$)	219	Neonaucleoside C
45	22.23	400	$C_{22}H_{28}N_2O_5$	10	Pos: 401.2090 ($[M+H]^+$, +1.9 mDa) Neg: –	Pos: 401 → 383.1953 ($C_{22}H_{26}N_2O_4$), 355.1652 ($C_{20}H_{22}N_2O_4$), 241.1699 ($C_{16}H_{20}N_2$), 239.1543 ($C_{16}H_{18}N_2$) Neg: –	212 280	Dihydroxycorynantheine
46	22.52	594	$C_{32}H_{50}O_{10}$	8	Pos: 595.3477 ($[M+H]^+$, − 3.1 mDa) Neg:593.3207 ($[M-H]^-$, +2.8 mDa)	Pos: 595 → 567.3522 ($C_{31}H_{50}O_9$), 536.2769 ($C_{32}H_{40}O_7$), 389.2051 ($C_{26}H_{28}O_3$) Neg: –	204	Atropuroside C
47	23.19	546	$C_{27}H_{34}N_2O_{10}$	12	Pos: 547.2286 ($[M+H]^+$, +3.8 mDa) Neg: 591.2195 ($[M+HCOO]^-$, +2.2 mDa)	Pos:547 → 385.1740 ($C_{21}H_{24}N_2O_5$), 367.1648($C_{21}H_{22}N_2O_4$), 349.1520 ($C_{21}H_{20}N_2O_3$), 335.1317 ($C_{20}H_{18}N_2O_3$) Neg: 591 → 383.1612 ($C_{21}H_{24}N_2O_5$)	202 217 279	3β-Isodihydrocadambine

Table 1 (continued)

No.	t_R (min)	MW	MF	DBE	MS	MS/MS	λ_{max} (nm)	Name
48	24.10	366	$C_{22}H_{26}N_2O_3$	11	Pos: 367.2016 ($[M+H]^+$, − 2.1 mDa) Neg: −	Pos:367 → 251.1628 ($C_{17}H_{18}N_2$), 236.1268 ($C_{13}H_{17}NO_3$), 224.1199 ($C_{12}H_{17}NO_3$), 192.1019 ($C_{11}H_{13}NO_2$) Neg: −	220 280	Corynantheine
49	24.40	810	$C_{42}H_{66}O_{15}$	10	Pos: 833.4294 ($[M+Na]^+$, − 3.6 mDa), 469.3306 ($C_{30}H_{44}O_4$) Neg: 809.4329 ($[M-H]^-$, − 2.5 mDa)	Pos:469 → 451.3204 ($C_{30}H_{42}O_3$), 423.3278 ($C_{29}H_{42}O_2$), 379.3331 ($C_{28}H_{42}$), 263.1778 ($C_{20}H_{22}$) Neg: 809 → 603.3873 ($C_{35}H_{56}O_8$)	207	Quinovic acid diglycoside
50	25.25	902	$C_{44}H_{58}N_2O_{18}$	17	Pos: 903.3714 ($[M+H]^+$, − 4.3 mDa) Neg: 901.3615 ($[M-H]^-$, +0.3 mDa)	Pos: 903 → 341.1434 ($C_{19}H_{20}N_2O_4$), 323.1406 ($C_{19}H_{18}N_2O_3$) Neg: −	221 280	Bahienoside B
51	25.70	368	$C_{22}H_{28}N_2O_3$	10	Pos: 369.2154 ($[M+H]^+$, +2.6 mDa) Neg: −	Pos:369 → 251.1179 ($C_{14}H_{22}N_2O_2$), 238.1458 ($C_{13}H_{19}NO_3$), 226.1418 ($C_{12}H_{19}NO_3$) Neg: −	220	Dihydrocorynantheine
52	27.51	956	$C_{48}H_{76}O_{19}$	11	Pos: 979.4895 ($[M+Na]^+$, +2.2 mDa) Neg: 955.4917 ($[M-H]^-$, +0.9 mDa)	Pos:979 → 935.434 ($C_{47}H_{76}O_{17}$), 773.4421 Neg: 955 → 749.4438 ($C_{41}H_{66}O_{12}$), 587.3923 ($C_{35}H_{56}O_7$), 441.3496	204	Quinovic acid triglycoside
53	27.74	366	$C_{22}H_{26}N_2O_3$	11	Pos: 367.2011 ($[M+H]^+$, − 0.5 mDa) Neg: −	Pos: 367 → 249.1363 ($C_{17}H_{16}N_2$) Neg: −	221 280	Geissoschizine methyl ether
54	29.76	382	$C_{22}H_{26}N_2O_4$	11	Pos: 383.1932 ($[M+H]^+$, − 3.3 mDa) Neg: −	Pos:367 → 223.1304 ($C_{15}H_{14}N_2$), 184.0878 ($C_{12}H_9NO$) Neg: −	206 224 348	Pubescin
55	30.02	366	$C_{22}H_{26}N_2O_3$	11	Pos: 367.2007 ($[M+H]^+$, − 0.9 mDa) Neg: −	Pos:367 → 251.1606 ($C_{17}H_{18}N_2$), 224.1386 ($C_{16}H_{17}N$) Neg: −	221 280	Hirsuteine
56	30.52	486	$C_{30}H_{46}O_5$	8	Pos: 487.3404 ($[M+H]^+$, − 1.4 mDa) Neg: −	Pos: 469 → 451.3117 ($C_{30}H_{42}O_3$), 423.3082 ($C_{29}H_{42}O_2$) Neg: −	202	Quinovic acid
57	31.84	368	$C_{22}H_{28}N_2O_3$	10	Pos: 369.2154 ($[M+H]^+$, − 1.9 mDa) Neg: −	Pos: 369 → 337.1945 ($C_{21}H_{24}N_2O_2$), 238.1481 ($C_{13}H_{19}NO_3$), 226.1380 ($C_{12}H_{19}NO_3$) Neg: −	221 280	Hirsutine

Peaks **20**, **22**, **28**, **32**, and **36** had the same molecular formula of $C_{21}H_{24}N_2O_4$, with a CH_2 less than corynoxeine. The MS^2 fragmentation from *m/z* 369 to 337 verified the presence of an OMe group. The abovementioned features pointed to the demethyl corynoxeine or its isomer. The decarbonylation and decarboxylation neutral losses of 28 Da and 46 Da were proved by the ions at *m/*z 309 and 291. By retrieving the corynoxine-type alkaloids isolated from this genus, the de-methyl derivates of corynoxeine, cisocorynoxeine (**20**), 18,19-dehydrocorynoxinic acid (**22**), 18,19-dehydrocorynoxinic acid B (**28**), demethylcorynoxeine (**32**), and demethylisocorynoxeine (**36**) were proposed [28].

2.1.1.5 Corynanthein-Type Alkaloids Peak **40** showed the protonated ion at *m/z* 355.1994, indicating the molecular formula of $C_{21}H_{26}N_2O_3$. The MS^2 profiles at *m/z* 224.1340 ($C_{12}H_{17}NO_3$), 212.1241 ($C_{11}H_{17}NO_3$), and 144.0792 ($C_{10}H_9N$) were indicative for sitsirikine [29]. Peaks **55** and **57** were assigned as hirsuteine and hirsutine, respectively, by reason of their molecular formula ($C_{22}H_{26}N_2O_3$ and $C_{22}H_{28}N_2O_3$) and MS^2 fragments. Peaks **48** and **53** with the same formula of $C_{22}H_{26}N_2O_3$ were determined to be corynantheine and geissoschizine methyl ether following their MS^2 fragments [30]. Similarly, peaks **45** and **51** were tentatively deduced to be the dihydroxy and dihydro derivatives of corynantheine [17].

2.1.1.6 Ajmalicine-Type Alkaloids Ajmalicine-type alkaloids maintain a pentacyclic heteroyohimbines framework showing similar UV absorption with corynanthein-type alkaloids. Peaks **39** and **54** were attributed with $C_{21}H_{24}N_2O_3$ and $C_{22}H_{26}N_2O_4$ with 11 double bond equivalents. The mass losses from *m/z* 352 to 321.1647 ($C_{20}H_{20}N_2O_2$), 222.1198 ($C_{12}H_{15}NO_3$), 210.1126 ($C_{11}H_{15}NO_3$), and 144.0798 ($C_{10}H_9N$) were in agree with ajmalicine [31]. Similarly, peak **54** was reasonably deduced to be pubescin from the MS^2 fragments at *m/z* 223.1304 ($C_{15}H_{14}N_2$) and 184.0878 ($C_{12}H_9NO$) [32].

2.1.2 Flavonoids

Flavonoids display characteristic UV absorptions at 220–280 (band II) and 300–400 (band I) nm, by which they can be easily characterized [33]. Peaks **4** and **8** with UV maximum absorption at 280 nm were designated with the molecular formula of $C_{30}H_{26}O_{12}$ with 18 unsaturation degrees. Consequent MS^2 experiment on $[M+H]^+$ ion generated fragments at *m/z* 409 ($C_{22}H_{16}O_8$), 301 ($C_{16}H_{12}O_6$), and 287 ($C_{15}H_{10}O_6$) indicating flavonoids dimers. Their relative retention time on ODS column were in accordance with procyanidin b1 (**4**) and procyanidin b2 (**8**) [34]. Peaks **5** and **10** were a pair of isomers with identical molecular formula of $C_{15}H_{14}O_6$. The MS^2 ion at *m/z* 139 ($C_7H_6O_3$) was ascribed to the $A^{1,3}$ retrocyclization fragment on ring C. Taking their UV absorptions at 280 nm and retention time into consideration, peaks **5** and **10** were reasonably determined as catechin (**5**) and epicatechin (**10**) [12]. Peaks **12** and **14** were isomers with the same molecular formula of $C_{30}H_{26}O_{11}$, suggesting flavonoids dimers. The MS^2 fragments at *m/z* 291.0856 ($C_{15}H_{14}O_6$) and 273.0778 ($C_{15}H_{12}O_5$) were attributed to fisetinidol and catechin moieties. From the above analyses, they were tentatively deduced to be fisetinidol-(4$\alpha \rightarrow$8)-epicatechin and fisetinidol-(4$\beta \rightarrow$8)-epicatechin [35]. Peak **13** with a formula of $C_{21}H_{24}O_{12}$ showed MS^2 information at *m/z* 317.0994 ($C_{16}H_{12}O_7$), corresponding to the loss of a C_5 part from the *C*-glycosyl moiety. From the above analyses, this peak was defined as gallocatechol *C*-glucoside [36, 37]. Peak **16** was designed with the molecular formula of $C_{27}H_{30}O_{16}$ with an additional $C_6H_{10}O_4$ part than **17** ($C_{21}H_{20}O_{12}$). In the MS^2 experiment, the same fragments at *m/z* 303 in positive mode and 301 in negative mode suggested the same aglycone in **16** and **17**. By retrieving the database, they were deduced as rutin (**16**) and hyperoside (**17**) [17]. Peak **29** gave $[M+H]^+$ ion at *m/z* 449.1068 and $[M-H]^-$ ion at *m/z* 447.0939, corresponding to the molecular formula of $C_{21}H_{20}O_{11}$. In the MS^2 experiment, the diagnostic MS^2 ions at *m/z* 301.0358 ($C_{15}H_{10}O_7$) and 271.0288 ($C_{14}H_8O_6$) in negative mode were indicative for the sequential loss of rhamnosyl and formaldehyde moieties. From the above analyses, this peak was deduced as quercetin 3-rhamnoside [38].

2.1.3 Chlorogenic Acids

Chlorogenic acid analogues are a type of caffeoyl quinic acids widely present in plants. In the UV spectrum, the maximum absorption at around 325 nm was due to the presence of caffeoyl group. In the MS^2 experiment, the product ions at *m/z* 163 ($C_9H_6O_3$) in positive mode and 191 ($C_7H_{12}O_6$) in negative mode were indicative for caffeic acid and quinic acid moieties. In this study, four isomers, namely, neochlorogenic acid (**3**), chlorogenic acid (**6**), cryptochlorogenic acid (**7**), and isochlorogenic acid (**9**) with identical formula of $C_{16}H_{18}O_9$ were detected and tentatively characterized by their retention time on ODS column [39]. Peak **30** was assigned with the molecular formula of $C_{25}H_{24}O_{12}$ with an additional quinoyl moiety compared to chlorogenic acid. This deduction was verified by the MS^2 ions at *m/z* 353.0882 ($C_{16}H_{18}O_9$) and 173.0401 ($C_7H_{10}O_5$) in negative mode. Thus, peak **30** was delineated as dicaffeoylquinic acid [40].

2.1.4 Triterpenoids

Peak **56** showing terminal absorption in UV spectrum was revealed with the molecular formula of $C_{30}H_{46}O_5$. The abovementioned features were indicative for a triterpenoid. The MS^2 fragments at *m/z* 469 ($C_{30}H_{44}O_4$), 451 ($C_{30}H_{42}O_3$),

and 423 ($C_{29}H_{42}O_2$) were in accordance with quinovic acid [41]. Peaks **49** and **52** were deduced to be diglycoside and triglycoside derivatives of quinovic acid by the additional two and three glycosyls which were verified by the sequential loss of $C_6H_{10}O_5$ parts in the MS^2 experiments. Thus, quinovic acid diglycoside and quinovic acid triglycoside were respectively determined [42].

2.1.5 Other Compounds.

Peak **1** was assigned as sucrose which was widely present in plants by the characteristic $[M+K]^+$ ion at *m/z* 381.0792. Peak **2** had a molecular formula of $C_{16}H_{24}O_{10}$ showing $[M+Na]^+$ ion at *m/z* 399.1258 and $[M-H]^-$ ion at *m/z* 375.1301. In the MS^2 experiment, the loss of glycosyl was verified by the ion at *m/z* 215.0678 ($C_{13}H_{10}O_3$). Thus, this peak was illustrated as loganic acid, the biosynthetic precursor of indole alkaloids [43].

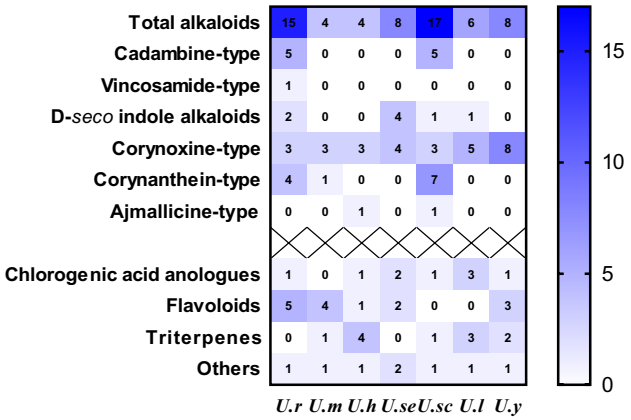


Fig. 2 Distribution of different types of compounds among seven *Uncaria* plants

2.2 Chemical Comparison

As shown in Figs. 2 and 3, a temporal and spatial distribution of chemical constituents in seven *Uncaria* plants provided a visual overview of their difference. The chemical profiles of *U. rhynchophylla* and *U. scandens* were similar in terms of either indole alkaloids or other types of compounds. Indole alkaloids as the characteristic constituents were more prolific in *U. rhynchophylla* and *U. scandens* when comparing to other *Uncaria* plants. Cadambine-type and corynanthein-type alkaloids were the characteristic constituents in *U. rhynchophylla* and *U. scandens*, whereas *corynoxine-type alkaloids* were widely distributed in all the seven *Uncaria* plants. Besides alkaloids, flavonoids were another type of constituent in *Uncaria* plants, which were mainly distributed in *U. rhynchophylla, U. macrophylla*, and *U. yunnanensis*. For the triterpenoids, *U. hirsuta* and *U. laevigata* showed more prolific than other plants.

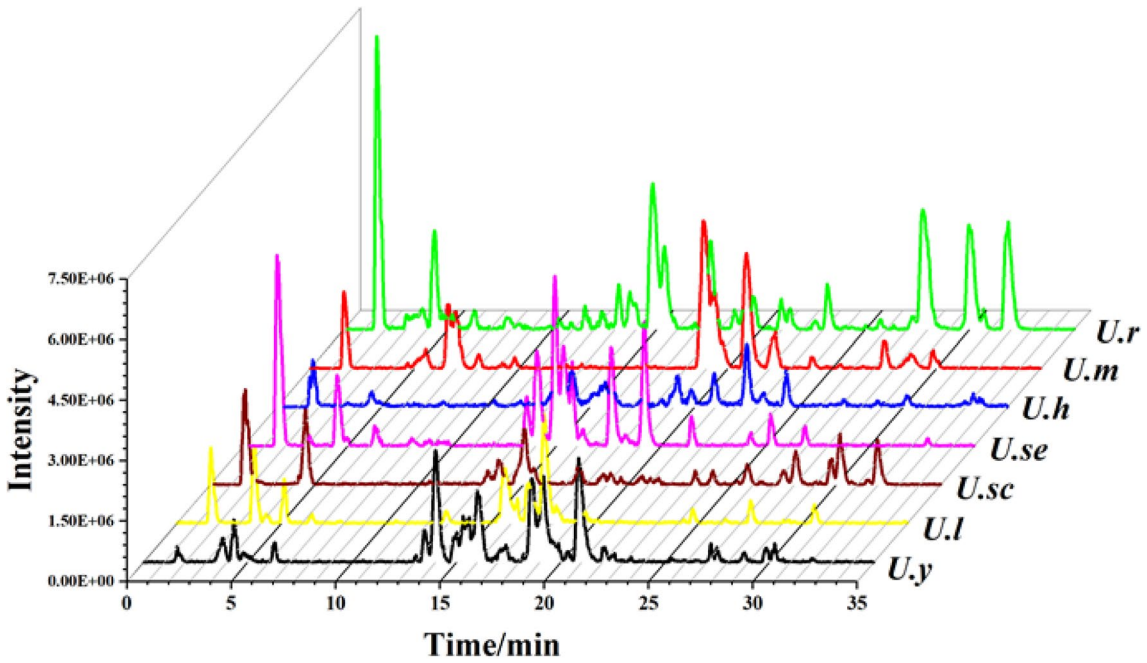


Fig. 3 Comparison of the BPCs (positive) of seven *Uncaria* plants

2.3 Biological Comparison on $MT_{1/2}$ and 5-$HT_{1A/2C}$ Receptors

Gou-Teng as a famous TCM are widely used for treating central nervous system (CNS) diseases in China. Therefore, four neurotransmitter receptors (MT_1, MT_2, 5-HT_{1A}, and 5-HT_{2C}) that are closely related to CNS diseases were used to evaluate the psychiatric-related effects of *Uncaria* plants. As shown in Fig. 4, three plants, *U. rhynchophylla*, *U. macrophylla*, and *U. yunnanensis* showed obviously agnostic activity on all the four receptors. As a comparison, *U. hirsuta, U. sessilifructus*, and *U. scandens* were moderate, and *U. laevigata* was less active. Specifically, *U. macrophylla* displayed the most potent activity on MT_1 receptor with an agonistic rate of 79.0%, then followed with *U. rhynchophylla* (71.9%), *U. yunnanensis* (41.5%), and *U. scandens* (26.1%), whereas *U. hirsuta*, *U. sessilifructus*, and *U. laevigata* were inactive. For MT_2 receptor, *U. yunnanensis* possessed the highest agonistic rate of 91.2%, and *U. macrophylla* and *U. rhynchophylla* exhibited moderate activity with agonistic rates of 54.2% and 44.8%; however, *U. scandens*, *U. sessilifructus*, *U. hirsuta*, *and U. laevigata* were weak or inactive. Similar with the MT receptors, *U. rhynchophylla*, *U. macrophylla*, and *U. yunnanensis* possessed significant activity on 5-HT_{1A} and 5-HT_{2C} receptors with agonistic rates higher that 60%. Interestingly, *U. scandens* was revealed with the highest activity on 5-HT_{2C} receptor (82.7%), almost threefold higher than 5-HT_{1A}, indicating the subtype selectivity.

3 Conclusion

Gou-Teng has long been recorded in ancient TCM books for the treatment of cardiovascular and mental disorders. According to the latest Chinese Pharmacopoeia, five *Uncaria* plants, *U. rhynchophylla*, *U. macrophylla*, *U. sinensis*, *U. hirsuta*, and *U. sessilifructus* are documented as the official resources of Gou-Teng. However, their chemical and biological difference as well as the discrepancy with other *Uncaria* plants are still disputed. Thus, the clinical application of Gou-Teng is confused owing to the prolific resources

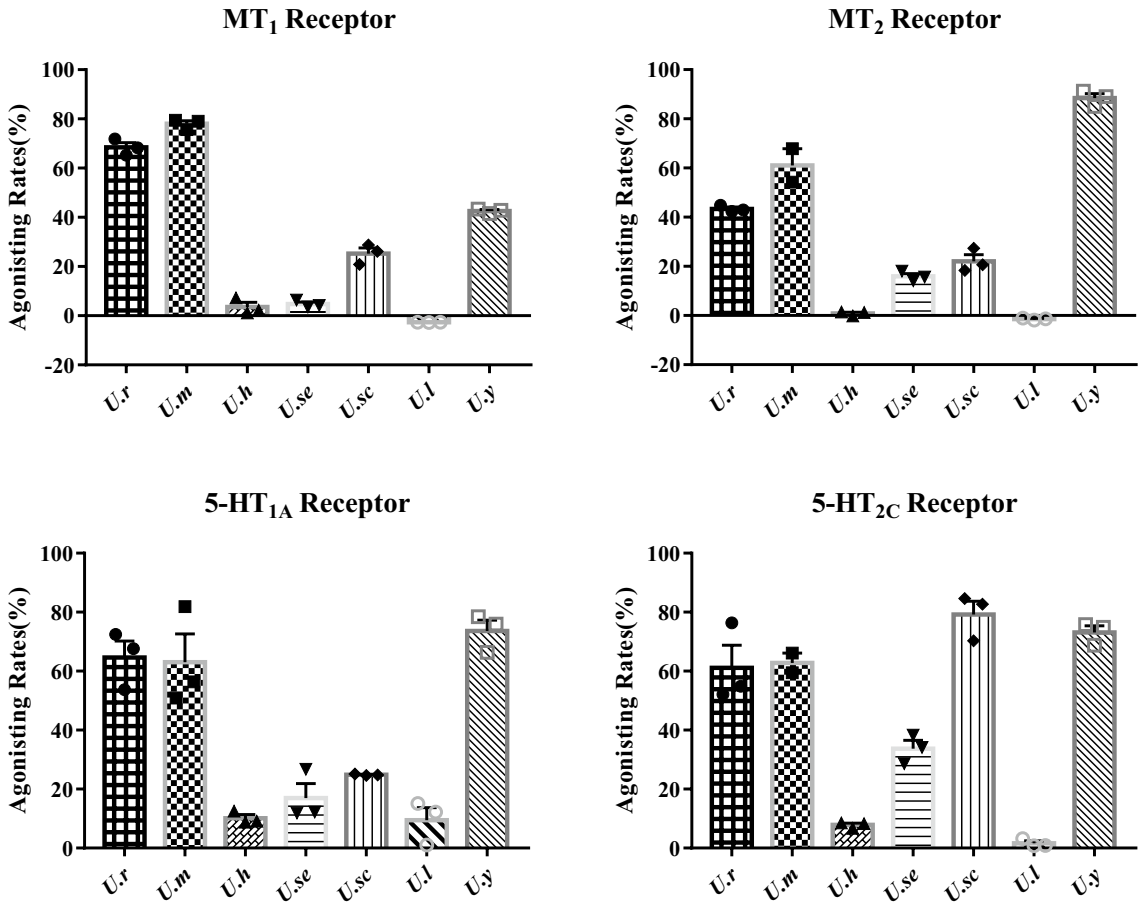


Fig. 4 Agonistic activities of seven *Uncaria* plants on $MT_{1/2}$ and 5-$HT_{1A/2C}$ receptors. The agonistic activities were expressed as X ± SEM ($n = 3$), which were obtained by comparing to the positive controls, melatonin (on MT receptors) and 5-hydroxytryptamine (on 5-HT receptors)

and morphological similarity between different species. In this investigation, seven *Uncaria* species involving four official, *U. rhynchophylla*, *U. macrophylla*, *U. hirsuta*, and *U. sessilifructus*, and three local species, *U. scandens*, *U. laevigata*, and *U. yunnanensis* were extensively compared based on LCMS and bioassay in vitro. In total, 57 constituents including 35 indole alkaloids, ten flavonoids, five triterpenoids, five chlorogenic analogues, and two other compounds were characterized based on their MS/MS patterns and UV absorptions. Cadambine-type and corynantheine-type alkaloids were exclusively present in *U. rhynchophylla* and *U. scandens*, whereas corynoxine-type alkaloids were commonly detected in all the seven *Uncaria* plants. Three *Uncaria* plants, *U. rhynchophylla*, *U. macrophylla*, and *U. yunnanensis* showed obviously agnostic activity on four receptors, suggesting their biological similarity regardless of the chemical difference. This investigation supported the synergistic effects of TCMs due to the complicated constituents and their complementarity in taking effects. This study provides valuable information for understanding the chemical and biological difference between different *Uncaria* plants and the "one-drug multi-source" theory.

4 Experimental

4.1 LCMS Analyses

LCMS analyses were performed on a Shimadzu UFLC/MS-IT-TOF apparatus (Shimadzu, Kyoto, Japan) equipped with a Welch Ultimate XB-C_{18} column (2.1 × 100 mm, *i.d.*, 1.8 μm). The mobile phase for LCMS consisted of water (0.05% formic acid, A) and acetonitrile (0.05% formic acid, B) with the flow rate of 0.2 mL/min. A binary gradient elution was performed as follows: linear gradient (B%) from 10 to 35% in 35 min, and fast increased to 100% in one min and maintained for three min. Re-equilibration duration was five min between individual runs. The injection volume was 2 μL for each LCMS analysis. The detailed MS parameters were set as previously reported [44]. The PDA profiles were recorded from 190 to 400 nm. The Shimadzu Composition Formula Predictor was used to speculate the molecular formula.

4.2 Plant Materials

Plants of *Uncaria rhynchophylla* (Miq.) Miq. ex Havil. (No. 2,016,090,001), *Uncaria macrophylla* Wall. (No. 2,016,090,002), *Uncaria hirsuta* Havil. (No. 2,016,090,003), *Uncaria sessilifructus* Roxb. (No. 2,016,090,004), *Uncaria scandens* (Smith) Hutchins. (No. 2,016,090,005), *Uncaria laevigata* Wall. ex G. Don (No. 2,016,090,006), and *Uncaria yunnanensis* K. C. Hsia (No. 2,016,090,007) were collected from Xishuangbanna Dai Autonomous Prefecture of Yunnan Province in China in September 2016, and authenticated by Dr. Li-Gong Lei (Kunming Institute of Botany, CAS). Voucher specimens (No. 2,016,090,001–2,016,090,007) were deposited in the Laboratory of Antivirus and Natural Medicinal Chemistry, Kunming Institute of Botany, CAS. The hook-bearing stems were dried at room temperature and kept in amber glass flasks until extraction. The powder of each sample (2.0 g) was extracted with ethanol–water (7:3, *v/v*, 10 mL) under ultrasonic for 30 min. The extraction was filtered through a PTFE micro-porous filter (0.22 μm, Jiangsu Hanbon Science & Technology Co., Ltd.) into 2 mL screw cap vials prior to LCMS analyses.

4.3 Agonistic Activities on $MT_{1/2}$ and 5-$HT_{1A/2C}$ Receptors

Bioassay for agonistic activities on melatonin and 5-hydroxytryptamine receptors was performed in accordance with the previous reports [20, 45]. In brief, HEK293 cells stably expressing human melatonin (MT_1 and MT_2) and 5-hydroxytryptamine (5-HT_{1A} and 5-HT_{2C}) receptors were maintained in DMEM containing 10% FBS. Cells were seeded at a density of 4×10^4 cells/well in pre-matrigel-coated 96-well black wall/clear bottom plates. After overnight incubation at 37 °C with 5% CO_2, the cells were dyed with 100 μL of HDB Wash Free Fluo-8 Calcium Assay kit at 37 °C. An hour later, the cells were transferred into FlexStation3 Benchtop Multi-Mode Microplate Reader (Molecular Devices, Sunnyvale, California, United States) for bioassay. The raw data from time sequence recording were normalized as percentage responses to melatonin and 5-hydroxytryptamine as the positive controls, and analyzed to fit the four-parameter logistic equation to assess the agonistic rates.

4.4 Statistical Analyses

All experiments were carried out in triplicate. Data were expressed as mean ± standard error of mean (Mean ± SEM). Statistical analysis was performed using GraphPad Prism 7 (GraphPad Software Inc., San Diego, CA) and Origin 2018 (OriginLab Corporation, Wellesley Hills, MA) software.

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Compliance with Ethical Standards

Conflict of interest The authors declare no conflict of interest.

References

1. Q. Zhang, J.J. Zhao, J. Xu, F. Feng, W. Qu, J. Ethnopharmacol. **173**, 48–80 (2015)
2. M.E. Heitzman, C.C. Neto, E. Winiarz, A.J. Vaisberg, G.B. Hammond, Phytochemistry **66**, 5–29 (2005)
3. A. Ndagijimana, X. Wang, G. Pan, F. Zhang, H. Feng, O. Olaleye, Fitoterapia **86**, 35–47 (2013)
4. J.Y. Zhou, S.W. Zhou, Fitoterapia **83**, 617–626 (2012)
5. K. Keplinger, G. Laus, M. Wurm, M.P. Dierich, H. Teppner, J. Ethnopharmacol. **64**, 23–34 (1999)
6. J.G. Zhang, J.J. Chen, C.A. Geng, Chin. J. Chin. Mater. Med. **44**, 685–695 (2019)
7. Chinese Pharmacopoeia Commission, *Pharmacopoeia of the People's Republic of China*, vol. 1 (China Medical Science Press, Beijing, 2015), p. 257
8. Y. Ogawa, Y. Fujii, R. Sugiyama, T. Konishi, J. Ethnopharmacol. **177**, 19–27 (2016)
9. T. Itoh, Y. Shimada, K. Terasawa, Mech. Ageing. Dev. **111**, 155–173 (1999)
10. I. Sakakibara, S. Terabayashi, M. Kubo, M. Higuchi, Y. Komatsu, M. Okada, K. Taki, J. Kamei, Phytomedicine **6**, 163–168 (1999)
11. L.C. Hsu, Y.J. Ko, H.Y. Cheng, C.W. Chang, Y.C. Lin, Y.H. Cheng, M.T. Hsieh, W.H. Peng, Evid. Based Complement. Altern. Med. **2012**, 497302 (2012)
12. C.A. Geng, T.H. Yang, X.Y. Huang, Y.B. Ma, X.M. Zhang, J.J. Chen, J. Ethnopharmacol. **232**, 39–46 (2019)
13. C. Von Gall, J.H. Stehle, D.R. Weaver, Cell. Tissue Res. **309**, 151–162 (2002)
14. R. Hardeland, D.P. Cardinali, V. Srinivasan, D.W. Spence, G.M. Brown, S.R. Pandi-Perumal, Prog. Neurobiol. **93**, 350–384 (2011)
15. J.D. McCorvy, B.L. Roth, Pharmacol. Ther. **150**, 129–142 (2015)
16. F. Artigas, Pharmacol. Ther. **137**, 119–131 (2013)
17. J.G. Zhang, C.A. Geng, X.Y. Huang, X.L. Chen, Y.B. Ma, X.M. Zhang, J.J. Chen, Eur. J. Mass Spectrom. **23**, 11–21 (2017)
18. M. Chandel, M. Kumar, U. Sharma, B. Singh, S. Kaur, Comb. Chem. High. Throughput. Screen. **20**, 760–772 (2017)
19. Y. Gai, H. Chen, C. Wu, F. Feng, Y. Wang, W. Liu, S. Wang, J. Sep. Sci. **36**, 3723–3732 (2013)
20. J.G. Zhang, X.Y. Huang, Y.B. Ma, X.M. Zhang, J.J. Chen, C.A. Geng, J. Sep. Sci. **41**, 1532–1538 (2018)
21. J. Qu, T. Gong, B. Ma, L. Zhang, Y. Kano, D. Yuan, Chem. Pharm. Bull. **60**, 23–30 (2012)
22. D. Martins, C.V. Nunez, Molecules **20**, 13422–13495 (2015)
23. C.L. Cardoso, I. Castro-Gamboa, D.H. Silva, M. Furlan, R.D. Epifanio, A.D. Pinto, C. de Moraes Rezende, J.A. Lima, V.D. Bolzani, J. Nat. Prod. **67**, 1882–1885 (2004)
24. A. Itoh, T. Tanahashi, N. Nagakura, T. Nishi, Phytochemistry **62**, 359–369 (2003)
25. J.H. Paul, A.R. Maxwell, W.F. Reynolds, J. Nat. Prod. **66**, 752–754 (2003)
26. S. Wei, Z. Luo, S. Cui, J. Qiao, Z. Zhang, L. Zhang, J. Fu, X. Ma, Molecules **24**, 175 (2019)
27. S. Xie, Y. Shi, Y. Wang, C. Wu, W. Liu, F. Feng, N. Xie, J. Pharm. Biomed. Anal. **81**, 56–64 (2013)
28. T.J. Kim, J.H. Lee, J.J. Lee, J.Y. Yu, B.Y. Hwang, S.K. Ye, L. Shujuan, L. Gao, M.Y. Pyo, Y.P. Yun, Biol. Pharm. Bull. **31**, 2073–2078 (2008)
29. X. Wei, L.P. Jiang, Y. Guo, A. Khan, Y.P. Liu, H.F. Yu, B. Wang, C.F. Ding, P.F. Zhu, Y.Y. Chen, Y.L. Zhao, Y.B. Chen, Y.F. Wang, X.D. Luo, Nat. Prod. Bioprospect. **7**, 413–419 (2017)
30. T. Pengsuparp, B. Indra, O. Nakagawasai, T. Tadano, Y. Mimaki, Y. Sashida, Y. Ohizumi, K. Kisara, Eur. J. Pharmacol. **425**, 211–218 (2001)
31. H.Q. Pan, W.Z. Yang, Y.B. Zhang, M. Yang, R.H. Feng, W.Y. Wu, D.A. Guo, Anal. Bioanal. Chem. **407**, 6057–6070 (2015)
32. M. Hesse, *Indolalkaloide in Tabellen*, vol. 105 (Springer, Berlin, 1964)
33. B. Zhao, Y. Huang, Q. Chen, Q. Chen, H. Miao, S. Zhu, C. Zeng, Biomed. Chromatogr. **32**, e4119 (2018)
34. S. Masumoto, S. Aoki, T. Miura, T. Shoji, Mol. Nutr. Food. Res. **62**, e1700867 (2018)
35. S. Zhang, X. Liu, Z.L. Zhang, L. He, Z. Wang, G.S. Wang, Molecules **17**, 13917–13922 (2012)
36. N. Lopez-Gutierrez, R. Romero-Gonzalez, P. Plaza-Bolanos, J.L. Martinez Vidal, A. Garrido Frenich, Food Chem. **173**, 607–618 (2015)
37. A. Singh, S. Kumar, V. Bajpai, T.J. Reddy, K.B. Rameshkumar, B. Kumar, Rapid Commun. Mass Spectrom. **29**, 1095–1106 (2015)
38. X.A. Yu, J. Teye Azietaku, J. Li, H. Wang, F. Zheng, J. Hao, Y.X. Chang, Evid. Based Complement. Altern. Med. **2018**, 4964291 (2018)
39. Y. Zhao, C.A. Geng, Y.B. Ma, X.Y. Huang, H. Chen, T.W. Cao, K. He, H. Wang, X.M. Zhang, J.J. Chen, J. Ethnopharmacol. **156**, 147–154 (2014)
40. Z. Wang, S. Wang, B. Qin, Biomed. Chromatogr. **31**, e3811 (2017)
41. C. Pavei, S. Kaiser, S.G. Verza, G.L. Borre, G.G. Ortega, J. Pharm. Biomed. Anal. **62**, 250–257 (2012)
42. P. Montoro, V. Carbone, D. de Quiroz, F. De Simone, C. Pizza, Phytochem. Anal. **15**, 55–64 (2004)
43. W. Liu, Q. Song, Y. Cao, N. Xie, Z. Li, Y. Jiang, J. Zheng, P. Tu, Y. Song, J. Li, J. Pharm. Biomed. Anal. **162**, 16–27 (2019)
44. C.A. Geng, H. Chen, X.L. Chen, X.M. Zhang, L.G. Lei, J.J. Chen, Int. J. Mass Spectrom. **361**, 9–22 (2014)
45. C.A. Geng, X.Y. Huang, Y.B. Ma, B. Hou, T.Z. Li, X.M. Zhang, J.J. Chen, J. Nat. Prod. **80**, 959–964 (2017)