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 (MT₁ and MT₂) and 5-hydroxytryptamine (5-HT₁A and 5-HT₂C) 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 (MT₁, MT₂, 5-HT₁A, and 5-HT₂C). 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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1 Introduction

Uncariae Ramulus Cum Uncis (Gou-Teng), the dried hook-bearing stems of Uncaria plants (Rubiaeaceae), 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. s), 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

Keywords Uncariae Ramulus Cum Uncis · Uncaria plants · LCMS-IT-TOF analyses · Melatonin and 5-hydroxytryptamine receptors
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), vinosamide-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 (NH₃) in the MS² 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 MS² ions at m/z 383.1612 (C₂₁H₂₂N₂O₅) and 351.1245 (C₂₀H₁₆N₂O₄), 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 C₂₇H₃₄N₂O₉ with two more hydrogens than 21. In the MS² spectra, the identical fragmentation at m/z 385 (C₂₁H₂₃N₂O₄) and 367 (C₂₁H₂₂N₂O₄) 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 Vinosamide-Type Alkaloids Peak 15 showing a molecular formula of C₁₈H₉₀N₂O₉ was deduced from the [M+H]⁺ ion at m/z 839.3054. In the positive MS² experiment, the sequential losses of three glycosyl moieties (C₆H₁₀O₅, 162 Da) suggested the presence of three glycosyl 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-hydroxyvinosamide 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 MS² ions attributed to the neutral loss of 17 Da (NH₃) from the precursor ions. Peaks 33 and 38 were assigned with the same molecular formula of C₂₇H₄₅N₂O₉ from the [M+H]⁺ ion at m/z 531. Their similar MS² fragmentations at m/z 514 (C₂₇H₃₃NO₆) and 352 (C₂₇H₂₃NO₅) 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 CH₂ (14 Da) less in the molecular formula. The MS² fragmentation ion at m/z 338.1568 implied the successive loss of 17 Da (NH₃) and 162 Da (C₇H₁₀O₃), by which this compound was assigned as strictosidinic acid [22]. The molecular formula of 25 was determined as C₃₈H₅₀N₂O₁₉ by the protonated ion ([M+H]⁺) at m/z 571.1896 and deprotonated ion ([M–H]⁻) at m/z 569.1780. In the MS² experiment, the sequential losses of 162 Da (C₂₁H₁₆O₅), 18 Da (H₂O), and 14 Da (CH₄) was consistent with the presence of glucosyl, hydroxyl, and methoxyl groups. From the above analyses, peak 25 was tentatively assigned as deoxy cordifoline 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 C₄₄H₅₄N₂O₂₀ and C₄₄H₅₈N₂O₁₈. The sequential losses of two 162 Da (C₁₉H₂₀O₂) 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 (C₁₄H₂₀N₅O₄) and 323.1406 (C₁₉H₁₄N₂O₅), 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 C₂₂H₂₈N₂O₄, which were determined by the [M+H]⁺ ion at m/z 385. The MS² 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 C₇-side chain. By comparing their relative retention time on octadecylsilyl (ODS) column, they were deduced as isorhynchophylline, corynoxine, and rhyphycolline [26]. Peaks 27 and 31 occupied the same molecular weight of 384, corresponding to the molecular formula of C₂₁H₂₄N₂O₅. Their MS² fragments at m/z 367, 351, and 335 accounting for the lost H₂O and two additional oxygen atoms indicated an oxygenated derivative of rhynchophyllinic acid. Likewise, peaks 24 and 35 were deduced as dehydro-derivatives of rhyphycolline, and peak 11 was proposed as the demethylated derivative of rhyphycolline [27].
### Table 1: Characterization of peaks in seven *Uncaria* plants by UFLC-DAD-MS/MS analyses

| No. | t<sub>r</sub> (min) | MW | MF | DBE | MS | λ<sub>max</sub> (nm) | Name |
|-----|-------------------|----|----|-----|----|----------------|------|
| 1   | 1.59              | 342| C<sub>12</sub>H<sub>22</sub>O<sub>11</sub> | 2  | Pos: 381.0792 ([M+K]<sup>+</sup>, − 0.2 mDa) Neg: 387.1170 ([M+HCOO]<sup>−</sup> + 2.6 mDa) | Pos: − Neg: 387 → 341.1091 (C<sub>12</sub>H<sub>22</sub>O<sub>11</sub>) | 201 Sucrose |
| 2   | 3.06              | 376| C<sub>16</sub>H<sub>24</sub>O<sub>10</sub> | 5  | Pos: 399.1258 ([M+Na]<sup>+</sup>, − 0.4 mDa) Neg: 375.1301 ([M−H]<sup>−</sup> + 0.4 mDa) | Pos: 399 → 377.1439(C<sub>16</sub>H<sub>24</sub>O<sub>10</sub>) Neg: 355 → 163.0406 (C<sub>4</sub>H<sub>2</sub>O<sub>2</sub>) | 234 Loganic acid |
| 3   | 3.71              | 354| C<sub>16</sub>H<sub>18</sub>O<sub>9</sub> | 8  | Pos: 355.1017 ([M+H]<sup>+</sup> + 0.7 mDa) Neg: 353.0877 ([M−H]<sup>−</sup> + 0.1 mDa) | Pos: 355 → 163.0406 (C<sub>4</sub>H<sub>2</sub>O<sub>2</sub>) Neg: 353 → 191.0565 (C<sub>7</sub>H<sub>12</sub>O<sub>6</sub>) | 221 Neochlorogenic acid |
| 4   | 3.80              | 578| C<sub>3</sub>H<sub>20</sub>O<sub>12</sub> | 18 | Pos: 579.1465 ([M+H]<sup>+</sup> + 3.2 mDa) Neg: 577.1327 ([M−H]<sup>−</sup> + 0.4 mDa) | Pos: 579 → 409.0915 (C<sub>16</sub>H<sub>24</sub>O<sub>10</sub>) Neg: 377 → 245.0817 | 279 Procyanidin B1 |
| 5   | 4.31              | 290| C<sub>15</sub>H<sub>18</sub>O<sub>9</sub> | 9  | Pos: 291.0841 ([M+H]<sup>+</sup> + 2.2 mDa) Neg: 289.0712 ([M−H]<sup>−</sup> + 0.6 mDa) | Pos: 291 → 273.0741 (C<sub>15</sub>H<sub>12</sub>O<sub>5</sub>) Neg: 273 → 145.0335 (C<sub>9</sub>H<sub>4</sub>O<sub>2</sub>) | 280 Catechin |
| 6   | 4.58              | 354| C<sub>16</sub>H<sub>18</sub>O<sub>9</sub> | 8  | Pos: 355.1016 ([M+H]<sup>+</sup> + 0.8 mDa) Neg: 353.0873 ([M−H]<sup>−</sup> + 0.5 mDa) | Pos: 355 → 163.0407 (C<sub>4</sub>H<sub>2</sub>O<sub>2</sub>) Neg: 273 → 139.0411 (C<sub>7</sub>H<sub>6</sub>O<sub>3</sub>) | 218 Chlorogenic acid |
| 7   | 5.10              | 354| C<sub>16</sub>H<sub>18</sub>O<sub>9</sub> | 8  | Pos: 355.1023 ([M+H]<sup>+</sup> + 0.1 mDa) Neg: 353.0887 ([M−H]<sup>−</sup> + 0.9 mDa) | Pos: 355 → 163.0401 (C<sub>4</sub>H<sub>2</sub>O<sub>2</sub>) Neg: 353 → 191.0565 (C<sub>7</sub>H<sub>12</sub>O<sub>6</sub>) | 325 Cryptochlorogenic acid |
| 8   | 5.57              | 578| C<sub>3</sub>H<sub>20</sub>O<sub>12</sub> | 18 | Pos: 579.1480 ([M+H]<sup>+</sup> + 1.7 mDa) Neg: 577.1330 ([M−H]<sup>−</sup> + 2.2 mDa) | Pos: 579 → 427.1024 (C<sub>15</sub>H<sub>12</sub>O<sub>5</sub>) Neg: 577 → 425.0911 (C<sub>16</sub>H<sub>12</sub>O<sub>5</sub>) | 280 Procyanidin B2 |
| 9   | 6.22              | 354| C<sub>16</sub>H<sub>18</sub>O<sub>9</sub> | 8  | Pos: 355.1013 ([M+H]<sup>+</sup> + 1.1 mDa) Neg: 353.0870 ([M−H]<sup>−</sup> + 0.8 mDa) | Pos: 355 → 163.0420 (C<sub>4</sub>H<sub>2</sub>O<sub>2</sub>) Neg: 353 → 191.0573 (C<sub>7</sub>H<sub>12</sub>O<sub>6</sub>) | 325 Isochlorogenic acid |
| 10  | 6.28              | 290| C<sub>15</sub>H<sub>18</sub>O<sub>9</sub> | 9  | Pos: 291.0841 ([M+H]<sup>+</sup> + 2.2 mDa) Neg: 289.0704 ([M−H]<sup>−</sup> + 1.4 mDa) | Pos: 291 → 139.0411 (C<sub>4</sub>H<sub>2</sub>O<sub>2</sub>) Neg: − | 325 Epicatechin |
| 11  | 6.43              | 370| C<sub>2</sub>H<sub>20</sub>N<sub>2</sub>O<sub>4</sub> | 10 | Pos: 371.1973 ([M+H]<sup>+</sup> + 0.8 mDa) Neg: − | Pos: 371 → 353.1889 (C<sub>16</sub>H<sub>24</sub>N<sub>2</sub>O<sub>4</sub>) Neg: − | 241 Corynoxinic acid |
| 12  | 7.00              | 562| C<sub>3</sub>H<sub>20</sub>O<sub>11</sub> | 18 | Pos: 563.1514 ([M+H]<sup>+</sup> + 3.4 mDa) Neg: 561.1392 ([M−H]<sup>−</sup> + 1.0 mDa) | Pos: 563 → 411.1049 (C<sub>16</sub>H<sub>24</sub>O<sub>10</sub>) Neg: − | 275 Fisetinol-(4α→8)-epicatechin |
| 13  | 7.87              | 468| C<sub>2</sub>H<sub>20</sub>O<sub>12</sub> | 10 | Pos: 469.1323 ([M+H]<sup>+</sup> + 1.8 mDa) Neg: − | Pos: 469 → 317.0994 (C<sub>15</sub>H<sub>10</sub>O<sub>6</sub>) Neg: − | 278 Gallocatechol C-glucoside |
| 14  | 8.09              | 562| C<sub>3</sub>H<sub>20</sub>O<sub>11</sub> | 18 | Pos: 563.1526 ([M+H]<sup>+</sup> + 2.2 mDa) Neg: 561.1400 ([M−H]<sup>−</sup> + 0.2 mDa) | Pos: 563 → 411.1014 (C<sub>16</sub>H<sub>24</sub>O<sub>10</sub>) Neg: − | 277 Fisetinol-(4β→8)-epicatechin |
| No. | t_R (min) | MW | MF | DBE | MS | MS/MS | λ_max (nm) | Name |  
|-----|----------|----|----|-----|----|-------|------------|------|  
| 15  | 11.59    | 838 | C_{38}H_{50}N_{2}O_{19} | 15  | Pos: 839.3054 ([M+H]^+, − 2.7 mDa) | Pos: 839 → 677.2546 (C_{18}H_{29}N_{3}O_{4}), 515.1975 (C_{18}H_{29}N_{3}O_{4}), 353.1502 (C_{18}H_{29}N_{3}O_{4}), 283.1411 (C_{18}H_{29}N_{3}O_{4}), Neg: 883 → 837.2849 (C_{18}H_{29}N_{3}O_{4}), 675.2278 (C_{18}H_{29}N_{3}O_{4}), 495.1688 (C_{18}H_{29}N_{3}O_{4}), 313.0865 (C_{18}H_{29}N_{3}O_{4}) | 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) | Pos: 611 → 543.0473 (C_{18}H_{15}O_{7}), 386.1677 (C_{18}H_{15}O_{7}), 245.1030 (C_{18}H_{15}O_{7}) | 253 | Rutin |  
| 17  | 12.40    | 464 | C_{21}H_{20}O_{12} | 12  | Pos: 465.1019 ([M+H]^+, − 0.9 mDa) | Pos: 465 → 303.0497 (C_{12}H_{10}O_{4}), 271.0146 (C_{12}H_{10}O_{4}) | 255 | Hyperoside |  
| 18  | 12.92    | 516 | C_{26}H_{32}N_{2}O_{9} | 12  | Pos: 517.2206 ([M+H]^+, + 2.5 mDa) | Pos: 517 → 338.1546 (C_{19}H_{11}O_{7}), 276.1250 (C_{19}H_{11}O_{7}) | 203 | Strictosidic acid |  
| 19  | 13.24    | 564 | C_{27}H_{32}N_{2}O_{9} | 11  | Pos: 565.2206 ([M+H]^+, − 0.7 mDa) | Pos: 565 → 497.1583 (C_{19}H_{11}O_{7}), 363.1222 (C_{19}H_{11}O_{7}) | 205 | 18,19-Dehydrocorynoxinic acid |  
| 20  | 13.55    | 368 | C_{21}H_{24}N_{2}O_{4} | 11  | Pos: 369.1800 ([M+H]^+, − 0.9 mDa) | Pos: 369 → 339.1347 (C_{15}H_{11}O_{7}), 297.0259 (C_{15}H_{11}O_{7}), 245.1030 (C_{15}H_{11}O_{7}) | 206 | Hydrated cadambine |  
| 21  | 13.86    | 544 | C_{22}H_{32}N_{2}O_{10} | 13  | Pos: 545.2108 ([M+H]^+, − 2.2 mDa) | Pos: 545 → 383.1612 (C_{19}H_{11}O_{7}), 351.1245 (C_{19}H_{11}O_{7}), 327.1093 (C_{19}H_{11}O_{7}) | 280 | Cadambine |  
| 22  | 14.08    | 368 | C_{21}H_{24}N_{2}O_{4} | 11  | Pos: 369.1800 ([M+H]^+, − 0.9 mDa) | Pos: 369 → 339.1347 (C_{15}H_{11}O_{7}), 297.0259 (C_{15}H_{11}O_{7}), 245.1030 (C_{15}H_{11}O_{7}), 213.0997 (C_{15}H_{11}O_{7}) | 206 | 18,19-Dehydrocorynoxinic acid |  
| 23  | 14.81    | 546 | C_{22}H_{34}N_{2}O_{10} | 12  | Pos: 547.2269 ([M+H]^+, − 1.7 mDa) | Pos: 547 → 385.1801 (C_{19}H_{11}O_{7}), 367.1688 (C_{19}H_{11}O_{7}), 345.1577 (C_{19}H_{11}O_{7}), 335.1372 (C_{19}H_{11}O_{7}), 317.1258 (C_{19}H_{11}O_{7}) | 220 | 3α-Dihydrocadambine |  
| 24  | 14.86    | 382 | C_{22}H_{32}N_{2}O_{4} | 11  | Pos: 383.1955 ([M+H]^+, − 1.0 mDa) | Pos: 383 → 351.1743 (C_{19}H_{11}O_{7}), 363.1222 (C_{19}H_{11}O_{7}), 327.1093 (C_{19}H_{11}O_{7}) | 205 | Isocorynoxeine |  
| 25  | 14.90    | 570 | C_{28}H_{36}N_{2}O_{11} | 15  | Pos: 571.1896 ([M+H]^+, − 2.6 mDa) | Pos: 571 → 409.1426 (C_{19}H_{11}O_{7}), 391.1250 (C_{19}H_{11}O_{7}), 377.1120 (C_{19}H_{11}O_{7}), 359.1064 (C_{19}H_{11}O_{7}), 341.0952 (C_{19}H_{11}O_{7}), 313.0973 (C_{19}H_{11}O_{7}), 285.0985 (C_{19}H_{11}O_{7}) | 216 | Deoxycordifoline |
| No. | t_R (min) | MW | MF | DBE | MS | MS/MS | λ_max (nm) | Name |
|-----|-----------|----|----|-----|----|-------|------------|------|
| 26  | 15.33     | 546 | C_{25}H_{31}N_{2}O_{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_{25}N_{2}O_{5}), 367.1697 (C_{21}H_{25}N_{2}O_{5}) | 218  | 3β-Dihydrocadambine |
| 27  | 15.38     | 384 | C_{25}H_{31}N_{2}O_{4} | 11 | Pos: 385.1739 ([M+H]^+, − 1.9 mDa) Neg: 429.1682 ([M+HCOO]^−, + 1.5 mDa) | Pos: 385 → 367.1698 (C_{21}H_{25}N_{2}O_{5}), 351.1696 (C_{21}H_{25}N_{2}O_{5}) | 206  | Oxocorynoxinic acid |
| 28  | 15.69     | 368 | C_{25}H_{31}N_{2}O_{4} | 11 | Pos: 369.1811 ([M+H]^+, + 0.2 mDa) | Pos: 369 → 337.1588 (C_{20}H_{22}N_{2}O_{5}), 309.1647 (C_{19}H_{22}N_{2}O_{4}) | 204  | 18,19-Dehydrocorynoxinic acid |
| 29  | 15.74     | 448 | C_{25}H_{31}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_{3}), 271.0288 (C_{14}H_{10}O_{3}) | 204  | Quercetin 3-rhamnoside |
| 30  | 16.28     | 516 | C_{25}H_{31}O_{12} | 14 | Pos: 517.1337 ([M+H]^+, − 0.4 mDa) Neg: 515.1187 ([M−H]^−, − 0.8 mDa) | Pos: – Neg: – | 247  | 3,5-Dicaffeoylquinic acid |
| 31  | 16.89     | 384 | C_{25}H_{31}N_{2}O_{4} | 11 | Pos: 385.1736 ([M+H]^+, − 0.2 mDa) Neg: 429.1686 ([M+HCOO]^−, + 1.9 mDa) | Pos: 385 → 367.1671 (C_{21}H_{25}N_{2}O_{5}), 351.1721 (C_{21}H_{25}N_{2}O_{5}) | 249  | Oxothynophylic acid |
| 32  | 17.59     | 368 | C_{25}H_{31}N_{2}O_{4} | 11 | Pos: 369.1817 ([M+H]^+, + 0.8 mDa) Neg: – | Pos: 369 → 337.1560 (C_{20}H_{22}N_{2}O_{5}), 309.1566 (C_{19}H_{22}N_{2}O_{4}) | 246  | Demethylcorynoxeine |
| 33  | 17.63     | 530 | C_{25}H_{31}N_{2}O_{9} | 12 | Pos: 531.2308 ([M+H]^+, − 2.9 mDa) Neg: 575.2225 ([M+HCOO]^−, − 2.1 mDa) | Pos: 531 → 514.2026 (C_{21}H_{19}N_{2}O_{5}), 352.1577 (C_{16}H_{19}N_{2}O_{5}) | 220  | 3-Epistrictosidine |
| 34  | 18.62     | 384 | C_{25}H_{31}N_{2}O_{4} | 10 | Pos: 385.2112 ([M+H]^+, − 1.0 mDa) Neg: – | Pos: 385 → 353.1852 (C_{21}H_{27}N_{2}O_{5}), 321.1618 (C_{18}H_{25}N_{2}O_{4}) | 206  | Isorhynchophylline |
| 35  | 19.16     | 382 | C_{25}H_{31}N_{2}O_{4} | 11 | Pos: 383.1959 ([M+H]^+, − 0.6 mDa) Neg: – | Pos: 383 → 351.1671 (C_{21}H_{27}N_{2}O_{5}), 319.1480 (C_{18}H_{25}N_{2}O_{4}) | 202  | Corynoxeine |
| 36  | 19.21     | 368 | C_{25}H_{31}N_{2}O_{4} | 11 | Pos: 369.1783 ([M+H]^+, − 2.6 mDa) Neg: – | Pos: 369 → 337.1539 (C_{20}H_{22}N_{2}O_{5}), 293.1347 (C_{18}H_{20}N_{2}O_{4}) | 207  | Demethylisocorynoxeine |
| No. | tR (min) | MW  | MF  | DBE | MS | MS/MS | λ\text{max} (nm) | Name                  |
|-----|---------|-----|-----|-----|----|-------|-------------------|-----------------------|
| 37  | 19.32   | 384 | C22H28N2O4 | 10  | Pos: 385.2133 ([M+H]+, + 1.1 mDa)  | Pos:385 → 353.1875 (C21H24N2O4), 321.1619 (C21H24N2O4), 265.1339 (C21H24N2O4), 241.1334 (C21H24N2O4), 187.0697 (C11H10N2O) | 210 Corynoxine        |
|     |         |     | Pos: −                      | Neg: −                                |                                     |
| 38  | 19.62   | 530 | C22H28N2O4 | 12  | Pos: 531.2311 ([M+H]+, − 2.6 mDa)  | Pos:531 → 514.2082 (C21H24N2O4), 352.1586 (C21H24N2O4), 334.1556 (C21H24N2O4) | 219 Strictosidine     |
|     |         |     | Neg: −                      | Neg: −                                |                                     |
| 39  | 19.89   | 352 | C22H28N2O4 | 11  | Pos: 353.1854 ([M+H]+, − 0.6 mDa)  | Pos:353 → 321.1474 (C21H24N2O4), 222.1198 (C21H24N2O4), 210.1126 (C11H10N2O) | 219 Ajmalicine         |
|     |         |     | Neg: −                      | Neg: −                                |                                     |
| 40  | 20.63   | 354 | C22H28N2O4 | 10  | Pos: 355.1994 ([M+H]+, − 2.2 mDa)  | Pos:355 → 224.1340 (C12H17NO3), 212.1241 (C12H17NO3), 144.0792 (C12H17NO3) | 220 Sitsirikine        |
|     |         |     | Neg: −                      | Neg: −                                |                                     |
| 41  | 20.96   | 580 | C22H28N2O4 | 8   | Pos: 581.3333 ([M+H]+, + 1.3 mDa)  | Pos:581 → 389.2065 (C16H10NO3)      | 202 Demythyl atropuroside C |
|     |         |     | Neg: −                      | Neg: −                                |                                     |
| 42  | 20.96   | 384 | C22H28N2O4 | 10  | Pos: 385.2101 ([M+H]+, − 2.1 mDa)  | Pos:385 → 304.1399 (C20H18N2O3), 144.0861 (C20H18N2O3) | 219 Geissoschizine     |
|     |         |     | Neg: −                      | Neg: −                                |                                     |
| 43  | 21.37   | 352 | C22H28N2O4 | 11  | Pos: 353.1829 ([M+H]+, − 3.1 mDa)  | Pos:353 → 204.1399 (C20H18N2O3), 222.1162 (C20H18N2O3) | 219 Neonaucleoside C   |
|     |         |     | Neg: −                      | Neg: −                                |                                     |
| 44  | 21.48   | 930 | C22H28N2O4 | 19  | Pos: 931.3357 ([M+H]+, + 1.4 mDa)  | Pos:931 → 769.2802 (C14H28N2O14), 719.2172 (C14H28N2O14), 607.2281 (C14H28N2O14), 557.1858 (C14H28N2O14) | 219 Neonaucleoside C   |
|     |         |     | Neg: −                      | Neg: −                                |                                     |
| 45  | 22.23   | 400 | C22H28N2O4 | 10  | Pos: 401.2090 ([M+H]+, + 1.9 mDa)  | Pos:401 → 383.1953 (C16H10NO3), 355.1652 (C16H10NO3), 241.1699 (C16H10NO3) | 212 Dihydroxycorynantheine |
|     |         |     | Neg: −                      | Neg: −                                |                                     |
| 46  | 22.52   | 594 | C22H28N2O4 | 8   | Pos: 595.3477 ([M+H]+, − 3.1 mDa)  | Pos:595 → 567.3522 (C16H18O3), 536.2769 (C16H18O3), 389.2051 (C16H18O3) | 204 Atropuroside C     |
|     |         |     | Neg: −                      | Neg: −                                |                                     |
| 47  | 23.19   | 546 | C22H28N2O4 | 12  | Pos: 547.2286 ([M+H]+, − 3.8 mDa)  | Pos:547 → 385.1740 (C16H18O3), 367.1648 (C16H18O3), 349.1520 (C16H18O3) | 202 3β-Isodihydrocadambine |
|     |         |     | Neg: −                      | Neg: −                                |                                     |
Table 1 (continued)

| No. | \(t_r\) (min) | MW | MF | DBE | MS | MS/MS | \(\lambda_{max}\) (nm) | Name |
|-----|---------------|----|----|-----|----|-------|------------------------|------|
| 48  | 24.10         | 366| C22H28N2O4 | 11 | Pos: 367.2016 ([M+H]+, − 2.1 mDa) | Pos-367 → 251.1628 (C17H18N2), 236.1268 (C13H12NO3), 224.1199 (C13H12NO3), 192.1019 (C11H11NO2) | 220  | Corynantheine |
|     |               |    |     |     | Neg: − | Neg: − |                        |      |
| 49  | 24.40         | 810| C44H68O15 | 10 | Pos: 833.4294 ([M+Na]+, − 3.6 mDa), 469.3306 (C31H34O3) | Pos-469 → 451.3204 (C29H24O2), 379.3331 (C25H24O2), 263.1778 (C20H22) | 207  | Quinovic acid diglycoside |
|     |               |    |     |     | Neg-809.4329 ([M−H]−, − 2.5 mDa) | Neg-809 → 603.3873 (C31H28O3) | 221  | Bahienoside B |
| 50  | 25.25         | 902| C44H58N2O18 | 17 | Pos: 903.3714 ([M+H]+, − 4.3 mDa) | Pos-903 → 341.1434 (C19H22N2O4), 323.1406 (C18H20N2O3) | 220  | Dihydrocorynantheine |
|     |               |    |     |     | Neg: − | Neg: − |                        |      |
| 51  | 25.70         | 368| C22H28N2O3 | 10 | Pos: 369.2154 ([M+H]+, + 2.6 mDa) | Pos-369 → 251.1179 (C17H18N2), 236.1458 (C13H12NO3), 224.1418 (C12H10NO3) | 207  | Quinovic acid triglycoside |
|     |               |    |     |     | Neg: − | Neg: − |                        |      |
| 52  | 27.51         | 956| C44H58O19 | 11 | Pos: 979.4895 ([M+Na]+, + 2.2 mDa) | Pos-979 → 935.434 (C31H28O3), 773.8421 | 207  | Quinovic acid triglycoside |
|     |               |    |     |     | Neg-955.4917 ([M−H]−, + 0.9 mDa) | Neg-955 → 749.4438 (C27H26O2), 587.3923 (C23H20O2), 441.3496 | 221  | Geissoschizine methyl ether |
| 53  | 27.74         | 366| C22H28N2O3 | 11 | Pos: 367.2011 ([M+H]+, − 0.5 mDa) | Pos-367 → 249.1363 (C17H18N2) | 221  | Quinovic acid triglycoside |
|     |               |    |     |     | Neg: − | Neg: − |                        |      |
| 54  | 29.76         | 382| C22H26N2O4 | 11 | Pos: 383.1932 ([M+H]+, + 3.3 mDa) | Pos-383 → 223.1304 (C15H14N2), 184.0878 (C12H8NO3) | 206  | Pubescin |
|     |               |    |     |     | Neg: − | Neg: − |                        |      |
| 55  | 30.02         | 366| C22H28N2O3 | 11 | Pos: 367.2007 ([M+H]+, + 0.9 mDa) | Pos-367 → 251.1606 (C17H18N2), 224.1386 (C13H12NO3) | 221  | Hirsuteine |
|     |               |    |     |     | Neg: − | Neg: − |                        |      |
| 56  | 30.52         | 486| C31H40O4 | 8  | Pos: 487.3404 ([M+H]+, + 1.4 mDa) | Pos-487 → 451.3117 (C30H36O3), 423.3082 (C29H24O3) | 207  | Quinovic acid |
|     |               |    |     |     | Neg: − | Neg: − |                        |      |
| 57  | 31.84         | 368| C22H28N2O3 | 10 | Pos: 369.2154 ([M+H]+, − 1.9 mDa) | Pos-369 → 337.1945 (C21H18N2O3), 238.1481 (C17H12NO3), 226.1380 (C12H8NO3) | 221  | Hirsutine |
Peaks 20, 22, 28, 32, and 36 had the same molecular formula of C_{21}H_{24}N_{2}O_{4}, with a CH_{2} less than corynoxeine. The MS\textsuperscript{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 decarboxylation and decarboxylation neutral losses of 28 Da and 46 Da were proved by the ions at m/z 309 and 291. By retrieving the corynoxeine-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_{23}H_{26}N_{2}O_{3}. The MS\textsuperscript{2} profiles at m/z 224.1340 (C_{15}H_{17}N_{2}O_{3}), 212.1241 (C_{11}H_{12}NO_{3}), and 144.0792 (C_{10}H_{9}N) 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_{2}O_{4} and C_{22}H_{28}N_{2}O_{3}) and MS\textsuperscript{2} fragments. Peaks 48 and 53 with the same formula of C_{22}H_{26}N_{2}O_{3} were determined to be corynantheine and geissoschizine methyl ether following their MS\textsuperscript{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_{2}O_{3} and C_{22}H_{26}N_{2}O_{4} with 11 double bond equivalents. The mass losses from m/z 352 to 321.1647 (C_{20}H_{20}N_{2}O_{3}), 222.1198 (C_{19}H_{15}NO_{3}), 210.1126 (C_{11}H_{13}NO_{3}), and 144.0798 (C_{10}H_{9}N) were in agree with ajmalicine [31]. Similarly, peak 54 was reasonably deduced to be pubescin from the MS\textsuperscript{2} fragments at m/z 223.1304 (C_{15}H_{14}N_{2}) and 184.0878 (C_{15}H_{2}NO) [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\textsuperscript{2} experiment on [M+H]\textsuperscript{+} ion generated fragments at m/z 409 (C_{30}H_{18}O_{6}), 301 (C_{19}H_{12}O_{5}), and 287 (C_{18}H_{10}O_{4}) 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_{19}H_{14}O_{6}. The MS\textsuperscript{2} ion at m/z 139 (C_{9}H_{4}O_{3}) was ascribed to the A\textsuperscript{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\textsuperscript{2} fragments at m/z 291.0856 (C_{19}H_{12}O_{6}) and 273.0778 (C_{17}H_{10}O_{5}) were attributed to fisetinidol and catechin moieties. From the above analyses, they were tentatively deduced to be fisetinidol-(4α→8)-epicatechin and fisetinidol-(4β→8)-epicatechin [35]. Peak 13 with a formula of C_{23}H_{25}O_{12} showed MS\textsuperscript{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 galocatechol C-glucoside [36, 37]. Peak 16 was designed with the molecular formula of C_{23}H_{30}O_{16} with an additional C_{6}H_{10}O_{4} part than 17 (C_{21}H_{20}O_{12}). In the MS\textsuperscript{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]\textsuperscript{+} ion at m/z 449.1068 and [M−H]\textsuperscript{−} ion at m/z 447.0939, corresponding to the molecular formula of C_{23}H_{29}O_{11}. In the MS\textsuperscript{2} experiment, the diagnostic MS\textsuperscript{2} ions at m/z 301.0358 (C_{19}H_{10}O_{7}) and 271.0288 (C_{13}H_{6}O_{4}) 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\textsuperscript{2} experiment, the product ions at m/z 163 (C_{6}H_{10}O_{4}) in positive mode and 191 (C_{5}H_{10}O_{3}) 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_{23}H_{25}O_{15} with an additional quinoyl moiety compared to chlorogenic acid. This deduction was verified by the MS\textsuperscript{2} ions at m/z 353.0882 (C_{19}H_{13}O_{5}) and 173.0401 (C_{7}H_{10}O_{3}) 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_{40}O_{5}. The abovementioned features were indicative for a triterpenoid. The MS\textsuperscript{2} fragments at m/z 469 (C_{30}H_{44}O_{4}), 451 (C_{30}H_{42}O_{3}),
33
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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_{6}H_{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_{5}). Thus, this peak was illustrated as loganic acid, the biosynthetic precursor of indole alkaloids [43].

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. 2 Distribution of different types of compounds among seven Uncaria 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.
and morphological similarity between different species. In this investigation, seven \textit{Uncaria} species involving four official, \textit{U. rhynchophylla}, \textit{U. macrophylla}, \textit{U. hirsuta}, and \textit{U. sessilifructus}, and three local species, \textit{U. scandens}, \textit{U. laevigata}, and \textit{U. yunnanensis} were extensively compared based on LCMS and bioassay in vitro. In total, 57 constituents including 35 indole alkaloids, 10 flavonoids, 5 triterpenoids, 5 chlorogenic analogues, and 2 other compounds were characterized based on their MS/MS patterns and UV absorptions. Cadambine-type and corynantheid-type alkaloids were exclusively present in \textit{U. rhynchophylla} and \textit{U. scandens}, whereas corynoxine-type alkaloids were commonly detected in all the seven \textit{Uncaria} plants. Three \textit{Uncaria} plants, \textit{U. rhynchophylla}, \textit{U. macrophylla}, and \textit{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 \textit{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-C18 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 a 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 \textit{Uncaria rhynchophylla} (Miq.) Miq. ex Havil. (No. 2,016,090,001), \textit{Uncaria macrophylla} Wall. (No. 2,016,090,002), \textit{Uncaria hirsuta} Havil. (No. 2,016,090,003), \textit{Uncaria sessilifructus} Roxb. (No. 2,016,090,004), \textit{Uncaria scandens} (Smith) Hutchins. (No. 2,016,090,005), \textit{Uncaria laevigata} Wall. ex G. Don (No. 2,016,090,006), and \textit{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 Fluoro-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.
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