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Rapid and systematic identification of indole alkaloids in Uncaria rhynchophylla by UPLC-Q-TOF-MS

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Research Article

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ABSTRACT

Uncaria rhynchophylla (UR), a well-known traditional Chinese medicine (TCM), is widely used to treat clinical cardiovascular diseases. Recently, some researches have been reported for pharmacology and photochemistry of UR. However, there are no reports on qualitative analysis previously. We used MDF function module of Agilent qualitative software and characteristic diagnostic fragmentation ions (CDFI) to identify Uncaria alkaloids. A total of thirty-two compounds were identified. Among them, twelve ingredients had never been reported in a qualitative way, including three ajmalicine-type (16,20,21), four yohimbine-type (4,5,15,27), 5α-carboxystrictosidine (6), gambireine (10), glabratine (12), villocarines D (17) and geissoschizine methyl ether (23).A rapid method integrated MDF and diagnostic ions is successfully established and applied to identify the alkaloids in UR.

INTRODUCTION

Uncaria rhynchophylla (UR) (also named Chinese Gou Teng), which belongs to Rubiacea family, is widely distributed in different localities of China. It has been demonstrated to be effective in the treatment of convulsions, hypertension, epilepsy, eclampsia and cerebral diseases [1,2]. UR is characterized by its high content of indole alkaloids and other active constituents such as phenols, flavonoids and terpenoids according to phytochemical studies [3,4]. Furthermore, the type of indole alkaloids is mainly heteroyohimbine and corresponding oxindole-types while pyridino-indoloquinolizidinones, roxburghines, and harmane are found to be limited distribution [5].

Recently, although high-performance liquid chromatography coupled with mass spectrometry (HPLC-MS) method has made valuable contribution to qualitative and quantitative identification of alkaloids in UR [6,7], there is hardly any research could provide an effective and accurate data processing method. Xie [8] has described a novel strategy based on characteristic diagnostic fragmentation ions (CDFI) to identify twenty nine compounds of tetracyclic monoterpenoid oxindole alkaloids. However, this approach was largely dependent on predefined fragments summarized from standards.

Mass defect filtering (MDF) technique is developed for metabolites detection based on a narrow and well-defined mass defect range (±50 mDa) between the parent drug and metabolites. Therefore, a significant number of background interference ions can be removed and the metabolite profile of biological samples can be obtained. MDF has also been successfully used for detecting homologues of herbal compounds. Yan et al. [9] presented a rapid and global method using MDF technique for the detection and characterization of aconitum alkaloids in Yin Chen Si Ni Tang, but false positive results still exist.

With the increasing development of technology of high performance liquid chromatography and high resolution mass spec-

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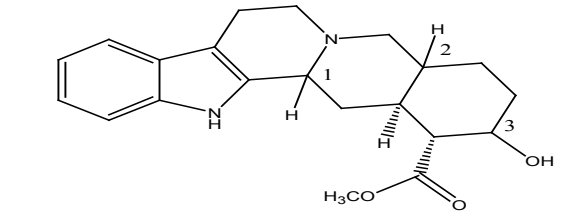
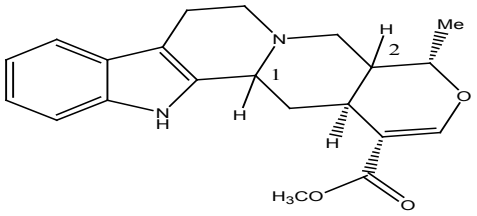
trometry, it is imperative to establish an efficient, accurate and simple data processing method. Therefore, we used MDF function module of Agilent qualitative software and characteristic diagnostic fragmentation ions (CDFI) to identify Uncaria alkaloids. A total of thirty-two compounds were identified. Among them, twelve ingredients had never been reported in a qualitative way, including three ajmalicine-type (16,20,21), four yohimbine-type (4,5,15,27), 5α-carboxystrictosidine(6), gambireine(10), glabratine(12), villocarines D(17) and geissoschizine methylether(23) (**Figure 1, Tables 1 and 2**).



I (a)



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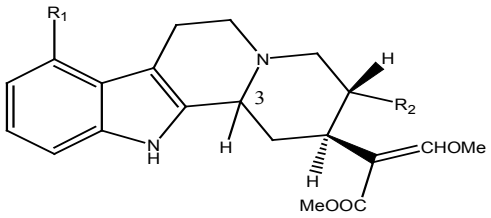

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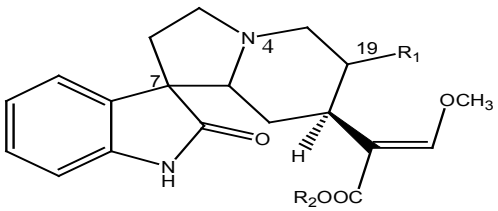
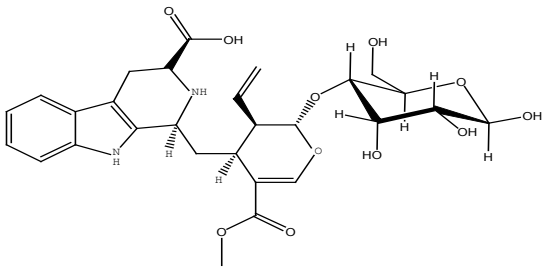
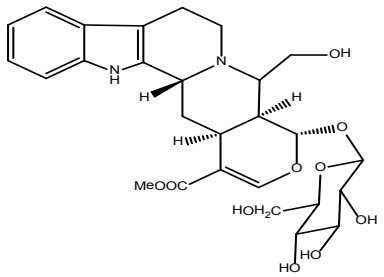


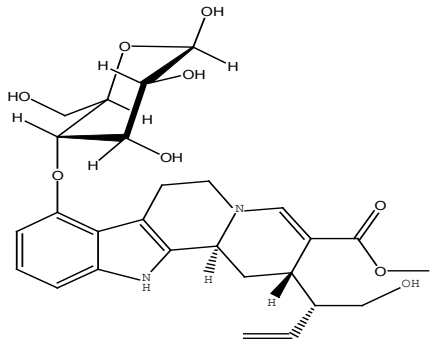
III



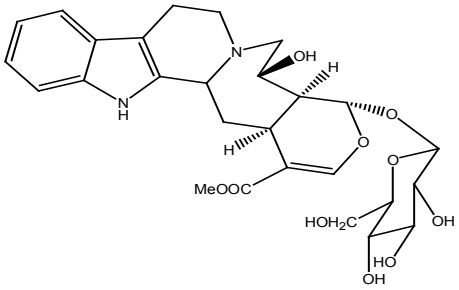
(6) 5α-carboxystrictosidine



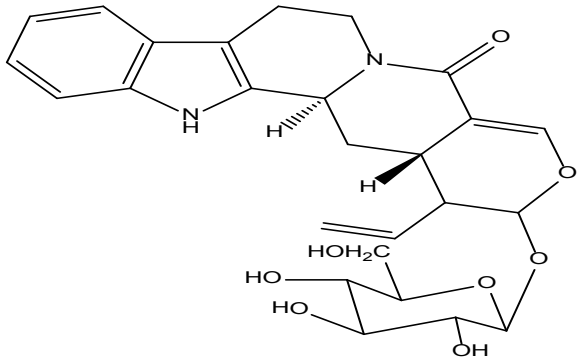
(8) 3β-isodihydrocadambine



(12) glabratine



(22) 3α-dihydrocadambine



(30)strictosamide

Figure 1. Core substructure of compounds identified in *Uncaria rhynchophylla*.

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Table 1. Chemical configurations of compounds identified in *Uncaria rhynchophylla.*

No	Compounds	Type	R1	R2	R3	C-3
4	Yohimbine	I(a)	S	R	R	-
5	Allo or epiallo yohimbine	I(a)	S or R	R	S or R	-
15	β-yohimbine	I(a)	S	S	R	-
16	Allo or epiallo ajmalicine	I(b)	S or R	R	-	-
20	Allo or epiallo ajmalicine	I(b)	S or R	R	-	-
21	ajmalicine	I(b)	S	R	-	-
27	Allo or epiallo yohimbine	I(a)	S or R	R	S or R	-
10	Gambireine	II	-OH	CH=CH2	-	S
23	Geissoschizine methyl ether	II	H	=CH-CH3	-	S
24	Epiallo-corynantheine	II	H	CH=CH2	-	R
25	Hisuteine or corynantheine	II	H	CH=CH2	-	R or S
28	Hisuteine or corynantheine	II	H	CH=CH2	-	R or S
32	Dihydrocorynantheine or hirsutine	II	H	CH2CH3	-	R or S

No	Compounds	Type	R1	R2	C-7	N-4	C-19
1	22-O-demethyl-22-O-β-D-glucopyranosylisocorynoxeine	III	CH=CH2	Glu	S	-	R
2	18,19-dehydrocorynoxinic acid	III	CH=CH2	H	S	-	S
3	18,19-dehydrocorynoxinic acid B	III	CH=CH2	H	R	-	S
7	Isorhynchophyllic acid	III	CH2CH3	H	S	-	R
9	Isocorynoxeine	III	CH=CH2	CH3	S	-	R
11	Rhynchophyllic acid	III	CH2CH3	H	R	-	R
13	Rhynchophylline	III	CH2CH3	CH_3	R	-	R
14	Corynoxeine	III	$CH=CH_2$	CH_3	R	-	R
17	Villocarines D	III	$=C-CH_3$	CH_3	R	-	R
18	Isorhynchophylline	III	CH_2CH_3	CH_3	S	-	R
19	Rhynchophylline N-oxide	III	CH_2CH_3	CH_3	R	-O	R
26	Isocorynoxeine N-oxide	III	$CH=CH_2$	CH_3	S	-O	R
29	Corynoxeine N-oxide	III	$CH=CH_2$	CH_3	R	-O	R
31	Isorhynchophylline N-oxide	III	CH_2CH_3	CH_3	S	-O	R

Table 2. Identification of 32 compounds from the UR by developed UHPLC--ESI−Q-TOF-MS.

Peak No.	Rt min	Formula	MDF (Da)	Mass defect tolerance (Da)	Experiment mass (m/z)	Error ppm	Product ions (m/z)	Identification
1	9.42	C27H34N2O9	0.2264	+0.0051, -0.01	531.2327 [M+H]+	1.90	369	22-O-demethyl-22-O-β-D -glucopyranosylisocorynoxeine
2	10.76	C21H24N2O4	0.1736	+0.01, -0.0051	369.1813[M+H]+	-1.12	351, 319, 267, 201, 160	18,19-Dehydrocorynoxinic acid
3	16.44	C21H24N2O4	0.1736	+0.01, -0.0051	369.1813[M+H]+	-1.12	351, 319, 267, 210, 160	18,19-Dehydrocorynoxinic acid B
4	16.89	C21H26N2O3	0.1934	+0.0041, -0.0009	355.201[M+H]	1.74	224, 212, 144, 108	yohimbine
5	18.27	C21H26N2O3	0.1934	+0.0041, -0.0009	355.201[M+H]+	1.74	338, 251, 224, 212, 169, 144, 108	allo or epiallo yohimbine
6	18.93	C28H34N2O11	0.2163	+0.0063, -0.0050	575.2234[M+H]+	0.24	558, 413, 396, 381, 343, 311, 231, 188, 144	5α-carboxystrictosidine
7	19.52	C21H26N2O4	0.1893	+0.0051, -0.0041	371.1965[M+H]+	0.1	353, 269, 212, 160	Isorhynchophyllic acid
8	20.11	C27H34N2O10	0.2213	+0.0050, -0.0051	547.2295[M+H]+	-1.60	385, 367, 144	3β-Dihydrocadambine
9	22.90	C22H26N2O4	0.1893	+0.0051, -0.0041	383.1966[M+H]+	-0.17	351, 267, 201, 160	Isocorynoxeine

10	24.30	C22H26N2O4	0.1893	+0.0051, -0.0041	383.1961[M+H]+	1.14	365, 351, 224, 184, 160	Gambireine
11	24.94	C21H26N2O4	0.1893	+0.0051, -0.0041	371.1962[M+H]+	0.91	267, 224, 212, 174, 160	Rhynchophyllic acid
12	25.46	C27H34N2O9	0.2264	+0.0051, -0.5541	531.2339 [M+H]+	0.77	514, 369, 352, 334, 144	Glabratine
13	25.48	C22H28N2O4	0.2049	+0.0047, -0.0051	385.2123[M+H]+	-0.3	353, 241, 160	Rhynchophylline
14	25.70	C22H26N2O4	0.1893	+0.0051, -0.0041	383.1958[M+H]+	1.93	351, 319, 267, 160	Corynoxeine
15	27.39	C21H26N2O3	0.1934	+0.0041, -0.0009	355.201[M+H]+	1.74	251, 224, 212, 158, 144	β-yohimbine
16	28.54	C21H24N2O3	0.1787	+0.0051, -0.0035	353.1862[M+H]+	0.76	336, 311, 289, 273, 251, 222, 210, 170, 144, 108	Isomer of ajmalicine (allo or epiallo type)
17	29.15	C22H26N2O4	0.1893	+0.0051, -0.0041	383.1958[M+H]+	1.93	353, 269, 160	Villocarines D
18	29.37	C22H28N2O4	0.2049	+0.0047, -0.0051	385.2114[M+H]+	2.05	353, 269, 160	Isorhynchophylline
19	30.19	C22H28N2O5	0.1998	+0.0055, -0.0004	401.2067[M+H]+	0.99	385, 239	Rhynchophylline N-oxide
20	30.52	C21H24N2O3	0.1787	+0.0051, -0.0035	353.1859[M+H]+	0.19	336, 292, 251, 222, 210, 170, 144, 108	Isomer of ajmalicine (allo or epiallo type)
21	33.37	C21H24N2O3	0.1787	+0.0051, -0.0035	353.1859[M+H]+	0.19	321, 222, 210, 178, 144, 108	Ajmalicine
22	35.97	C27H34N2O10	0.2213	+0.0050, -0.0051	547.2282[M+H]+	0.78	385, 367, 144	3α-Dihydrocadambine
23	37.02	C22H26N2O3	0.1943	+0.0009, -0.0055	367.202[M+H]+	-1.05	335, 251, 236, 224, 192, 170, 144, 129, 108	Geissoschizine methylether
24	40.92	C22H26N2O3	0.1943	+0.0009, -0.0055	367.202[M+H]+	-1.05	350, 335, 251, 236, 224, 170, 144, 129	Epiallo corynantheine
25	42.32	C22H26N2O3	0.1943	+0.0009, -0.0055	367.202[M+H]+	-1.05	335, 251, 236, 224, 170, 144	Hisuteine or corynantheine
26	42.34	C22H26N2O5	0.1842	+0.0035, -0.0051	399.1913[M+H]+	0.37	383, 365, 335, 282, 224, 160	Isocorynoxeine N-oxide
27	43.22	C21H26N2O3	0.1934	+0.0041, -0.0009	355.201[M+H]+	1.74	337, 224, 212, 170, 144	allo or epiallo yohimbine
28	45.59	C22H26N2O3	0.1943	+0.0009, -0.0055	367.202[M+H]+	-1.05	335, 298, 236, 224, 199, 170, 144	Hisuteine or corynantheine
29	45.66	C22H26N2O5	0.1842	+0.0035, -0.0051	399.1909[M+H]+	1.37	236, 224, 108, 383	Corynoxeine N-oxide

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30	47.40	C26H30N2O8	0.2002	+0.0004, -0.0047	499.2080[M+H]+	-1.01	337, 267, 171, 144	Strictosamide
31	47.57	C22H28N2O5	0.1998	+0.0055, -0.0004	401.2067[M+H]+	0.99	385, 280, 239, 226, 208	Isorhynchophylline N-oxide
32	47.60	C22H28N2O3	0.2100	+0.0051, -0.0063	369.2166[M+H]+	1.81	337, 226, 144	Dihydrocorynantheine or hirsutine

Experimental

Chemicals

Solvents used for plant extractions were of analytical grade (SDS, Peypin, France). Formic acid and acetonitrile of HPLC grade were purchased from Tedia (America) and Fisher Scientific (Pittsburgh, PA, USA). Water for all preparations were deionized and further purified by a Mill-Q Plus water purification system (Millipore, Milford, MA, USA) and then was filtered through a 0.22 µm filter prior.

Plant material

The plant material was provided by Pharmacy Institute of Tianjin University of Chinese Medicine. Species identification was confirmed by Prof. Tianxiang Li, Tianjin University of Chinese Medicine, China.

Sample preparation

Air dried powder of UR (0.4g) was accurately weighed and extracted with 80% (v/v) methanol (10 mL) at room temperature for 40 min. The solution was allowed to stand for 24 hours. A volume of 500 µL of upper layer was diluted with 500 µL water, filtered through a 0.45 µm membrane filter and then centrifuged at 14,000 rpm for 10 min before UPLC analysis.

UPLC

Analyses were performed on an Agilent 1290 UHPLC instrument (Agilent Corp., Milford, MA, USA) consisting of a binary pump, a diode array detector (DAD), an auto-sampler and a column thermostat. The samples were separated on a Waters Acquity BEH C18 column (150 mm × 2.1 mm, 1.7 µm) connected to a Waters Van Guard BEH C_{18} Guard column (2.1 × 5 mm, 1.7 µm). The column temperature was set at 25°C. The mobile phase consisted of CH_3CN (solvent B) and H2O containing 0.1% formic acid (v/v) (solvent A). Linear gradients between the time points were as follows: 0 min, 3% B; 5 min, 10% B; 20 min, 15% B; 30 min, 18% B; 35 min, 20% B; 45 min, 36% B; 50 min, 40% B; 55 min, 70% B; 60 min, 100% B. The injection volume was 5 µL and the flow rate was set at 0.4 mL/min. The column effluent was monitored at a range of 254 to 280 nm with the acquisition of full spectra.

Spectrophotometry

An Agilent 6520 Q-TOF mass spectrometer (Agilent Corp., Santa Clara, CA) was connected to the Agilent 1290 UPLC instrument via electrospray ionization (ESI) interface. Detection was performed in positive mode in the m/z range of 100 to 1700, with an acquisition time of 1.4 s in centroid mode. The ESI conditions were as follows: capillary voltage, 3500 V; collision chamber voltage, 16 V; fragment or voltage, 175 V; capillary temperature, 200°C; dry gas (nitrogen), 8.0 L/min at 35°C; gas flow rate, 10 mL/min and nebuliser, 35 psi. Typical chromatograms are shown in (**Figure 2**).

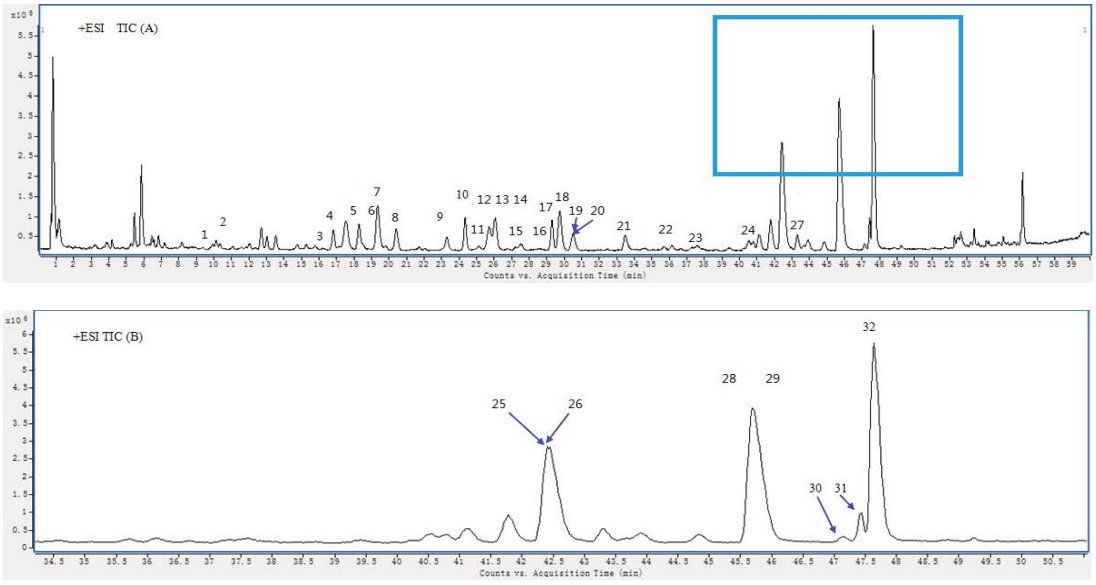

Figure 2. The total ion current (TIC) chromatogram of *Uncaria rhynchophylla* in positive mode (A-B).

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MDF function module and CDFI strategy

MDF method was operated on a function module provided by Masshunter Qualitative Analysis Software V.3.0 (Agilent Corp., Santa Clara, CA). We used to choose the function of molecular feature extraction to find the corresponding compounds directly, however some minor components of low response were submerged. The mass defect filtering plug-in enabled us to get desired mass defect by entering the formula of target compounds. Meanwhile, we need to set mass defect tolerance equal to the difference of adjacent mass defect value of representative formula for compounds in each category to narrow the scope of candidates. In general, the obtained TIC which was extracted by precursor ions included all peaks with those ions. The method was optimized to filter out target compounds with the same formula through adding three parameters of mass defect.

As another theoretical basis of this strategy, it is well known that compounds with the same core substructure always produced similar MS fragmentation ions which were used to rapidly identify a same family of compounds [10]. In this study, the diagnostic fragmentation ions were all from the published literatures.

According to the theory above, the first step was to establish a compound library based on all structures of the published components. The second step was to preliminarily screen the total ion chromatograms (TIC) on the mass defect filtering (MDF) level showing a series of eligible accurate mass weight and related retention time. The third step was to import the previous processing results into the compound library and then search for the potential target ingredients. The last step was to ensure the specific configuration of compounds by comparing the diagnostic fragmentation ions with possible target components fragmention ions which were extracted on MS2 level (**Figure 3**).

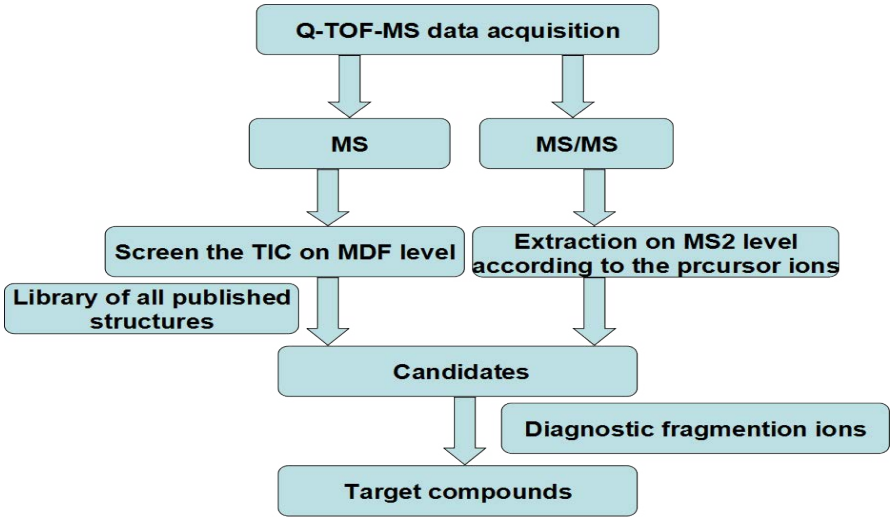

Figure 3. The procedure of MDF and CDFI Integration strategy.

Results and discussion

The UR alkaloids had primarily been classified as monoterpene alkaloids, two terpene indole alkaloids and sesquiterpene indole alkaloids on the basis of common core structure of tryptamine prephenate formaldehyde. The vast majority of monoterpene alkaloids were divided into pentacyclic (I), tetracyclic heteroyohimbine (II) and corresponding oxindole types (III) (**Figure 1**) in view of the ring system and the bonding order of tryptamine prephenate formaldehyde [11].

Taking peaks 4, 5, 15 and 27 as an example to explain the application of the completed integrated strategy. First of all, obtain a list of accurate mass weight and related retention time of four candidates. We set three parameters as followed: formula ($C_{21}H_{26}N_2O_3$), desired mass defect (0.1934) and mass defect tolerance (+0.0041 to -0.0009) in method editor mentioned before. Compared with the compound database, a range of potential ingredients within the permissible error limits (<10 ppm) were selected. Finally, compound 4, 5, 15 and 27 all showed the same [M+H]+ ion at m/z 355, which gave a series of fragment at m/z 144 with lesser signals at m/z 212 and 224 (**Figure 4**). According to the literature[12], these characteristic ions implied that the four components should belong to yohimbine type alkaloids. It was well known that yohimbine alkaloids were classified into four types as normal, pseudo, allo and epiallo according to the relative configurations of the C-3, C-15 and C-20 chiral centers [13]. In addition, a D/E cis-quinolizidine ring junction was pointed to the allo and epiallo yohimbine. MS spectrometry could be used to distinguish cis D/E from trans D/E isomers in the closed E ring heteroyohimbine series [14]. As a rigid structure, the Trans structure was stable than cis type which could produce configuration isomers by turning ring. Hence, the D/E ring of allo and epiallo isomers was easier to disconnect and exhibit m/z 169 or 170 fragment ion. Compound 5 and 27 were assigned as allo or epiallo type yohimbine, meanwhile, compound 4 and 15 should be yohimbine and β-yohimbine, which was the only other naturally occurring yohimbine indole alkaloid with the 'normal' conformation that had been reported [15].

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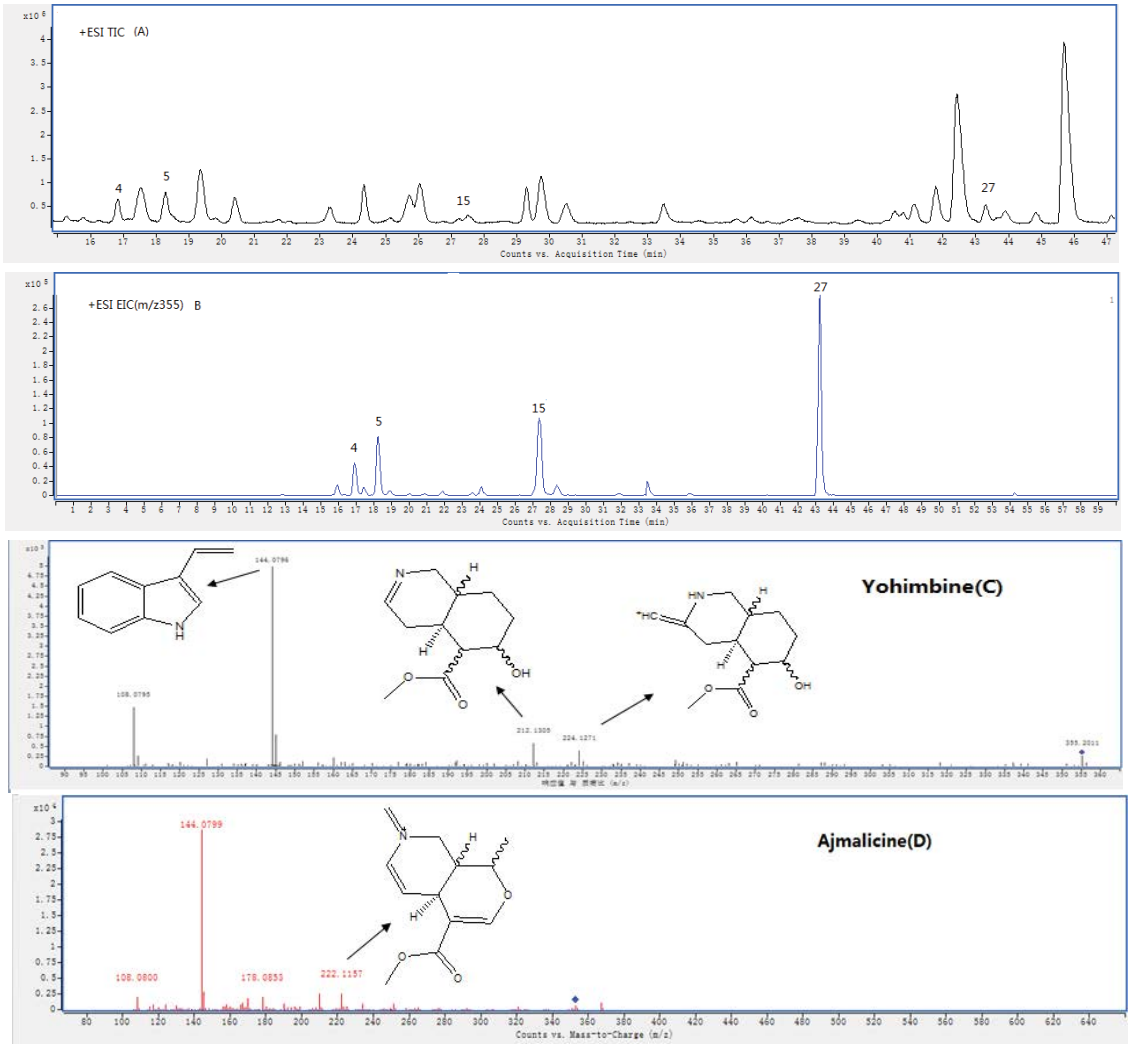

Figure 4. Partial enlarged (+ESI) TIC chromatogram of Uncaria rhynchophylla (A); extract ion chromatogram (EIC) at m/z 355 for MDF detection of yohimbine and its isomers (B). Characteristic diagnostic fragmentation ions of yohimbine (C) and ajmalicine (D).

Identification of other structures are complied with the above steps while setting parameters (formula, desired mass defect and mass defect tolerance) mentioned in **Table 1**, so only elaborated on the last step. Peaks 16, 20 and 21 showed the same [M+H]+ ion at m/z 353, which was 2 Da lighter than that seen for yohimbine and its isomers. Based on the literature [16], the diagnostic fragmentation ions at m/z 222, 210, 178 and 144 of compound 20 was proved to be ajmalicine (**Figure 4**). Ar-unsubstituted closed E ring alkaloids of the ajmalicine type had the same regulations with yohimbine type. Thus, compound 16 and 21 were identified as isomers of ajmalicine (allo or epiallo type).

When it came to the tetracyclic heteroyohimbine, compound 10 showed its [M+H]+ ion at m/z 383, which produced an ion at m/z 184, suggesting Gambireine [17]. Compound 23, 24, 25 and 28 all showed the same ions at m/z 367 and a characteristic product ion at m/z 224, 170 and 144. According to the literature [18], peak 24 was preliminarily judged as epiallo corynantheine due to its product ion m/z 170 was less intense than others. Then others were respectively identified as geissoschizine methylether, hisuteine or corynantheine owing to the differences of peak time [19]. Compound 32 showed the [M+H]+ ion at m/z 369 which not only had the same characteristic fragments at m/z 144 and 170 pointing out that the structure was belonged to corynantheine type alkaloids but also had other pieces that were 2 Da more than corynantheine. Therefore, compound 32 was speculated as dihydrocorynantheine or hirsutine.

The oxindole alkaloids can readily be distinguished from the heteroyohimbines since they produced a diagnostic ion at m/z 160 [8]. Moreover, a series of ions at [M+H-14]+, [M+H-Me+162]+ and [M+H+16]+ arose from the difference of C-15 (tetracyclic), C-16 (pentacyclic) or N-4 substituted groups (-COOH, -Glucose, N-Oxide).

Compounds 13 and 18 each showed the [M+H]+ ion at m/z 385 and a product ion at m/z 353 ([M+H-32]+) due to the loss of a methoxy group. It was found that compounds 13 and 18 were identified as rhynchophylline and isorhychophylline compared with their fragmentation and retention time between mass spectral data and literature [8]. Compounds 2 and 3 showed the [M+H]+ ion at m/z 369, they were respectively taken as 18, 19-dehydrocorynoxinic acid and 18, 19-dehydrocorynoxinic acid B according to the literature [20]. Compounds 7 and 11 each showed the same [M+H]+ ion at m/z 371, which was 14 Da lighter than that of rhynchophylline and isorhychophylline. Compounds 7 and 11 were thereby identified as isorhynchophyllic acid and rhynchophyllic

acid, respectively [8]. Compounds 9, 14 and 17 showed same [M+H]+ ion at m/z 383 and fragmentations at m/z 351, 267 which was 2 Da lighter than that of rhynchophylline and isorhychophylline. Thus, compounds 9, 14 and 17 were assigned as isocorynoxeine, corynoxeine [8] and villocarines D [21], respectively.

Similar UV spectra and MS fragmentation patterns were observed for compounds 19, 26, 29 and 31 which suggested they share a common skeleton. Compounds 26 and 29 showed the same [M+H]+ ion at m/z 399, implying the incorporation of an additional oxygen atom was inserted into the structure of isocorynoxeine or corynoxeine ([M+H]+ m/z 383). Compounds 19 and 31 showed a common [M+H]+ ion at m/z 401, suggesting the incorporation of an additional oxygen atom was inserted into rhynchophylline or isorhynchophylline ([M+H]+ m/z 385). Besides, it had been reported that the chromatographic behavior of N-oxides is akin to that of their non-oxide counterparts [7]. Therefore, according to the order of the retention times in the chromatograms, compounds 19, 26, 29 and 31 were tentatively identified as isocorynoxeine N-oxide, rhynchophylline N-oxide, corynoxeine N-oxide and isorhynchophylline N-oxide, respectively.

Compounds 8 and 22 both showed the [M+H]+ ion at m/z 547 which gave a series of ions at 385 and 367. The only difference between two candidates was 3α-Dihydrocadambine with a hydroxyl group and 3β-Dihydrocadambine with a hydroxymethyl group displayed as a seven-membered ring as screening from the database. According to the literature [22], the abundance of fragments at m/z 367 of 3β-dihydrocadambine was much higher than 3α-dihydrocadambine, as the seven-membered ring with a hydroxymethyl group was more stable than that with a hydroxyl group. Thus, compounds 8 and 22 were characterized as 3β-dihydrocadambine and 3α-isodihydrocadambine.

Compounds 1 and 12 showed the same product ion peak at m/z 369 ([M+H-162]+) corresponding to the loss of a hexose from a precursor ion at m/z 531 ([M+H]+) [23]. In addition, compound 12 provided a peak at m/z 144 which remarkably described to be a yohimbine type alkaloid. Therefore, compounds 1 and 12 were predicated as 22-O-demethyl-22-O-β-D-glucopyranosyl isocorynoxeine and glabratine.

Compound 6 showed its [M+H]+ ion at m/z 575, which was obtained only one candidate named 5α-carboxystrictosidine by the above steps. However, the researches focused on it were very few and limited on the synthesis [24] and pharmacology [25]. We concluded the fragmentation pathways of 5α-carboxystrictosidine consisted of secologanin and L-tryptophan [26] (**Figure 5**). Compound 30 showed its [M+H]+ ion at m/z 499 which produced a major fragment at m/z 337 due to the loss of a glucose. Besides, the parent structure and the fragment at m/z 337 could both produce 4, 9-dihydro-3H-beta-carboline at m/z 171 through further fragmentation [27]. Therefore, compound 30 was rapidly characterized as strictosamide.

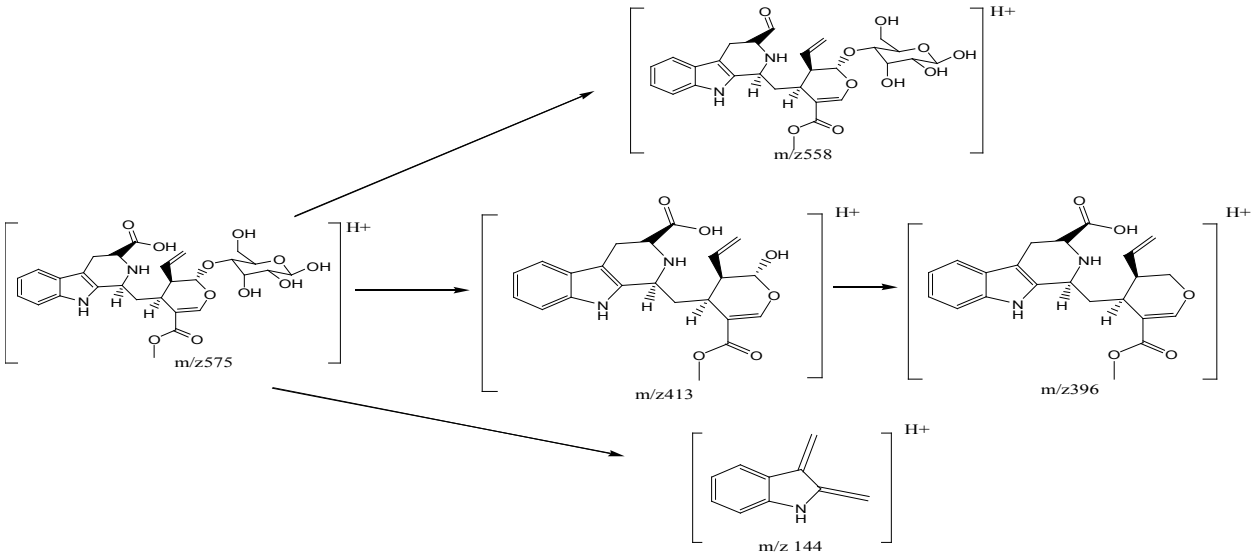

Figure 5. The possible fragmentation pathways of 5α-carboxystrictosidine.

This method showed a deep excavation of the qualitative software. A comprehensive utilization of MDF and diagnostic ions for rapid identification was established and applied to identify alkaloids in UR. Compared with traditional manual inspection, this method could be used for analyzing data more accurately by reducing the interferences of false positive results. Three ajmalicine type, four yohimbine type and five novel alkaloids had been identified except typical tetracyclic monoterpenoid oxindole alkaloids.

Conclusion

In this study, a comprehensive utilization of MDF and diagnostic ions for rapid identification was established and applied to identify alkaloids in UR. A total of thirty-two compounds were identified. Among them, twelve ingredients had never been reported in a qualitative way, including three ajmalicine-type (16,20,21), four yohimbine-type (4,5,15,27), 5α-carboxystrictosidine(6), gambireine(10), glabratine(12), villocarines D(17) and geissoschizine methylether(23) (**Figure 1, Tables 1 and 2**).

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