Comparative Study of Fourteen Alkaloids from *Uncaria rhynchophylla* Hooks and Leaves Using HPLC-Diode Array Detection-Atmospheric Pressure Chemical Ionization/MS Method

Jialin Qu,^a Tianxing Gong,^b Bin Ma,^a Lin Zhang,^a Yoshihiro Kano,^a and Dan Yuan^{*a}

^aSchool of Traditional Chinese Medicine, Shenyang Pharmaceutical University; 103 Wenhua Rd., Shenyang 110016, China; and ^bUniversity of British Columbia; 2329 West Mall, Vancouver, B.C. V6T 1Z1, Canada.

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The purpose of the study is to compare alkaloid profile of *Uncaria rhynchophylla* hooks and leaves. Ten oxindole alkaloids and four glycosidic indole alkaloids were identified using HPLC-diode array detection (DAD) or LC-atmospheric pressure chemical ionization (APCI)-MS method, and a HPLC-UV method for simultaneous quantification of major alkaloids was validated. The hooks are characterized by high levels of four oxindole alkaloids rhynchophylline (R), isorhynchophylline (IR), corynoxine (C) and isocorynoxine (IC), while the leaves contained high level of two glycosidic indole alkaloids vincoside lactam (VL) and strictosidine (S). The presented methods have proven its usefulness in chemical characterization of *U. rhynchophylla* hooks and leaves.

Key words oxindole alkaloid; glycosidic alkaloid; *Uncaria rhynchophylla*; alkaloid profile; HPLC; LC-atmospheric pressure chemical ionization-MS

China has an abundant resource of *Uncaria rhynchophylla* (MIQ.) JACKS. The hooks on this plant are used in the treatment of infantile convulsion, headache, dizziness, hypertension and stroke in Traditional Chinese Medicine and Japanese Kampo Medicine.^{1,2} In addition, the leaves, as a candidate part of the hooks,³ are used in the manufacture of an anti-hypertensive remedy in China.⁴ Oxindole alkaloids such as rhynchophylline (R) and isorhynchophylline (IR) are major components of *U. rhynchophylla*,⁵ responsible for the cardio-cerebral vascular effects, including hypotension,⁶ vasodilation,⁷ anti-platelet aggregation⁸ and protective effect against neuronal damage.⁹

Concerning quality assessment of *U. rhynchophylla* hooks, HPLC-UV method may be practical for determination of oxindole alkaloids due to the characteristic absorption of indole ring at 245 nm and 290 nm.¹⁰ The alkaloid profile of the hooks was studied using HPLC-UV technique in the previous literatures.¹¹ LC-MS technique is able to provide both analytical separation and structural determination of unknown bioactive compounds, which was applied to chemically characterize the oxindole alkaloids from *Uncaria tormentosa*¹² and the metabolites of R and IR.^{13,14} Yamanaka *et al.* determined ten tertiary alkaloids in various parts of *U. rhynchophylla*, indicating that the alkaloid profile of the leaves is similar to that of the hooks.¹⁵ Recently, we reported the isolation of several oxindole alkaloids and glycosidic indole alkaloids from *U. rhynchophylla* leaves, together with their inhibitory effects on microglial activation. R, IR, corynoxine (C), isocorynoxine (IC), strictosidine (S) and vincoside lactam (VL) are found to be potentially active.^{16–18} However, the chemical analysis of S and VL of *U. rhynchophylla* has not been reported yet.

In the present study, ten oxindole alkaloids and four glycosidic alkaloids from *U. rhynchophylla* hooks and leaves were identified by HPLC-diode array detection (DAD) and LC-MS technologies. Moreover, an accurate and sufficiently sensitive HPLC-UV method was validated to simultaneously determine these alkaloids. Utilizing optimized methods, the comprehensive alkaloid profiles of the hooks and leaves of *U. rhynchophylla* have been clarified.

Experimental

Chemicals and Reagents The standard compounds of fourteen alkaloids used in the qualitative and quantitative determination were isolated from *U. rhynchophylla* leaves in our previous studies.^{16,17} They included ten oxindole alkaloids, 18,19-dehydrocorynoxinic acid (DCA), 18,19-dehydrocorynoxinic acid B (DCAB), isocorynoxine *N*-oxide (IC-NO), corynoxine *N*-oxide (C-NO), isorhynchophylline *N*-oxide (IR-NO), rhynchophylline *N*-oxide (R-NO), IC, IR, C and R, and four glycosidic alkaloids, 11-hydroxy-2'-*O*- β -D-glucopyranosyl vincoside lactam (HGV), 22-*O*- β -D-glucopyranosyl isocorynoxine acid (GICeA), S and VL (see Fig. 1). The identity of these compounds was confirmed by melting point, UV, IR, ¹H- and ¹³C-NMR and MS.^{16,17} Their purities that were evaluated with HPLC-UV were more than 95%. Other chemical reagents were of analysis or HPLC grades. MQ-water daily prepared with a Milli-Q water purification system (Millipore, Molsheim, France) was used in the mobile phase.

Plant Materials Eight aerial samples were directly collected from some growing regions in China (see Table 6 for details), all of which were authenticated as *Uncaria rhynchophylla* (MIQ.) JACKS. by Professor Zerong Jiang, Shenyang Pharmaceutical University, China. Voucher specimens were kept in the reference library with the medicinal herbs at Shenyang Pharmaceutical University. The hook and leaf samples were separated from the aerial parts in our laboratory, respectively.

Instrumentation and Chromatographic Conditions Qualitative and quantitative analyses were performed on a Kromasil C₁₈ column (4.6×200 mm, 5 μ m, Tianjin Scientific Instruments Co., Ltd., China) at 45°C. The mobile phase consisted of a gradient system of solution A, methanol containing 0.03% diethylamine, and solution B, water containing 0.03% diethylamine, at the flow rate of 1.0 mL/min. The gradient program was as follows: solution A—B by linear gradient from 30:70 (v/v) to 36:64 (v/v) in 6 min, held at 36:64 (v/v)

* To whom correspondence should be addressed. e-mail: yuandan_kampo@163.com

Fig. 1. Alkaloid Compounds Identified in *U. rhynchophylla* Hooks and Leaves

in 8 min, by linear gradient to 45:55 (v/v) in 11 min, to 50:50 (v/v) in 25 min, and finally to 65:35 (v/v) in 35 min. The injection volume was 10 μ L.

Alkaloid identification by LC-MS was preformed on Shimadzu QP8000 α LC-MS spectrometer equipped with atmospheric pressure chemical ionization (APCI) interface (Shimadzu, Japan). APCI-MS was performed in the positive or negative modes under the following operating parameters: scanning mode: total ion current (TIC)/selective ion monitoring (SIM), scanning range (m/z): 100–1000, capillary voltage: 1.8 kV, CDL temperature: 250°C, APCI temperature: 400°C, flow rate of dry nitrogen: 2.5 L/min. The online UV spectra were obtained with a Waters HPLC system (Waters, U.S.A.) consisting of model 510 pump, automated gradient controller, model 2996 photodiode array detector, and Millennium32 software. They were recorded in the range of 200–400 nm.

Shimadzu LC-2010AHT HPLC system equipped with CLASS-VP workstation (Shimadzu, Japan) was used for quantitative determinations, and the detective wavelength was set at 245 nm.

Preparation of Standard Solutions Primary stock solutions of fourteen alkaloid references at a final concentration of 0.2 mg/mL were separately prepared by dissolving the accurately weighed reference compounds in MeOH-CHCl₃ (70:30). The stock solutions were then serially diluted until desired concentrations to obtain calibration curves. All of the solutions were stored at 4°C prior to use.

Sample Preparation The ground plant materials (0.3 g, passing through a 500 μ m mesh sieve) were double-extracted in MeOH-H₂O (70:30, 30 mL each) by sonication at room temperature for 20 min. The mixtures were centrifuged at 3000 rpm for 10 min. The combined supernatant and washing liquid were evaporated to dryness *in vacuo*. The residues were reconstituted in 10 mL of MeOH-CHCl₃ (70:30), and then filtered through a 0.45 μ m membrane filter prior to use.

Stability Trial The reference standards VL, ICO, IR, CO, and R in proper amount were dissolved in 10 mL of MeOH or MeOH-CHCl₃ (70:30) at the concentration of 0.1 mg/mL, respectively. Their stability was determined during 30-d storage

at 4°C. They were taken on day 8, 16, 24 and 30, and subsequently subjected to HPLC analysis. For the stability trial, the mobile phase consisting of the solutions A and B was run in isocratic mode in the ratio of 50:50 (v/v). Other HPLC conditions were the same as those described in the qualitative and quantitative determination.

The concentrations of five standard solutions were calculated using the following calibrations, R: $y=1.790 \times 10^7 x + 3.509 \times 10^5$ ($r=0.9993$), IR: $y=1.922 \times 10^7 x - 9.464 \times 10^4$ ($r=1.000$), C: $y=2.192 \times 10^7 x + 2.399 \times 10^4$ ($r=0.9993$), IC: $y=1.435 \times 10^7 x + 8.471 \times 10^5$ ($r=1.000$), VL: $y=1.457 \times 10^7 x - 1.885 \times 10^5$ ($r=0.9994$). The linear range for these calibrations was from 0.04 mg/mL to 0.16 mg/mL. The control data described as day 0 was obtained from the samples just before the stability trial.

Data Analysis Each sample solution was injected in triplicate, and the content of the analytes was calculated according to the corresponding calibration curves. The full identification of all marker compounds was performed by comparing the retention time, MS fragments, and UV spectrum of their peaks with corresponding standards.

Results and Discussion

Identification of Fourteen Alkaloids by HPLC-DAD and LC-APCI-MS Methods In order to characterize the major alkaloids from *U. rhynchophylla*, a hook samples (Sample #1) were subjected to HPLC-UV and LC-MS analyses (see Fig. 2). Fourteen specific peaks (labeled Peaks 1–14, Fig. 2) were characterized by typical UV absorptions of oxindole ring at 242–249 nm or indole ring at 281–296 nm. By direct comparison of the characteristic molecular ions, UV spectra as well as retention time with those of authentic standards, the alkaloids corresponding to Peaks 1–14 were identified as DCA, DCAB, HGVL, IC-NO, C-NO, IR-NO, R-NO, GICeA, S, VL, IC, IR, C and R, respectively (see Table 1). They were satisfactorily separated under the HPLC-UV condition. The detective wavelength for quantification of these alkaloids was chosen at 245 nm by comparison of the chromatograms of sample solutions at different wavelengths.

The APCI-MS analysis was operated in TIC and SIM scan

modes detecting positive or negative ions. As shown in Fig. 2, for the oxindole alkaloids with one basic nitrogen atom, the positive-ion mode produced a stronger ion current. Glycosidic alkaloids with acidic hydroxyl groups gave stronger negative ions. Overall, the signal intensity of all the alkaloids in LC-APCI-MS chromatograms was stronger than that achievable from UV detector. Since background level running in APCI-SIM mode is far less, SIM analysis allowed for minor alkaloids such as DCA, DCAB, IC-NO, C-NO, IR-NO, R-NO and

GICeA to be efficiently detected and measured.

Extraction of Marker Alkaloids In order to optimize the extraction of various alkaloids from the plant materials, we compared several extractive procedures. As shown in Table 2, a direct ultrasonic extraction for 20 min with MeOH–H₂O (70:30) resulted in a more effective extraction of IC and C and a similar extraction of VL, IR and R from *U. rhyncho-phylla* leaf (Sample #12), compare with the same extractive manner but with MeOH or MeOH–10% HOAc (70:30).¹⁹ Also a reflux extraction with MeOH–H₂O (70:30) resulted in similar extraction of major alkaloids to an ultrasonic extraction plus a successive reflux extraction with the same solvent. This result indicates that a great increase in the amount of VL, C and R resulted from the reflux extraction should be ascribed to the transformation or isomerization of the alkaloid compounds during the heat process.²⁰ A single ultrasonic extraction with MeOH–H₂O (70:30) is, therefore, preferred to oxindole and glycosidic indole alkaloids.

The isomerization of rhynchophylline-type alkaloids occurs at *spiro* center C-7 during the heat process, or in alkaline or polar solvents.²¹ In order to understand the stability of these alkaloid compounds and to select a proper solvent for inhibition of their isomerization, we undertook a stability trial for five reference standards VL, IC, IR, C and R. They were dissolved in MeOH or MeOH–CHCl₃ (70:30), respectively, and subsequently analyzed during 30 d of storage at 4°C. The representative HPLC chromatograms of the stability trial are shown in Fig. 3, and the course of the isomerization is depicted in Fig. 4. The content levels of R, IR, C and IC decreased 17.6%, 8.1%, 7.1%, 9.2% in MeOH, respectively, on day 30 after storage at 4°C, while they were rather stable in MeOH–CHCl₃ (70:30) under the same storage condition. The decomposition products of the four alkaloids, which were identified by their UV spectra as well as co-chromatographic procedures with the reference standards, were their respective 7-configurational isomers. The 7*S* isomers seem to be more stable than the 7*R* isomers. The result of stability trial is consistent with Laus's study.²¹ In addition, VL retained stable content level in both MeOH and MeOH–CHCl₃ (70:30). MeOH–CHCl₃ (70:30) is, therefore, a preferable solvent for these oxindole alkaloids.

Validation of HPLC Method Good linear calibration

Fig. 2. Chromatograms of Alkaloids Occurring in *U. rhyncho-phylla* Hooks (Sample #1)

(a) HPLC chromatogram detected at 245 nm. (b) and (c) TIC chromatograms in positive and negative ion modes. (d) and (e) SIM chromatograms in positive and negative ion modes. See Table 1 for the peak numbers, and see Experimental section for HPLC-MS conditions.

Table 1. The Alkaloids Identified from *U. rhyncho-phylla* Hooks and Leaves by HPLC-DAD and LC-APCI-MS Methods

Compounds (Peak No.)	<i>t_R</i> (min)	UV ($\lambda_{\max}$, nm)	Molecular ion (<i>m/z</i>)	MS approach
DCA (1)	10.6	210, 242	369.1 [M+H] ⁺	TICp, SIM
DCAB (2)	15.5	212, 242	369.1 [M+H] ⁺	SIMp
HGVL (3)	21.0	227, 296	675.0 [M–H] [–]	TICn, SIMn
IC-NO (4)	25.3	210, 247	401.1 [M+H] ⁺	TICp, SIM
C-NO (5)	26.0	210, 247	401.1 [M+H] ⁺	TICp, SIM
IR-NO (6)	28.1	210, 248	398.9 [M+H] ⁺	TICp, SIM
R-NO (7)	30.2	208, 243	399.1 [M+H] ⁺	TICp, SIM
GICeA (8)	34.7	210, 249	529.1 [M–H] [–]	TIC, SIM
S (9)	57.9	228, 281	528.8 [M–H] [–]	TICn, SIM
VL (10)	61.8	228, 282	497.0 [M–H] [–]	TICn, SIM
IC (11)	69.0	208, 245	383.1 [M+H] ⁺	TIC, SIM
IR (12)	73.1	209, 245	385.1 [M+H] ⁺	TIC, SIM
C (13)	77.9	209, 245	382.9 [M+H] ⁺	TIC, SIM
R (14)	84.1	210, 247	385.1 [M+H] ⁺	TIC, SIM

TIC: total ion current, SIM: selective ion monitoring, and p or n: in positive or negative mode.

Table 2. Optimization of Extraction of Major Alkaloids

Extractive procedures, solvents, time and temperature	Content (mg/g)				
	VL	IC	IR	C	R
Ultrasonic/20°C/20 min					
MeOH	2.62	1.07	1.02	0.58	0.56
MeOH–H ₂ O (70:30)	2.62	1.26	1.24	0.66	0.59
MeOH–10% HAC (70:30)	2.61	1.22	1.26	0.65	0.59
MeOH–H ₂ O (70:30)					
Reflux/90°C/20 min	4.39	0.98	1.05	0.86	0.92
Ultrasonic/20°C/20 min	2.62	1.29	1.23	0.69	0.59
+ reflux/90°C/20 min					

Sample #12 was used in the experiment.

curves and the linear range were obtained for all the alkaloid standards except for DCAB and C-NO (see Table 3). The *r* values were more than 0.999—1.000. The calibrations for

DCAB was not established because it was either absent or present in trace amounts in all the samples. Because of a great difference in the contents of S and VL between the hooks and leaves, two calibrations were established to serve for the low and high content levels.

The limit of detection (*S/N*=3) of these alkaloid compounds were *ca.* 0.09—0.28 µg/mL. The limit of quantitation (*S/N*=10) of these alkaloid compounds were *ca.* 0.31—0.74 µg/mL. The precision and recovery tests were done only for major alkaloid compounds HGVL, S, VL, IC, IR, C and R. This method had good reproducibility with intra-day variations (RSD) of less than 1.3% and inter-day variations (RSD) of less than 2.1% (see Table 4), respectively. Recovery data was obtained from the comparison of the responses between the test samples and the spiked samples. The recovery for the seven alkaloids ranged from 97.3 to 101.3%, with RSD ranging from 0.9 to 2.9% (see Table 5). Thus, this analytical procedure is accurate and sufficiently sensitive for the quantitative determination of major alkaloids from *U. rhynchophylla* hooks and leaves.

Quantification of Thirteen Alkaloids of *U. rhynchophylla* Hooks and Leaves Chemical profiling and quantification of the thirteen alkaloids from *U. rhynchophylla* hooks and leaves using HPLC-UV method were carried out. Fig. 5 presents HPLC chromatograms of the methanolic extracts of a hook and a leaf as well as a mixture of fourteen alkaloid standards. Under the chromatographic condition, DCA (peak 1) may not

Fig. 3. HPLC Chromatograms of Freshly Prepared MeOH–CHCl₃ (70:30) Solution of IC (a), C (c), IR (e) and R (g), and the Solutions on Day 30 of Storage at 4°C (b, d, f, h)

The four tested solution are at the concentration of 0.1 mg/mL. See Table 1 for the peak numbers, and see stability trial of Experimental for HPLC condition.

Fig. 4. Course of the Content Variations of VL, IC, IR, C and R in MeOH or MeOH–CHCl₃ (70:30) during 30 d Storage at 4°C

For stability trials, see Experimental.

Table 3. Calibration and Detection Limit for Twelve Alkaloids

Compounds	Calibration curve	<i>r</i>	Linear range (mg/mL)	LOD ($\mu\text{g/mL}$)	LOQ ($\mu\text{g/mL}$)
HGVL	$Y=1.073\times 10^7x-7.682\times 10^4$	0.999	4.30×10^{-3} — 1.70×10^{-1}	0.21	0.43
IC-NO	$Y=1.996\times 10^7x-5.788\times 10^3$	0.999	4.25×10^{-4} — 8.50×10^{-3}	0.13	0.39
C-NO	$Y=1.734\times 10^7x-3.477\times 10^3$	1.000	4.29×10^{-4} — 8.57×10^{-3}	0.12	0.37
IR-NO	$Y=1.963\times 10^7x-5.364\times 10^3$	1.000	3.80×10^{-4} — 7.60×10^{-3}	0.10	0.31
R-NO	$Y=1.097\times 10^7x-2.484\times 10^3$	0.999	1.29×10^{-3} — 5.10×10^{-3}	0.09	0.37
GICeA	$Y=6.654\times 10^6x-1.148\times 10^4$	1.000	1.05×10^{-3} — 4.20×10^{-3}	0.14	0.33
S	$Y=3.127\times 10^6x-2.790\times 10^4$	0.999	8.10×10^{-3} — 1.61×10^{-1}	0.28	0.63
	$Y=4.526\times 10^6x-3.417\times 10^5$	0.999	8.07×10^{-2} —1.61		
VL	$Y=1.657\times 10^7x-1.376\times 10^5$	0.999	8.80×10^{-3} — 1.75×10^{-1}	0.11	0.53
	$Y=1.372\times 10^7x-9.850\times 10^5$	0.999	8.75×10^{-2} —1.75		
IC	$Y=2.190\times 10^7x-2.923\times 10^4$	0.999	3.50×10^{-3} — 1.40×10^{-1}	0.16	0.48
IR	$Y=2.001\times 10^7x-1.061\times 10^4$	1.000	2.50×10^{-3} — 9.92×10^{-2}	0.23	0.68
C	$Y=2.306\times 10^7x-8.344\times 10^3$	0.999	1.70×10^{-3} — 6.60×10^{-2}	0.18	0.55
R	$Y=1.780\times 10^7x-1.378\times 10^3$	0.999	3.27×10^{-3} — 6.54×10^{-2}	0.28	0.74

LOD: limit of detection, LQD: limit of quantification.

Table 4. Intra- and Inter-Day Variations of the HPLC Method for Determination of Major Alkaloids

Compounds	Intra-day precision		Inter-day precision	
	Content ($\mu\text{g/g}$)	RSD (%)	Content ($\mu\text{g/g}$)	RSD (%)
HGVL	622 ± 4	0.57	620 ± 12	1.94
S	2305 ± 30	1.32	2291 ± 43	1.88
VL	3038 ± 33	1.10	3100 ± 66	2.12
IC	1933 ± 5	0.24	1923 ± 30	1.58
IR	1860 ± 7	0.37	1848 ± 24	1.29
C	655 ± 4	0.63	659 ± 13	2.00
R	741 ± 7	1.00	732 ± 14	1.92

Sample #12 was used in the experiment.

be precisely quantified due to lack of the perfect detection. We found that the four oxindole alkaloids (IC, IR, C and R) and two glycosidic indole alkaloids (S and VL) are major components of hooks and leaves (see Fig. 5, Table 6). However, the content ratio of the oxindole alkaloids to the glycosidic alkaloids in both parts is quite different from each other. In the hooks, the total amount of four oxindole alkaloids (2.60—13.82 mg/g) is much more than that of two glycosidic alkaloids (0.15—1.50 mg/g), while in the leaves, the total amount of the oxindole alkaloids (2.18—7.11 mg/g) is similar to that of the glycosidic alkaloids (3.73—7.16 mg/g). The data also demonstrated that two glycosidic indole alkaloids S and VL are in greater abundance in the leaves than in the hooks. Otherwise, DCAB, IC-NO, C-NO, IR-NO, R-NO and GICeA were not detected at all or detected in small or trace amounts in both the hooks and leaves. Several studies on HPLC analysis of oxindole alkaloids of *Uncaria* sp. are reported.^{22,23} Most of them focused on RIN and IRN. Yamanaka *et al.* reported the HPLC analysis of four alkaloids (including IC, IR, C and R) and six indole alkaloids (including corynantheine, akuanmagine, dihydrocorynoxine, hirsutine, hirsutine and geissoschizine methyl ether) in hooks and leaves of *U. rhynchophylla*,¹⁵ indicating that the pattern of the ten alkaloids in hooks is similar to that in leaves. Additionally, HPLC-DAD or HPLC-ESI/MS determination of six penta-cyclic oxindole alkaloids, isopteropodine, pteropodine, isomitraphylline, uncarline F, milraphyllin, and speclophylline as well as IC, IR, C and R in *U. tomentosa* was reported.²⁴ In our present study, two glycosidic oxindole alkaloids, S and VL, were determined for the first time due to

Fig. 5. HPLC Chromatograms of a Mixed Standard (a) and Typical Extracts of *U. rhynchophylla* Hooks (b, Sample #1) and Leave (c, Sample #2)

See Table 1 for the peak numbers, and see Experimental for quantification condition.

their existence in both hooks and leaves, together with four minor *N*-oxide oxindole alkaloids.

In our previous study, IC, IR, C, R, S and VL demonstrated

Table 5. Accuracy of the HPLC Method for Determination of Major Alkaloids

Compounds	Added (mg)	Observed (mg)	Recovery (%)	Mean (%)	RSD (%)
HGVL	0.1036	0.1018	98.3	99.5	1.6
	0.1036	0.1028	99.2		
	0.1036	0.1060	102.3		
	0.1036	0.1041	100.5		
	0.1036	0.1020	98.5		
	0.1036	0.1018	98.3		
S	0.3820	0.3984	104.3	100.5	2.9
	0.3820	0.3839	100.5		
	0.3820	0.3778	98.9		
	0.3820	0.3728	97.6		
	0.3820	0.3958	103.6		
	0.3820	0.3736	97.8		
VL	0.5080	0.4917	96.8	98.8	1.4
	0.5080	0.5095	100.3		
	0.5080	0.4999	98.4		
	0.5080	0.5034	99.1		
	0.5080	0.5095	100.3		
	0.5080	0.4978	98.0		
IC	0.3195	0.3125	97.8	98.8	1.6
	0.3195	0.3131	98.0		
	0.3195	0.3083	96.5		
	0.3195	0.3211	100.5		
	0.3195	0.3208	100.4		
	0.3195	0.3185	99.7		
IR	0.3333	0.3392	101.8	101.4	1.7
	0.3333	0.3369	101.1		
	0.3333	0.3341	100.2		
	0.3333	0.3486	104.6		
	0.3333	0.3361	100.9		
	0.3333	0.3332	100.0		
C	0.1067	0.1085	101.7	101.3	0.9
	0.1067	0.1093	102.4		
	0.1067	0.1082	101.4		
	0.1067	0.1068	100.1		
	0.1067	0.1085	101.7		
	0.1067	0.1069	100.2		
R	0.1217	0.1187	97.5	97.3	1.1
	0.1217	0.1204	98.9		
	0.1217	0.1170	96.1		
	0.1217	0.1179	96.9		
	0.1217	0.1191	97.9		
	0.1217	0.1176	96.6		

Sample #12 was used in the experiment.

the potent inhibitory activity against NO release from microglia, with the other minor alkaloids being of less or weak activity.^{16,17)} The comprehensive chromatographic profiles in this study demonstrate not only the similarity in the alkaloid composition but also the difference in quantities of the major components between the hooks and the leaves of *U. rhynchophylla*. Therefore, the leaves are a valuable candidate for the hooks due to the abundance in the active alkaloids as well as rich botanical nature.

Conclusions

The present study is the first report on HPLC-DAD and HPLC-APCI/MS methods for the chemical profiling of fourteen alkaloids from *U. rhynchophylla* hooks and leaves. Both $[M+H]^+$ ions of oxindole alkaloids and $[M-H]^-$ ions

of glycosidic alkaloids, obtained by APCI-MS determination, showed higher signal intensity than their peaks achievable with HPLC-UV method, thus allowing a more efficient identification of these alkaloids from *U. rhynchophylla*. Moreover, a validated HPLC method was successfully applied for simultaneous determination of them with good accuracy and precision. The results demonstrate the similarity and differences in alkaloids composition between *U. rhynchophylla* hooks and leaves.

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Table 6. Contents (mg/g) of fourteen alkaloids of different parts of *U. rhynchophylla*

Sample items	Collection region/date	Oxindole				Glycoside						N-Oxide				T _A		
		DCAB	IC	IR	C	R	T _O	HGVL	GlCeA	S	VL	T _G	IC-NO	C-NO	IR-NO		R-NO	T _{NO}
1 hook	Fujian/051011	n.d.	4.63	4.55	2.47	2.17	13.82	0.20	0.21	1.08	0.42	1.90	0.07	0.02	0.04	tr.	0.13	15.85
2 leaf		tr.	2.31	3.15	0.69	0.96	7.11	0.22	tr.	2.43	3.98	6.62	tr.	n.d.	tr.	0.15	0.15	13.87
3 hook	Hunan/040917	n.d.	4.52	3.95	2.18	2.21	12.86	0.15	tr.	0.57	0.35	1.08	tr.	tr.	tr.	tr.	tr.	13.95
4 leaf		tr.	2.24	2.43	0.75	0.80	6.22	0.48	n.d.	3.84	3.22	7.53	0.08	tr.	tr.	n.d.	0.08	13.83
5 hook	Hunan/040914	n.d.	1.59	2.38	1.05	1.73	6.75	n.d.	0.10	0.18	0.28	0.56	tr.	tr.	0.19	0.10	0.29	7.60
6 leaf		tr.	1.34	2.29	0.49	0.81	4.93	0.10	tr.	2.61	3.45	6.16	0.06	n.d.	0.07	tr.	0.13	11.22
7 hook	Hunan/041108	n.d.	0.66	0.99	0.40	0.76	2.80	0.11	tr.	0.13	0.11	0.35	tr.	tr.	0.15	0.12	0.27	3.42
8 leaf		tr.	0.94	1.36	0.35	0.69	3.34	0.33	0.05	1.30	2.56	4.25	tr.	tr.	tr.	tr.	tr.	7.58
9 hook	Jiangxi/041125	n.d.	1.40	1.37	0.75	1.48	5.01	0.12	tr.	0.51	0.26	0.89	0.05	0.02	tr.	tr.	0.07	5.97
10 leaf		n.d.	0.71	0.81	0.27	0.39	2.18	0.15	tr.	1.44	2.29	3.88	tr.	tr.	tr.	tr.	tr.	6.06
11 hook	Jiangxi/041021	n.d.	0.74	0.87	0.49	0.50	2.60	0.08	tr.	0.07	0.08	0.22	0.04	0.02	0.05	tr.	0.11	2.93
12 leaf		n.d.	1.93	1.89	0.65	0.74	5.21	0.62	n.d.	2.36	3.07	6.05	0.08	tr.	0.07	tr.	0.15	11.41
13 hook	Zhejiang/040929	n.d.	1.30	1.09	0.89	0.69	3.97	0.19	tr.	0.37	0.14	0.70	0.05	n.d.	0.06	tr.	0.11	4.77
14 leaf		tr.	2.11	1.96	0.82	0.72	5.61	0.86	tr.	2.79	3.60	7.25	n.d.	tr.	0.20	tr.	0.20	13.06
15 hook	Hubei/041016	n.d.	1.03	1.61	0.49	0.84	3.97	0.07	tr.	0.19	0.19	0.45	0.05	tr.	0.07	tr.	0.12	4.54
16 leaf		n.d.	1.53	2.48	0.46	0.84	5.31	0.09	tr.	3.24	3.92	7.25	0.05	n.d.	0.05	tr.	0.10	12.66

All the samples are botanically identified as *U. rhynchophylla*. T_A, T_O, T_G and T_{NO} represent the sum quantities of thirteen alkaloids, oxindole alkaloids, glycosidic alkaloids and alkaloidal N-oxides, respectively. n.d., not detectable (< limit of detection). tr., trace (> limit of detection, and < minimum of the linear range), as described in the section of method validation.

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