MALAYSIAN JOURNAL OF ANALYTICAL SCIENCES
Published by The Malaysian Analytical Sciences Society
ISSN 1394 - 2506

CHEMICAL PROFILING AND IDENTIFICATION OF ALKALOIDS AND FLAVONOIDS IN *Uncaria lanosa* var. *ferrea* VIA UHPLC-ORBITRAP MS

(Profil Kimia dan Pengenalpastian Alkaloid dan Flavonoid dalam *Uncaria lanosa* var. *ferrea* melalui UHPLC - Orbitrap MS)

Nursyaza Husna Shaharuddin[1, 2], Nor Hadiani Ismail[1, 2], Nurhuda Manshoor[1, 3], Fatimah Salim[1, 2], Rohaya Ahmad[1, 2]*

[1]*Faculty of Applied Sciences,*
Universiti Teknologi MARA, 40450 Shah Alam, Selangor, Malaysia
[2]*Atta-ur-Rahman Institute for Natural Products Discovery*
[3]*Faculty of Pharmacy*
Universiti Teknologi MARA, 42300 Bandar Puncak Alam, Selangor, Malaysia

**Corresponding author: rohayaahmad@salam.uitm.edu.my*

Received: 24 February 2015; Accepted: 27 October 2015

Abstract

Our previous studies on Malaysian *Uncaria* (Rubiaceae) have yielded over 20 compounds including alkaloids and flavonoids with some compounds showing interesting biological activities. In the search of new bioactive compounds from the genus, a phytochemical investigation on *Uncaria lanosa* (Wall.) locally known as “gegambir paya” or “gegambir hitam” was carried out via metabolite profiling. The plant is reported to be used as an infusion for intestine inflammation and a decoction for cleaning wounds and ulcers. Metabolite profiling was carried out using ultrahigh-performance liquid chromatography coupled with orbitrap mass spectrometry detectors (UHPLC-Orbitrap MS). Ten alkaloids and six flavonoids previously isolated from other *Uncaria* species were used as reference compounds. Phytochemical analysis of the stem extracts of the plant found the presence of a flavonoid and three alkaloids whose identities were obtained through the comparison of their mass and fragmentation pattern as well as the retention times of the reference compounds. The developed LC-MS method is expected to lead to a more rapid and reliable approach in discovery of new or novel compounds in *Uncaria* genus. The development of the MS database will also aid in the metabolite profiling of other medicinal plants in natural product research.

Keywords: *Uncaria*, *Uncaria lanosa*, metabolite profiling, UHPLC-Orbitrap MS

Abstrak

Kajian kami sebelum ini ke atas *Uncaria* (Rubiaceae) Malaysia telah menghasilkan lebih 20 sebatian termasuk alkaloid dan flavonoid dengan beberapa sebatian yang menunjukkan aktiviti biologi yang menarik. Dalam usaha mencari sebatian bioaktif baru dari genus ini, siasatan fitokimia ke atas *Uncaria lanosa* (Wall.) yang dikenali sebagai "gegambir paya" atau "gegambir hitam" telah dijalankan melalui profil metabolit. Tumbuhan ini dilapor digunakan sebagai infusi untuk radang usus dan sebagai rebusan untuk membersihkan luka dan ulser. Pemprofilan metabolit telah dijalankan dengan menggunakan kromatografi cecair ultra tinggi dengan pengesan spektrometri jisim orbitrap (UHPLC-Orbitrap MS). Sepuluh alkaloid dan enam flavonoid yang sebelum ini diasingkan daripada spesies *Uncaria* lain telah digunakan sebagai bahan rujukan. Analisis fitokimia ekstrak daripada batang tumbuhan tersebut menemui satu flavonoid dan tiga alkaloid yang dikenalpasti melalui perbandingan jisim, corak fragmentasi serta masa tahanan berbanding sebatian rujukan. Kaedah LC-MS yang dibangunkan dijangka membawa kepada pendekatan yang lebih pantas dan boleh dipercayai dalam penemuan sebatian baru atau novel dalam genus *Uncaria.* Pembangunan pangkalan data MS juga akan membantu pemprofilan tumbuhan ubatan lain dalam penyelidikan produk semulajadi.

Kata kunci: *Uncaria*, *Uncaria lanosa*, profil metabolit, UHPLC-Orbitrap MS

Introduction

Uncaria genus (Rubiaceae) which comprises 34 species worldwide has been used to treat various diseases including treatment of cancer, Parkinson's disease, Alzheimer's disease, chirrhosis, dizziness, hypertension, convulsion, asthma, rheumatism and many other diseases [1, 2]. Fourteen species are available in Malaysia. In our previous studies on Malaysian *Uncaria*, over 20 compounds including alkaloids and flavonoids have been isolated [3]. Some of the tested compounds have shown interesting biological activities. However, isolation of compounds using conventional chromatographic techniques can be tedious and time-consuming and may yield uninteresting or known compounds. In the search of new compounds from the genus, we have carried out a phytochemical investigation on *Uncaria lanosa* Wall which is locally known as "gegambir paya" or "gegambir hitam". The plant is reported to be used as an infusion for intestine inflammation and a decoction for cleaning wounds and ulcers [4]. According to Kam et al. [5], there are 14 species of *Uncaria* identified in Peninsular Malaysia and for Malaysian *Uncaria lanosa* var. *ferrea*, only two alkaloids, isopteropodine and pteropodine have been recorded.

Materials and Methods

Chemicals and raw materials

Stems of *U. lanosa var. ferrea* (Blume) Ridsd. with voucher specimen number HTBP4324 were collected from Pahang, Malaysia and standards were previously isolated from other *Uncaria* species. Ultrapure water was obtained from a PURELAB Ultra Laboratory Water Purification Systems and HPLC grade acetonitrile and methanol were purchased from Merck. Solvents used for fractionation and isolation were of industrial grade and were distilled prior to use.

Sample preparation

Pure compounds previously isolated from *Uncaria* extracts were dissolved separately in methanol. For sample preparation, dried and ground stems of *Uncaria lanosa* was extracted through 3-days maceration with methanol. The mixture was filtered and evaporated off under reduced pressure. The crude extract was further extracted by liquid–liquid extraction using two types of solvents; non-polar solvent (hexane) and medium polar solvent (dichloromethane, DCM). Each mixture was filtered separately and evaporated off under reduced pressure. 1 mg of DCM extract was weighed accurately and dissolved in 1 mL of methanol: water (30:70, v/v). Samples were filtered through a 0.22 µm PTFE filter into 2 ml screw cap vials prior to LC-MS analysis.

Instrumentation

UHPLC-Orbitrap MS was conducted using Thermo Scientific Q Exactive Hybrid Quadrupole – Orbitap Mass Spectrometer with ESI source system, a dual pump, an automatic injector (20 µL loop), a degasser unit and column heater. Data processing was performed using Xcalibur software.

High performance liquid chromatography

Samples were separated using GL Sciences - Inertsil Column C-18 (250 mm x 4.6 mm, i.d., 5µm) at a column temperature 40 °C. Flowrate was 0.6 ml/min and injection volume was 5 µl. After each injection the needle was washed with 100 % ACN. The mobile phase consisted of water containing 0.01 % formic acid (A) and acetonitrile (B). A gradient elution program was used as follows: 5 – 55 % B (0.00 – 30 min) and 55 – 95 % B (30 – 50 min).

Mass spectrometry condition for LC-MS

The LC/MS profiling of the extract and standards were identified using UHPLC-Orbitrap MS. Nitrogen was used as the sheath and auxiliary gas at flow rate of 45 and 25 ml/min, respectively. The optimum source (ESI) condition were: spray voltage 4.5 and 3.7kV for positive and negative respectively, S-Lens RF Level at 55.00, ESI capillary temperature 320 °C and probe heater temperature 30 °C.

Results and Discussion

Four chemical structures of compounds identified in DCM extract of *U. lanosa* var. *ferrea* was illustrated in Figure 1.

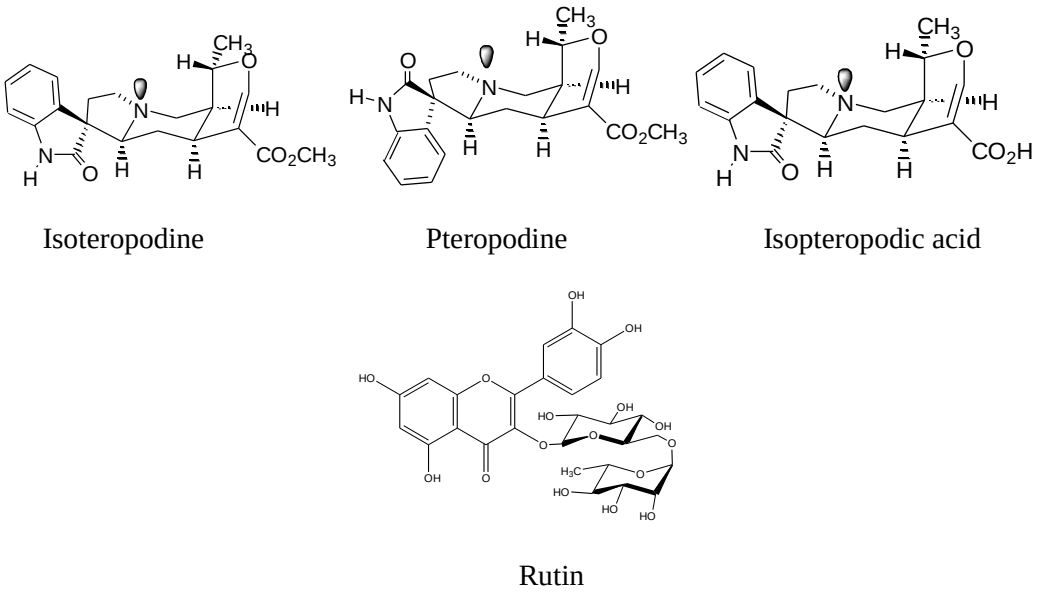

Figure 1. Chemical structures of compounds identified in DCM extract of *U. lanosa* var. *ferrea*

Development of database

The LC-ESI-MS profiles of previously isolated compounds from *Uncaria* species was added into MS database and percent error (ppm) were calculated. The LC-MS spectra of each compound was stored in the MS libary and to be used as reference for the identification of compounds from LC-MS analysis of crude extracts and fractions of *Uncaria* species. The LC-MS profiles are shown in Table 1 and Table 2, respectively.

Table 1. Characterization of reference compound by developed UHPLC-Orbitrap MS method for positive mode

No. of reference compounds	Compound (RT (min))	Proposed formula	Observed mass $[M+H]^+$	Calculated mass $[M+H]^+$	Error (Δ ppm)
1	Catechin (15.18)	$C_{15}H_{14}O_6$	291.08636	291.0868	-1.512
2	Formosaninol (15.19)	$C_{21}H_{26}N_2O_5$	387.19180	387.1920	-0.517
3	(-)Epi-catechin (16.49)	$C_{15}H_{14}O_6$	291.08646	291.0868	-1.168
4	Isoformaninol (16.52)	$C_{21}H_{26}N_2O_5$	387.19180	387.1920	-0.517
5	Rutin (18.27)	$C_{27}H_{30}O_{16}$	611.16058	611.1612	-1.014
6	(-)Epi-afzelechin (18.80)	$C_{15}H_{14}O_5$	275.09158	275.0919	-1.163
7	2-Oxosecologanine (20.31)	$C_{11}H_{15}NO_4$	226.10739	226.1079	-2.256
8	Pteropodine (21.37)	$C_{21}H_{24}N_2O_4$	369.18097	369.1814	-1.165
9	Isopteropodic acid (22.96)	$C_{20}H_{22}N_2O_4$	355.16537	355.1658	-1.211

Table 1 (cont'd). Characterization of reference compound by developed UHPLC-Orbitrap MS method for positive mode

No. of reference compounds	Compound (RT (min))	Proposed formula	Observed mass $[M+H]^+$	Calculated mass $[M+H]^+$	Error (Δ ppm)
10	Rauniticine-allo-acid B(22.97)	$C_{20}H_{22}N_2O_4$	355.16550	355.1658	-0.845
11	Uncariechin (24.88)	$C_{18}H_{14}O_6$	327.08624	327.0868	-1.712
12	Quercetin (27.27)	$C_{15}H_{10}O_7$	303.04993	303.0505	-1.881
13	Isopteropodine (29.83)	$C_{21}H_{24}N_2O_4$	369.18109	369.1814	-0.84
14	Uncarine F (29.92)	$C_{21}H_{24}N_2O_4$	369.18094	369.1814	-1.246
15	Speciophylline (29.98)	$C_{21}H_{24}N_2O_4$	369.18085	369.1814	-1.49
16	Rauniticine-allo-oxindole B (30.01)	$C_{21}H_{24}N_2O_4$	369.18079	369.1814	-1.652
10	Rauniticine-allo-acid B(22.97)	$C_{20}H_{22}N_2O_4$	355.16550	355.1658	-0.845

Table 2. Characterization of reference compound by developed UHPLC-Orbitrap MS method for negative mode

No. of reference compounds	Compound (RT (min))	Proposed formula	Observed mass $[M-H]^-$	Calculated mass $[M-H]^-$	Error (Δppm)
1	Catechin (15.13)	$C_{15}H_{14}O_6$	289.07184	289.0712	2.21
2	Formosanin-17-ol (15.18)	$C_{21}H_{26}N_2O_5$	385.17816	385.1764	4.57
3	(-)Epi-catechin (16.52)	$C_{15}H_{14}O_6$	289.07199	289.0712	2.73
4	Isoformosanin-17-ol (16.53)	$C_{21}H_{26}N_2O_5$	385.17810	385.1764	4.41
5	Rutin (18.26)	$C_{27}H_{30}O_{16}$	609.14783	609.1456	3.66
6	(-)Epi-afzelechin (18.83)	$C_{15}H_{14}O_5$	273.07706	273.0763	2.78
7	Longiflorine	$C_{11}H_{15}NO_4$	-	224.0923	-
8	Pteropodine (21.40)	$C_{21}H_{24}N_2O_4$	367.16754	367.1658	4.74
9	Isopteropodic acid (22.97)	$C_{20}H_{22}N_2O_4$	353.15201	353.1502	5.13
10	Rauniticinic-allo Acid B	$C_{20}H_{22}N_2O_4$	-	353.1502	-
11	Uncariechin (24.87)	$C_{18}H_{14}O_6$	325.07230	325.0712	3.38
12	Quercetin (27.22)	$C_{15}H_{10}O_7$	301.03546	301.0349	1.86
13	Isopteropodine (29.84)	$C_{21}H_{24}N_2O_4$	367.16760	367.1658	4.90
14	Uncarine F (29.91)	$C_{21}H_{24}N_2O_4$	367.16751	367.1658	4.66
15	Speciophylline (30.03)	$C_{21}H_{24}N_2O_4$	367.16751	367.1658	4.66
16	Rauniticine-allo-oxindole B (29.95)	$C_{21}H_{24}N_2O_4$	367.16739	367.1658	4.33

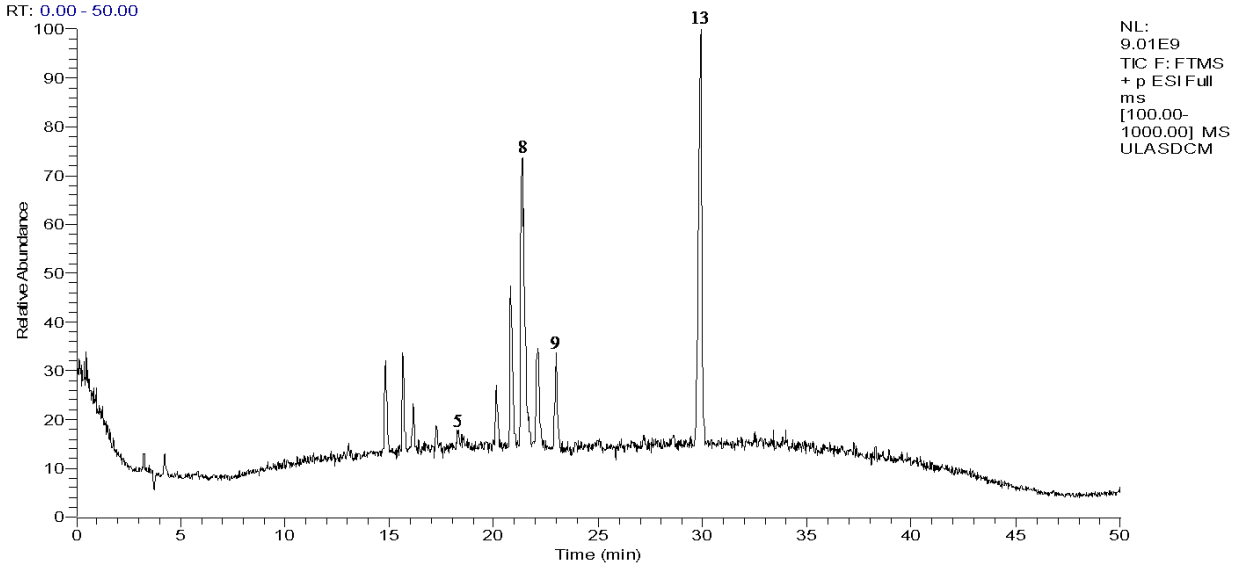

Figure 2**.** Chromatogram of DCM extract of *U. lanosa*; the total ion chromatogram (TIC) in positive mode; the number assigned to the identified peak is according to the number of the reference compound as shown in Table 1

Qualitative analysis of compounds in *Uncaria lanosa* extract

The UHPLC-Orbitrap MS method was employed for the metabolite profiling and identification of the chromatographic peaks of the DCM stem extract of *Uncaria lanosa*. From the analysis, the positive mode affords higher sensitivity for most of the peaks compared to the negative mode. All of the reference compounds can be detected in positive mode but two of the reference compounds cannot be detected in negative mode. Therefore this mode was chosen and applied in the analysis. By comparing the retention time and *m/z* value with the reference compounds, four peaks have been identified, including one flavonoid and three alkaloids which were unambiguously identified as rutin (5), pteropodine (8), isopteropodic acid (9) and isopteropodine (13). The structures of the identified compounds are shown in Figure 1. As shown in Figure 2, peak (5) shows a $[M+H]^+$ ion at 611.16058, peak (8) shows a $[M+H]^+$ ion at 369.18097, peak (9) shows a $[M+H]^+$ ion at 355.16537 and peak (13) shows a $[M+H]^+$ ion at 369.18109. It was found that the Orbitrap MS detection provided accurate molecular weight (±7 ppm). To date, metabolite profiling using mass spectrometry has only been reported for *Uncaria tomentosa* (cat's claw), *Uncaria rhynchophylla* and *Uncaria sinensis* [6 - 9].

Conclusion

In this paper, the proposed UHPLC-Orbitrap MS method was used for the qualitative analysis of alkaloids and flavonoids in *Uncaria lanosa*. Sixteen previously isolated compounds comprising six flavonoids and ten alkaloids were used as standards and were characterized by their retention time and *m/z* values. Four peaks in the extract of *U. lanosa* were identified as rutin, pteropodine, isopteropodic acid and isopteropodine. The developed LC-MS method is expected to lead to a more rapid and reliable approach in the discovery of new or novel compounds from the *Uncaria* genus.

Acknowledgement

This research was supported by Science Fund (MOSTI) (100-RMI/SF 16/6/2 (23/2012). NHS would like to thank to Ministry of Science, Technology and Innovation Malaysia and UiTM for financial support and fellowship award. We also would like to express our appreciation to Thermo Fisher Scientific Malaysia and Technology Park Malaysia for allowing access to UHPLC-Orbitrap MS.

References

1. Heitzman, M. E., Neto, C. C., Winiarz, E., Vaisberg, A. J. and Hammond, G. B. (2005). Ethnobotany, phytochemistry and pharmacology of *Uncaria* (Rubiaceae). *Phytochemistry*, 66: 5 – 29.
2. Zhang, Q., Zhao, J. J., Xu, J., Feng, F. and Qu, W. (2015). Medicinal uses, phytochemistry and pharmacology of the genus *Uncaria*. *Journal of Ethnopharmacology*. 173: 48 – 80.
3. Salim, F., Zain, M. M., Ridzuan, M. S. M., Langat, M. K., Mulholland, D. A. and Ahmad, R. (2013). Flavan-3-ols from the leaves of Malaysian *Uncaria longiflora* var. pteropoda (Miq.) Ridsd. *Phytochemistry Letters.* 6: 236 – 240.
4. Burkill, I. H. (1966). A Dictionary of the Economic Products of the Malay Peninsula. Vols 2. London: Crown Agents to the Colonies. (Reprint 1966, Kuala Lumpur. Ministry of Agriculture and Cooperative). 2236 – 2245.
5. Kam, T., Lee, K. and Goh, S., (1992). Alkaloid distribution in Malaysian *Uncaria. Phytochemistry*, 31: 2031 – 2034.
6. Pavei, C., Kaiser, S., Verza, S. G., Borre, G. L. and Ortega, G. G. (2012). HPLC-PDA method for quinovic acid glycosides assay in Cat's claw (*Uncaria tomentosa*) associated with UPLC/Q-TOF-MS analysis. *Journal of Pharmaceutical and Biomedical Analysis*. 62: 250 – 257.
7. Xie, S., Shi, Y., Wang, Y., Wu, C., Liu, W., Feng, F. and Xie, N. (2013). Systematic identification and quantification of tetracyclic monoterpenoid oxindole alkaloids in *Uncaria rhynchophylla* and their fragmentations in Q-TOF-MS spectra. *Journal of Pharmaceutical and Biomedical Analysis*. 81-82: 56 – 64.
8. Zhang, Y., Yang, W., Yao, C., Feng, R., Yang, M., Guo, D. and Wu, W. (2014). New triterpenic acids from *Uncaria rhynchophylla*: Chemistry, NO-inhibitory activity, and tandem mass spectrometric analysis. *Fitoterapia*. 96: 39 – 47.
9. Tan, S. N., Yong, J. W. H., Teo, C. C., Ge, L., Chan, Y. W. and Hew, C. S. (2011). Determination of metabolites in *Uncaria sinensis* by HPLC and GC–MS after green solvent microwave-assisted extraction. *Talanta*. 83: 891 – 898.