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Constituents from the Stems of Malaysian *Uncaria tomentosa*

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Abstract: *Uncaria tomentosa* (Willd. ex Schult.) DC. is well known as a medicinal plant that has been used for various treatments. This tropical vine grows up to 30 m tall and commonly found in natural forest. It has hook-like thorns that resemble the claws of a cat. The leaves are elliptic with a smooth edge, and grow in opposite whorls of two. Although there has not been any literature found on the availability of this species locally, we have discovered it in several parts of Peninsular Malaysia. A preliminary investigation of the stems of the plant has yielded three compounds, scopoletin, isopteropodine along with ß-sitosterol. The structures of the isolated compounds were characterized by analysis of spectral characteristics (mostly 1D, 2D NMR and mass spectrometry) and comparison with literature.

Keywords: *Uncaria tomentosa*, scopoletin, isopteropodine, pteropodine, β-sitosterol.

INTRODUCTION

Uncaria is a genus of flowering plants in the family of *Rubiaceae* and also described as a woody climber [1]. *Uncaria tomentosa* was given the name *una de gato* (cat's claw) because of hook-like thorns that resemble the claws of a cat. This plant has been used for various treatment including allergies, arthritis, asthma, cancer, chemotherapy side effects, contraception, disease prevention, fevers, gastric ulcers, inflammation, menstrual irregularity, recovery from child birth, skin impurities, urinary tract inflammation, viral infections, weakness, wounds and others [2]. Phytochemical studies of the Peruvian *U. tomentosa* have led to the isolation of numerous constituents including quinovic acid glycosides, oxindole alkaloid, proanthocyanidins, polyphenols, triterpenes and numerous sterols [3-5]. The extracts of the plant are also widely studied compared to other species of the genus and they have been found to display multiple biological activities including anti-inflammatory, antioxidant, cytotoxicity, immunostimulant and antibacterial [6]. Although there was no literature on the existence of the species in Malaysia, we have discovered the species in our forests. Hence, in the interest of the biochemical systematics of the *Uncaria* genus, this study reports on a preliminary phytochemical study of Malaysian *U.tomentosa.* The structures of the isolated compounds were characterized on the basis of their physical, chemical and spectral characteristics (1D, 2D NMR and mass spectrometry) and comparison with those reported in the literature.

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MATERIALS AND METHODS

General Procedures

Column chromatography was conducted using silica gel 60, 70-230 mesh ASTM (Merk 7734 packed by slurry packing method. Radial chromatography was carried out using glass plate with Merck's silica gel (Kieselgel 60 PF_{254} Merck Art 7749). Preparative TLC was performed using glass supported silica gel 60 F254 (1.0 mm thickness). TLC used to monitor the eluates was performed using pre-coated aluminium-backed supported silica gel 60 F254 (0.2 mm thickness). Identification of the spots on the TLC plate was carried out by spraying Dragendorff's reagent for alkaloid and *P*-anisaldehyde-sulfuric acid for terpenes, sterols and steroids. Spots and bands for compounds on TLC and PTLC were viewed individually under UV light (254 and 365 nm). The mass spectrum was recorded with Agilent1100 series LC/MS ion trap. The ultraviolet (UV) spectra were obtained in ethanol on a Shimadzu UV-Vis 160i. The infrared (IR) data was obtained on a Perkin Elmer model FT-IR spectrometer as KBr disc. NMR spectra were recorded with a Bruker 300 Ultra shield NMR spectrometer measured at 300 MHz.

Plant Material

The plant material was collected from Pasir Raja, Dungun, Terengganu in December 2011. The plant sample was identified by En. Shamsul Khamis of Universiti Putra Malaysia. The plant materials were air-dried in the dark at room temperature before being ground with a mechanical grinder.

Extraction and Isolation

The fine powders of the stems (5.0 kg) were sequentially extracted with hexane (non-polar), dichloromethane

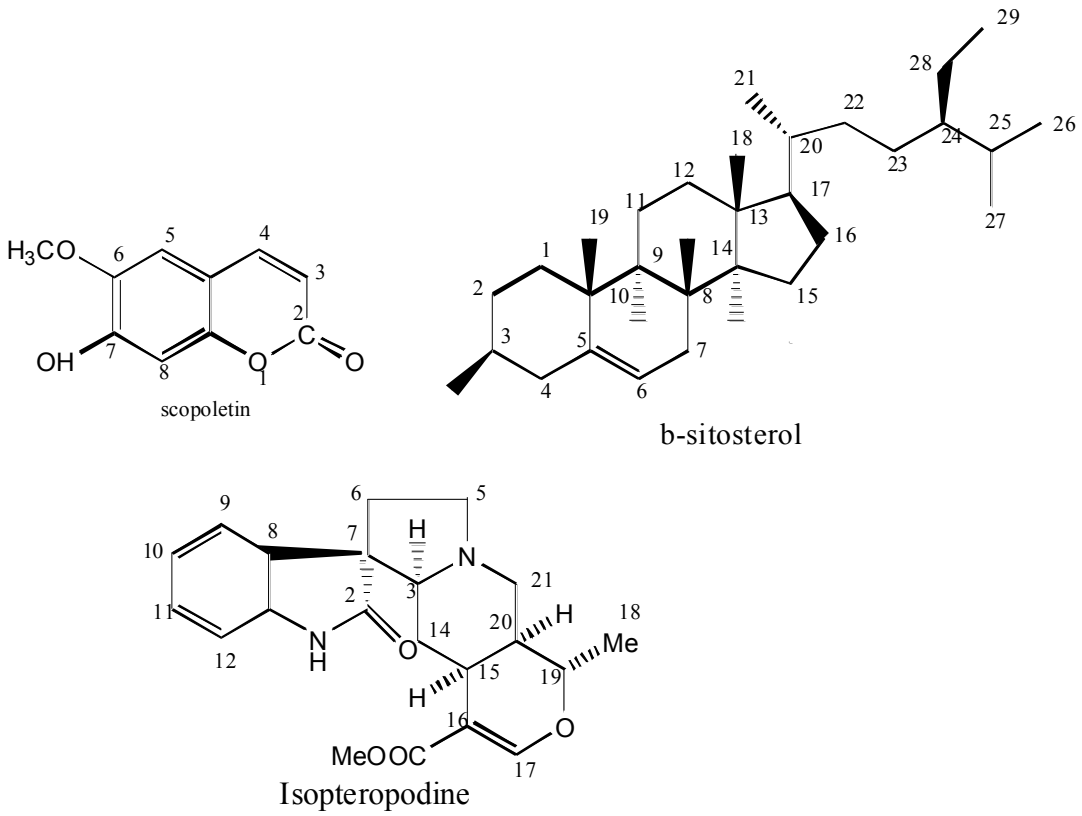

Fig. (1). The chemical structure of isolated compounds.

(medium polar) and 80% ethanol (polar) solvent by continuous hot extraction technique with a soxhlet extractor. The solvents were then evaporated to dryness under reduced pressure. The stems of this plant yielded an alkaloid mixture (3 g) and a non-alkaloid mixture (18g), by conventional acid-base extraction of the dichloromethane extract. The alkaloid and non-alkaloid mixture were then subjected to column chromatography over silica gel, eluted with hexane: dichloromethane (1:0-0:1) and subsequently dichloro-methane: methanol (1:0-0:1) yielding 30 and 9 fractions, respectively. Based on their TLC profiles, the fractions from the alkaloid mixture were combined together into 9 major subfractions (Sfr.1-Sfr.9). Further purification of Sfr 3 (0.006g) by preparative TLC on silica gel yielded compound 1 (2.7 mg). Subfraction 7 (0.04g) was subjected to radial chromatography using dichloromethane and increasing polarity with ethyl acetate to give compound 2 (15 mg). Column chromatography on the targeted fraction of non alkaloid mixture using hexane: dichloromethane (9.5:0.5) solvent system successfully yielded compound 3 (9.4 mg). The structure of isolated compounds was determined by (^{1}H and ^{13}C) NMR and mass spectral analysis as well as by comparing with the literature. Compounds 1, 2 and 3 were identified as scopoletin, isopteropodine and β-sitosterol.

RESULTS AND DISCUSSION

Repeated chromatographic techniques and purification on the dichloromethane extracts of the stems of *Uncaria tomentosa* afforded a total of three compounds, scopoletin (1); isopteropodine (2) and β-sitosterol (3). The structures were determined by extensive spectral analysis (mostly 1D, 2D NMR and mass spectrometry) as well as by comparison of their spectral data with the literature. The structures of the compounds are shown in Fig. (**1**).

Scopoletin (1) was obtained as whitish colorless needle and has a sweet odor. The mass spectral data of the compound showed molecular ion peak at *m/z* 192.0418 $[M+H]^+$, corresponding to the molecular formula $C_{10}H_8O_4$ which was supported by the ^{13}C NMR spectral data. The IR spectrum showed absorption at 3350 cm^{-1} (OH); 1670 cm^{-1}(OH); 1670 cm^{-1}(CO); 1615 cm^{-1}(C=C); 1564 cm^{-1} and 1449 cm^{-1}(aromatic C-C) [7]. ^{1}H NMR spectra (Table **1**) of the compound showed the presence of four aromatic protons (δ_H 6.22, 6.77, 7.86, and 7.18 ppm) also one methoxy group appeared at δ_H 3.92. The spectra were compared with published data of Zhang *et al.* [8] works. Ten carbons from ^{13}C-NMR showed the chemical structure of the compound (Fig. **1**).

Isopteropodine (2) was isolated as a white powder. This compound belongs to allo-group of heteroyohimbine-type oxindole alkaloid. The mass spectral data showed molecular ion peak at m/z 368$[M+H]^+$, suggesting a molecular formula of $C_{21}H_{24}N_2O_4$. The IR spectrum showed a strong NH absorption peak at 3368 cm^{-1} and two carbonyl absorptions at 1718 cm^{-1} (ester), 1657 cm^{-1} (amide) [9]. Examination of the ^{1}H NMR spectrum of the compound indicated the signals of 15-H appeared at δ 2.4-2.5 also the coupling constants between14 β-H and 15-H are in the range of 10Hz which is attributed to the allo-type alkaloid. Furthermore, it also revealed another characteristic of allo-group, which is the

Table 1. ^{1}H NMR Data for Compound 1-3

	Scopoletin (δH)		Isopteropodine (δH)		β-sitosterol (δH)	
Position	**Reference (Zhang, 2011)**	**Compound 1**	**Reference (Salim, 2010)**	**Compund 2**	**Reference (Md Abdul Muhit, 2010)**	**Compound 3**
1			7.83(*br,s*,1H,NH)	8.3 (*s*,1H,NH)		
2	6.21 (*d*,1H, *J*=9.3Hz)	6.22 (*d*, 1H, *J*=9.3Hz)	-	-		
3	7.92 (*d*,1H, *J*=9.3Hz)	7.86 (*d*,1H, *J*=9.3Hz)	2.60-2.34 (m)	2.59-2.32 (m)	3.51 (m,1H)	3.54 (m)
4			-	-		
5	7.21 (*s*,1H)	7.12 (*s*,1H)	*α* = 2.6-2.3 (m) *β*= 3.33-3.21(m)	*α*=2.59-2.3(m) *β*=3.3-3.2		
6			*α* = 2.01(m) *β* = 2.6-2.34(m)	*α*=2.00 (m) *β*=2.30 (m)	5.35 (m,1H)	5.37 (*t*,1H,*J*=5.4Hz)
7			-	-		
8	6.78 (*s,*1H)	6.79(*s*,1H)	-	-		
9			7.29 (*d*,1H, *J*=7.5 Hz)	7.29 (*d*, 1H, *J*=7.5Hz)		
10	3.87 (*s*,3H)	3.92 (*s*,3H)	7.04 (*ddd*,1H *J*=7.5,1.2Hz)	7.03 (*ddd,*1H, *J*=7.5,1.2Hz)		
11			7.21(*ddd*,1H, *J*=7.5,1.2 Hz)	7.2 (*ddd,* 1H, *J*=7.5,1.2Hz)		
12			6.88 (*d*,1H,*J*=7.5Hz)	6.89 (*d,* 1H, *J*=7.5Hz)		
13			-	-		
14			*α* = 1.67-1.69(m) *β* = 0.88 (m)	*α*=1.6 *β*=0.89		
15			2.6-2.34 (m)	2.48 (m)		
16				-		
17			7.43 (*s*,1H)	7.43 (*s* ,1H)		
18			1.43 (*d*,3H, *J*=6Hz)	1.43 (*d*,3H,Me, *J*=6Hz)	0.67 (*s*,3H)	0.75 (*s*,3H)
19			4.36 (*q*,1H,*J*=6Hz)	4.36(m)	1.0 (*s*,3H)	1.02 (*s*,3H)
20			1.67-1.69 (m)	1.59(m)		
21			2.60-2.34 (m)	*α*=2.4, *β*=3.3		
22			-	-		
23			3.62 (*s*,3H,OMe)	3.6 (*s*, 3H,OMe)		
24						

Table 1. contd….

	Scopoletin (δH)		Isopteropodine (δH)		β-sitosterol (δH)	
Position	**Reference (Zhang, 2011)**	**Compound 1**	**Reference (Salim, 2010)**	**Compund 2**	**Reference (Md Abdul Muhit, 2010)**	**Compound 3**
25						
26					0.81 (*d*,3H,*J*=7.4)	0.85 (*d*,3H, *J*=6.3Hz)
27					0.85 (*d*,3H,*J*=7.4)	0.88(*d*,3H,*J*=6.3Hz)
28						
29					0.91 (*d*,3H,*J*=7.4)	0.94 (*t*,3H,*J*=6.6Hz)

existence signal of 3-H at δ2.5-2.6 [10]. 1H NMR signals also observed at [δ 8.3 (1H, s, NH); (δ 7.42 (1H, s, H-17); δ 7.03 (1H, ddd, H-10); δ 7.2 (1H, ddd, H-11); δ 7.29 (1H, dd, H-9); δ 6.89 (1H, d, H-12)] which indicated the presence of an oxindole moiety [11]. The 13 C NMR spectrum data showed signals at [δ 181.25 (C2); δ140.96 (C13); δ127.58 (C11); δ122.04 (C10); δ 124.07 (C9); δ109.43 (C12); δ57.09 (C7); δ 133.69 (C8) which supported the characteristic of a heteroyohimbine-type oxindole alkaloid of this compound. The above spectral features are in close agreement to those observed for isopteropodine by Salim *et al*. (2010).

β-sitosterol (3) was isolated as colorless needles. The mass spectral data of the compound showed a molecular ion peak at m/z 414$[M+H]^+$, corresponding to the molecular formula $C_{29}H_{50}O$. For the IR spectrum data, the observed absorption bands are 33381 cm^{-1}, that is characteristic of O-H stretching; 2934-2833 cm^{-1}, is due to aliphatic C-H stretching; 1668 cm^{-1} resulted for C=C stretching and 1450 cm^{-1} is a bending frequency for cyclic CH_2 [12]. The ^1H NMR showed a multiplet peak at δ 3.54 which indicates H-3 proton of the steroid nucleus. It also revealed the existence of a signal for H-6 olefinic proton of the steroidal skeleton which appeared as a multiplet at δ 5.37. The spectrum showed signals at δ 0.75 and δ 1.03 (3H each) corresponding to the methyl groups connected to the C-13 and C-10, respectively. Moreover two doublets at δ 0.88 (J = 6.3 Hz) and δ 0.85 (J = 6.3 Hz) could be assigned to two methyl groups at C-25. The ^{13}C NMR spectrum data showed twenty nine carbon signal including six methyls, eleven methylenes, ten methine and three quaternary carbons. It also showed recognizable signals at δ 140.76 and δ 121.73 ppm which are attributed to C5 and C6 double bond respectively. Thus, the assignments are in close agreement for the structure of β-sitosterol in the literature [13].

CONCLUSION

A preliminary study of Malaysian *Uncaria tomentosa* stems extract has yielded pentacyclic oxindole alkaloid isopteropodine as the major compound as well as scopoletin and β-sitosterol isolated from dichloromethane extract. The structures of the isolated compounds were characterized on the basis of their physical, chemical and spectral characteristics (1D, 2D NMR and mass spectrometry) and comparison with those reported in the literature.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

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