

CHEMICAL CONSTITUENTS FROM TWO WEED SPECIES OF *SPERMACOCE* (RUBIACEAE)

(Kandungan Kimia daripada Dua Spesies Rerumput *Spermacoce* (Rubiaceae))

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Abstract

Spermacoce articularis and *Spermacoce exilis* are weeds commonly growing in wastelands but widely used as traditional medicines. The separation works on the two plant species had been carried out using various solvents and chromatographic methods. The structures of the isolated compounds were determined by using spectroscopic methods such as IR, MS, ¹H NMR, ¹³C NMR, 2D-NMR and by comparison with the data reported previously. Extracts from *Spermacoce articularis* gave two compounds, identified as ursolic acid (**1**) and stigmaterol, while extracts from *Spermacoce exilis* yielded four compounds, ursolic acid (**1**), benzo[g]isoquinoline-5,10-dione (**2**), stigmaterol and hexadecanoic acid (**3**). There was no previous phytochemical investigation on *Spermacoce exilis*.

Keywords : weed; *Spermacoce*; Rubiaceae; ursolic acid

Abstrak

Spermacoce articularis dan *Spermacoce exilis* adalah rerumput yang selalunya tumbuh di tanah terbiar tetapi banyak digunakan meluas sebagai ubatan tradisional. Proses pemisahan telah dijalankan ke atas dua spesies tumbuhan tersebut menggunakan pelbagai pelarut dan kaedah kromatografi. Struktur sebatian yang telah dipencilkan ditentukan dengan kaedah spektroskopi seperti IM, JS, RMN ¹H, RMN ¹³C, RMN-2D serta membandingkan dengan data yang dilaporkan sebelumnya. Ekstrak daripada *Spermacoce articularis* memberikan dua sebatian, dikenali sebagai asid ursolik (**1**) dan stigmaterol. manakala ekstrak daripada *Spermacoce exilis* memberikan empat sebatian, asid ursolik (**1**), benzo[g]isokuinolin-5,10-dion (**2**), stigmaterol and asid heksadekanoik (**3**). Tiada siasatan mengenai fitokimia bagi spesies *Spermacoce exilis* dilaporkan selama ini.

Katakunci: rerumput; *Spermacoce*; Rubiaceae; asid ursolik

Introduction

Rubiaceae is one of the larger plant families and comprises of some 650 genera and 10,500 species. Most of the members are mainly distributed in the tropical and subtropical regions, with a few exceptions in temperate regions. There are 80 genera and 555 species in the Malay Peninsular. Several famous members of this family of economic importance are coffee (*Coffea*) and quinine (*Cinchona*), as well as *Gardenia*, cultivated for its fragrant flowers. *Spermacoce* is a relatively large genus of herbs or half-shrubby plants. This genus consists of about 100 species distributed throughout the tropics. Several of them are united with *Borreria*, so that some of the Malayan species have synonyms [1].

Spermacoce articularis (shaggy button weed) is a diffused herb found in tropical Asia and Peninsular Malaysia. It is commonly seen growing in sandy wastelands often in the coastal areas. A poultice of this plant is used to heal leg ulcers, wounds, headaches, toothaches and found to be highly antioxidative [2]. Previous phytochemical studies on *S. articularis* originated from India indicated the presence of flavonoids [3], triterpenoids [4] and ursolic acid [5]. *Spermacoce exilis* is also found throughout Peninsular Malaysia. It commonly grows in wastelands and garden paths. It is used to treat headache, fever and ulcers. This herb contains coumarins and is used in Java for poultice. Despite all the applications in the medicinal plant practices, the chemical constituents of this plant have never been investigated. Thus, here we report the phytochemical studies on *Spermacoce articularis* and *Spermacoce exilis*.

Experimental

General

Melting points were determined using Kohfler melting points apparatus and were uncorrected. The IR spectra were recorded using KBr discs on Perkin Elmer FTIR spectrophotometer 1650. ¹H and ¹³C NMR spectra were obtained on JEOL spectrometer at 400 and 100 MHz, respectively with tetramethylsilane (TMS) as internal standard. Mass spectra were recorded on an AE1-MS12 spectrometer. Separation by column chromatography was carried out using silica gel (Merck 7749 and 9385), while silica gel 60PF₂₅₄ was used for TLC analysis.

Plant material

Both *Spermacoce articularis* and *Spermacoce exilis* were collected from Sungkai Reverse Forest in Perak in 2005 and identified by Mr. Shamsul Khamis, Institute of Bioscience (IBS), Universiti Putra Malaysia and voucher specimens were deposited at the IBS Herbarium. The voucher specimen numbers of *Spermacoce articularis* is SK 607/03 while *Spermacoce exilis* is SK 608/03.

Extraction and isolation

The finely ground air-dried *Spermacoce articularis* (600 g) was extracted with 90% ethanol for 72 hours to give 30.5 g of crude extract. The extract was partitioned with various solvents and concentrated under reduced pressure to give hexane (12.5 g), dichloromethane (3.75 g) and ethyl acetate (12.6 g) extracts. Ten gram of hexane extract was subjected to column chromatography eluted with mixtures of hexane, ethyl acetate and ethanol as eluents to give 25 fractions. Purification by trituration with MeOH of fraction 8 and fraction 12 gave stigmasterol (18 mg) and compound **1** (1.45 g), respectively. While the finely ground air-dried *Spermacoce exilis* (600 g) was similarly extracted with 90% ethanol for 72 hours to give 21.1 g of crude extract. The extract was partitioned with various solvents and concentrated under reduced pressure to give hexane (1.1 g), dichloromethane (4.5 g) and ethyl acetate (0.98 g) extracts. One gram of hexane extract was subjected to column chromatography eluted with mixtures of hexane, ethyl acetate and ethanol as eluents to give 41 fractions. Fraction 4 was further purified by using smaller scale column chromatography to give compound **2** (8 mg) and stigmasterol (23 mg). Recrystallization of solid obtained from fraction 7 gave compound **3** (15 mg). Further isolation work on 4 gram of dichloromethane extract gave compound **1** (0.30 g).

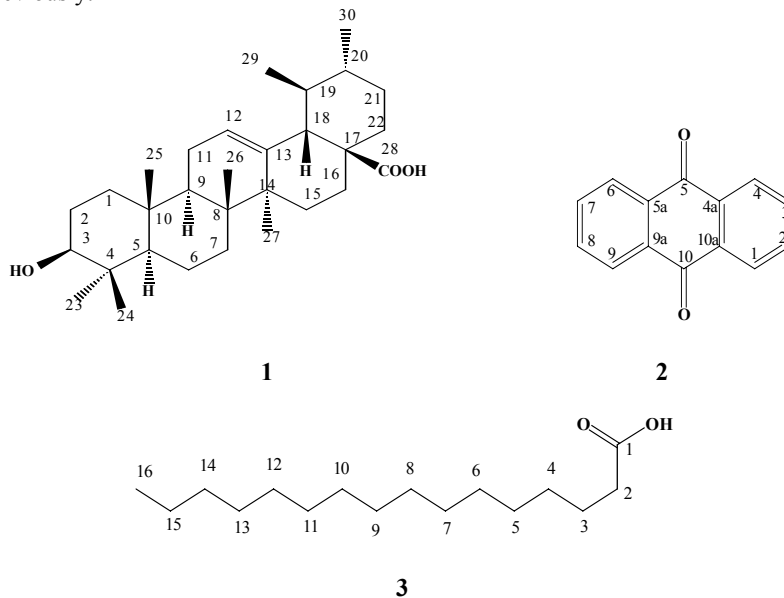
Ursolic acid (**1**); colourless crystals, C₃₀H₄₈O₃, m.p 270-272 °C (lit. [6], m.p. 276-279 °C). IR (cm⁻¹, KBr) ν_{max}: 3446, 2392, 1698, 1456, 1382, 1244, 1036, 998, 664. ¹H NMR (400 MHz, DMSO) δ: 5.14 (1H, *m*, H-12), 3.05 (1H, *dd*, *J*=11.0, 4.6 Hz, H-3), 2.11 (1H, *d*, *J*=11.9 Hz, H-18), 1.94 (1H, *m*, H-16α), 1.85 (2H, *m*, H-11), 1.83 (1H, *m*, H-15α), 1.59 (1H, *m*, H-22), 1.53 (1H, *m*, H-16β), 1.50 (2H, *m*, H-2), 1.49 (1H, *m*, H-6α), 1.47 (1H, *m*, H-9), 1.45 (1H, *m*, H-7α), 1.44 (1H, *m*, H-21α), 1.36 (1H, *m*, H-21β), 1.30 (1H, *m*, H-19), 1.27 (1H, *m*, H-6β), 1.26 (1H, *m*, H-7β), 1.03 (3H, *s*, H-27), 0.93 (1H, *m*, H-15β), 0.92 (1H, *m*, H-20), 0.88 (2H, *m*, H-1), 0.88 (3H, *d*, *J*=6.4 Hz, H-30), 0.87 (3H, *s*, H-23), 0.86 (3H, *s*, H-25), 0.83 (3H, *d*, *J*=6.4 Hz, H-29), 0.76 (3H, *s*, H-26), 0.69 (3H, *s*, H-24), 0.69 (1H, *d*, *J*=6.4 Hz, H-5). ¹³C NMR, (100 MHz, DMSO) δ: 181.7 (C-28), 139.6 (C-13), 126.9 (C-12), 79.7 (C-3), 56.7 (C-5), 54.4 (C-18), 49.2 (C-9), 48.6 (C-17), 43.2 (C-14), 40.8 (C-8), 40.4 (C-19), 40.4 (C-20), 40.0 (C-4), 39.8 (C-1), 38.1 (C-10), 38.1 (C-22), 34.3 (C-7), 31.8 (C-21), 29.2 (C-23), 28.8 (C-15), 27.8 (C-2), 25.3 (C-16), 24.3 (C-27), 24.0 (C-11), 21.6 (C-30), 19.5 (C-6), 17.8 (C-29), 17.7 (C-26), 16.4 (C-24), 16.0 (C-25). EI-MS *m/z* (% intensity): 456 (M⁺, 3), 438 (1), 423 (1), 248 (100), 219 (18), 203 (42).

*Benzo[*g*]isoquinoline-5, 10-dione* (**2**); green needle-shaped crystals, C₁₃H₇NO₂, m.p 178-180 °C (lit [7], m.p. 178-179 °C). IR (cm⁻¹, KBr) ν_{max}: 3582, 2920, 2850, 1678, 1578, 1302, 1142, 1022, 946, 922, 858, 792, 702. ¹H NMR (400 MHz, CDCl₃) δ: 9.61 (1H, *s*, H-1), 9.15 (1H, *d*, *J*=7.5 Hz, H-3), 8.37 (1H, *m*, H-9), 8.37 (1H, *m*, H-6), 8.11 (1H, *d*, *J*=7.5 Hz, H-4), 7.91 (2H, *m*, H-7/8). ¹³C NMR (100 MHz, CDCl₃) δ: 182.8 (C-10), 182.8 (C-5), 155.7 (C-3), 150.0 (C-1), 138.7 (C-4a), 135.3 (C-8), 134.9 (C-7), 133.3 (C-5a), 133.3 (C-9a), 127.7 (C-6), 127.6 (C-9), 126.6 (C-10a), 119.3 (C-4). EI-MS *m/z* (% intensity): 209 (M⁺, 100), 181 (57), 153 (99), 126 (71), 99 (12).

Hexadecanoic acid (**3**); white crystal, C₁₆H₃₂O₂, m.p 38-40 °C (lit [8], m.p. 38-40 °C). IR (cm⁻¹, KBr) ν_{max}: 3438, 2920, 2850, 1738, 1468, 1174, 724. ¹H NMR (400 MHz, CDCl₃): 2.32 (2H, *t*, *J*=7.3 Hz, H-2), 1.60 (2H, *m*, H-3), 1.27 (2H, *m*, H-15), 1.26 (22H, *br s*, H-4 to H-14), 0.85 (3H, *t*, *J*=7.3 Hz, H-16). ¹³C NMR (100 MHz, CDCl₃) δ: 179.2 (C-1), 34.0 (C-2), 31.9 (C-14), 29.0-29.7 (C-4 to C-13), 24.7 (C-3), 22.7 (C-15), 14.1 (C-16). EI-MS *m/z* (% intensity): 256 (M⁺, 44), 242 (2), 227 (88), 185 (22), 129 (67), 85 (42), 55 (100).

Results and Discussion

Extraction and fractionation works on the extracts of *Spermacoce articularis* and *Spermacoce exilis* have afforded ursolic acid (**1**) as the major constituent besides benzo[*g*]isoquinoline-5,10-dione (**2**), hexadecanoic acid (**3**) and stigmasterol. The structures of the compounds were elucidated using spectroscopic techniques. The isolations works on *Spermacoce articularis* and *Spermacoce exilis* originated from Perak, Malaysia have never been reported previously.



The EI-MS spectrum of compound **1** showed molecular ion peak at m/z 456 that corresponds to molecular formula $C_{30}H_{48}O_3$. IR spectrum showed the presence of hydroxyl group as broad peak at 3346 cm^{-1} , while peak at 1698 cm^{-1} revealed the existence of carbonyl group. Peak at 1382 cm^{-1} exhibited the presence of trisubstituted olefinic group. The ^1H NMR spectrum showed the presence of 48 protons. Higher field signals in the region of δ 0.69 to δ 2.11 indicated the characteristic of triterpene skeleton. An olefinic proton peak at δ 5.14 was assigned to H-12 which correlated to C-14 in HMBC spectrum. A signal represented H-3 was observed at lower field at δ 3.05 due to the attachment of a hydroxyl group to C-3. The COSY spectrum displays that H-15 α (δ 1.83) showed cross peak with H-16 α (δ 1.53) while H-15 β (δ 0.93) showed cross peak with H-16 β (δ 1.94). Besides that, the COSY spectrum also showed that H-2 coupled to H-3 and H-18 coupled to H-19. The ^{13}C NMR spectrum accounted of 30 carbons. The peaks at δ 181.7 indicated the presence of a carbonyl group, assigned as C-28. A pair of sp^2 carbon (C-12 and C-13) was indicated at δ 126.9 and 139.6. The prominent peaks at higher field of δ 29.2, 16.4, 16.0, 17.7, 24.4, 17.8 and 21.6 were attributed to methyl carbons of C-23, C-24, C-25, C-26, C-27, C-29 and C-39, respectively. Some of the important HMBC correlations were shown by the cross peak between methyl proton peaks at δ 0.87 (H-23) and δ 0.69 (H-24) with carbon signal at δ 40.0 (C-4). Proton signals at δ 0.83 (H-29) and δ 0.88 (H-30) also correlated to carbon signal at δ 40.4 (C-19 and C-20). The complete assignments of NMR data are displayed in Table 1. Thus, compound **1** was determined as ursolic acid [5]. Ursolic acid was previously isolated and elucidated from *Ilex paraguariensis* [9] and also reported as the main compound present in apple (*Malus domestica*) cuticular wax as well as in the leaves [10].

Table 1: NMR spectral data for ursolic acid (**1**)

Position, C	^{13}C (δ)	^1H (δ)	HMBC Correlation	COSY Correlation
1	39.8	0.88, <i>m</i>	C-2, C-5	
2	27.9	1.50, <i>m</i>	C-10	H-3

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3	79.7	3.05, <i>dd</i> , $J=11.0$, 4.6 Hz		H-2
4	40.0			
5	56.7	0.69, <i>d</i> , $J=6.4$ Hz		
6	19.5	1.27, <i>m</i> 1.49	C-10	
7	34.3	1.26, <i>m</i> 1.45	C-14, C-26	
8	40.8			
9	49.2	1.47, <i>m</i>	C-10, C-11, C-14	
10	38.1			
11	24.1	1.85, <i>m</i>	C-9, C-13	H-12
12	126.9	5.14, <i>m</i>	C-14	H-11
13	139.6			
14	43.2			
15	28.8	0.93, <i>m</i> 1.83	C-13, C-17	H-16
16	25.3	1.53, <i>m</i> 1.94	C-14	H-15
17	48.6			
18	54.4	2.11, <i>d</i> , $J=11.9$ Hz	C-13, C-17, C-19 C-14, C-20	H-19
19	40.4	1.30, <i>m</i>		H-18
20	40.4	0.92, <i>m</i>		
21	31.8	1.36, <i>m</i> 1.44		
22	38.1	1.59, <i>m</i>		
23	29.2	0.87, <i>s</i>	C-3, C-4, C-5, C-24	
24	16.4	0.69, <i>s</i>	C-4, C-3, C-5, C-23	
25	16.0	0.86, <i>s</i>	C-10, C-5	
26	17.7	0.76, <i>s</i>	C-7	
27	24.4	1.03, <i>s</i>	C-13	
28	181.7			
29	17.8	0.83, <i>d</i> , $J=6.4$ Hz	C-19, C-18, C-20	
30	21.6	0.88, <i>d</i> , $J=6.4$ Hz	C-20, C-19	

Compound **2** exhibited a molecular formula of $C_{13}H_7NO_2$ from its EI-MS. The IR spectrum exhibited the presence of C=O stretching peak at 1678 cm^{-1} , while 1578 cm^{-1} was due benzene skeleton ring. Peaks at 1302 and 1142 cm^{-1} indicated the presence of C=N and C-N groups. The ^1H NMR spectrum exhibited a total of seven protons. The aromatic region of the ^1H NMR spectrum indicated the presence of an A_2B_2 system in ring A. Multiplets appearing at δ 8.37 and δ 7.91 integrating each for two protons were attributed to H-6/9 and H-7/8, respectively. A set of doublet at δ 9.15 ($J=7.5\text{ Hz}$) and δ 8.11 ($J=7.5\text{ Hz}$) were due to *ortho*-coupled H-3 and H-4 protons, which also showed correlation in COSY spectrum. In addition, one singlet integrated for one proton at δ 9.61 was assigned to H-1. The ^{13}C NMR spectrum gave absorption peaks representing all aromatic and carbonyl carbons. The presence of two carbonyl groups were indicated by overlapping peak at δ 182.8 (C-5/10). The locations of the C=O were suggested by HMBC correlation between H-4 and H-6 with C-5, and between H-9 with C-10. Other aromatic carbons of rings A and C were represented by signals between δ 119.3 and 155.7. Among the prominent HMBC cross peaks representing 2J correlations were observed between H-6 and C-7, C-5a; H-7 and C-6; H-8 and C-9 and between H-9 and C-8, C-9a. The complete NMR data are summarized in Table 2. Based on the comparison of these spectral and physical data, compound **2** has thus been identified as benzo[*g*]isoquinoline-5,10-dione, which was previously isolated from *Mitracarpus scaber* [7]. This is the first report on the isolation of the compound from this plant species.

Table 2: NMR spectral data for benzo[*g*]isoquinoline-1,5-dione (**2**)

Position, C	^{13}C (δ)	^1H (δ)	HMBC Correlation	COSY Correlation
1	150.0	9.61, <i>s</i>	C-10a, C-3, C-4a	
3	155.7	9.15, <i>d</i> , $J=7.5\text{ Hz}$	C-4, C-1, C-4a	H-4
4	119.3	8.11, <i>d</i> , $J=7.5\text{ Hz}$	C-3, C-5, C-10a	H-3
4a	138.7			
5	182.8			
5a	133.3			
6	127.7	8.37, <i>m</i>	C-7, C-5a, C-5, C-8, C-9a	H-7
7	134.9	7.91, <i>m</i>	C-6, C-5a, C-9	H-6
8	135.3	7.91, <i>m</i>	C-6, C-9, C-9a	H-9
9	127.6	8.37, <i>m</i>	C-8, C-9a, C-7, C-10	H-8
9a	133.3			
10	182.8			
10a	126.6			

The EI-MS spectrum of compound **3** exhibited molecular ion at m/z 256, which corresponds to a molecular formula of $C_{16}H_{32}O_2$. The IR spectrum indicated the presence of asymmetric and symmetric methylene stretching bonds at 2920 and 2850 cm^{-1} , respectively. Two absorptions at 1738 and 1468 cm^{-1} represent carbonyl and methylene groups, respectively. The ^1H NMR spectrum showed the characteristic of aliphatic carboxylic acid at region between δ 0.80 to 2.40. The spectrum indicated the presence of a long chain methylene protons representing eleven methylene groups overlapped as broad peak at δ 1.26. The ^{13}C NMR spectrum showed the presence of seven peaks. The terminal methyl proton C-16 appeared at δ 14.1 attached to methylene carbon C-15 (δ 22.7). Another methylene carbon resonates at δ 31.9 (C-14), while methylene carbons C-14 and C-13 overlapped at between δ 29.7 – 29.0. The existence of carbonyl signal was observed at δ 179.2. The NMR

data are shown in Table 3. The structure of compound **3** was therefore characterized as hexadecanoic acid. This fatty acid had been reported as one of constituents that was isolated from *Lysimachia fordiana* [11].

Table 3: NMR spectral data for hexadecanoic acid (**3**)

Position, C	¹³ C (δ)	¹ H (δ)	HMBC Correlation
1	179.2		
2	34.0	2.32, <i>t</i> , <i>J</i> =7.3 Hz	C-1, C-3, C-4
3	24.7	1.60, <i>m</i>	C-1, C-2, C-4
4-13	29.0-29.7	1.26, <i>br s</i>	
14	31.9		C-15
15	22.7	1.27, <i>m</i>	C-13, C-14
16	14.1	0.85, <i>t</i> , <i>J</i> =7.3 Hz	

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