

## Alkaloid Constituents of *Haplophyllum laeviusculum* (Rutaceae) (Kandungan Alkaloid daripada *Haplophyllum laeviusculum* (Rutaceae))

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### ABSTRACT

The investigation of the aerial parts of *Haplophyllum laeviusculum* (Rutaceae), a perennial herb native to Iran, afforded three alkaloids, skimmianine (1), lunamarine (2), ribalinidine (3) and two common sterols,  $\gamma$ -sitosterol and campesterol. The structures of compounds were identified by using spectroscopic methods by using UV, IR, NMR, MS spectra and also by comparison with previous works. There has been no detail phytochemical investigation report on the plant carried previously. This is the first report on the identification of skimmianine (1), lunamarine (2) from genus *Haplophyllum*.

**Keywords:** *Haplophyllum laeviusculum*; lunamarine; ribalinidine; skimmianine

### ABSTRAK

Kajian terhadap bahagian atas *Haplophyllum laeviusculum* (Rutaceae), tumbuhan herba yang berasal dari Iran, telah menghasilkan tiga alkaloid, skimmianina (1), lunamarina (2), ribalinidina (3) dan dua sterol umum,  $\gamma$ -sitosterol and kampesterol. Struktur bagi sebatian ini telah dikenalpasti dengan menggunakan kaedah spektroskopi UV, IR, NMR dan SJ dan juga perbandingan dengan laporan kajian sebelum ini. Tiada kajian fitokimia terperinci telah dilakukan sebelum ini ke atas tumbuhan *H. laeviusculum*. Ini adalah laporan pertama kehadiran skimmianina (1), lunamarina (2) daripada tumbuhan genus *Haplophyllum*.

**Kata kunci:** *Haplophyllum laeviusculum*; lunamarina; ribalinidina; skimmianina

### INTRODUCTION

The genus *Haplophyllum* of the family Rutaceae made up of about 70 species worldwide. This genus grows naturally around the Mediterranean section of Europe and in Western Asia up to Siberia (Townsend 1986). Iran is an important autochthonous region for diversity of *Haplophyllum* species, where 30 species are found. The common Persian name of this plant is “Sodaby” (Mozaffarian 1996). Many species of this genus are used in traditional medicine for the treatment of herpes, warts, stomachache, erysipelas, toothache, skin diseases (Bessonova et al. 1989) and in the treatment of testicular cancer (Somphary et al. 2008). Previous work on *Haplophyllum* species revealed the presence of a number of aromatic compounds such as lignans (Gozler et al. 1996, Parhoodeh et al. 2011), coumarins, flavonoids (Yuldashev 2002) and several classes of alkaloids (Staerk et al. 2009). *Haplophyllum laeviusculum* is a perennial herb that is endemic in West Azarbaijan Province in Iran locally known as “Suddabby miyaneey”. There has been no phytochemical investigation previously undertaken on the plant. In continuation of our work on Rutaceous plants (Komala et al. 2006; Rahmani et al. 2003; Susidarti et al. 2009), we wish to report the identification of three alkaloids, skimmianine (1), lunamarine (2) and ribalinidine (3) together with two common sterols,

$\gamma$ -sitosterol (5) and campesterol (6) from the aerial parts of *Haplophyllum laeviusculum*.

### METHODS

#### GENERAL

All melting points were measured on a Kofler hot stage apparatus and are uncorrected. Infrared (IR) spectra were recorded using KBr discs or NaCl cell on Perkin Elmer FTIR spectrophotometer. Mass spectra were recorded by EIMS using a Direct Injection Probe in a Shimadzu GCMS-QP5050 spectrometer at 70 eV. The UV spectra were recorded on a Shimadzu UV-Vis 160A spectrophotometer. The  $^1\text{H-NMR}$  and  $^{13}\text{C-NMR}$  spectra were obtained on a JOEL ECA-400 spectrometer operating at 400 MHz and 100 MHz for  $^1\text{H}$  and  $^{13}\text{C}$  probes, respectively. Tetramethylsilane (TMS) was used as an internal standard and chemical shifts are shown in  $\delta$  values..

#### PLANT MATERIAL

The aerial parts of *Haplophyllum laeviusculum* were collected in West Azerbaijan Province, Iran in May 2008. Voucher specimen for the plant was deposited in the Shiraz University herbarium, Shiraz, Iran (No.3598). Collected plants were shade dried and powdered.

## EXTRACTION AND ISOLATION

The dried powder aerial parts of *Haplophyllum laeviusculum* (920 g) were soaked sequentially for 72 h with petroleum ether, ethyl acetate and methanol. The filtrated solvents were concentrated under reduced pressure by using rotary evaporator at a maximum temperature of 45°C to yield 12.2, 11.5 and 30 g crude extracts, respectively. The ethyl acetate extract (10.5 g) was initially subjected to column chromatography eluted with petroleum ether, chloroform, EtOAc and methanol of increasing polarity to give 89 fractions of 100 mL each. From fractions 19-25, the two common sterols,  $\gamma$ -sitosterol (5) (65 mg) and campesterol (6) (43 mg) were obtained as white crystals with m.p. 134-136°C and 155-157°C, respectively. Fractions 42-53 were combined washed and recrystallized from chloroform to give skimmianine (1) (3.35 g). Fraction 79-82 was further purified by column chromatography and eluted with mixture of chloroform, ethyl acetate and methanol to give white solid. The material was washed with hexane and recrystallised with chloroform to give white needle-shaped crystals (1.52 g) of lunamarine (2). Similar column chromatographic separation procedure was carried out on the methanol extract (20 g) to give 105 fractions of 200 mL each. Fractions 70-73 were combined and the solid obtained was washed with hexane and chloroform, and recrystallised with methanol to yield ribalinidine (3) (1.88 g) as colourless needle crystals, m.p. 257-259°C (Corral et al. 1973, m.p. 257-258°C). This compound showed one fluorescent blue spot when visualized under long wave UV light (366 nm). The extract also furnished another batch of skimmianine (1) (234 mg) and lunamarine (2) (75 mg).

Skimmianine (1): colorless prism crystals, m.p. 178-179°C (Rahmani et al. 2010, m.p. 179-181°C). The compound showed  $R_f$  value of 0.81 (20% EtOAc: 80% CHCl<sub>3</sub>). UV  $\lambda_{\max}$  nm (log  $\epsilon$ ), CHCl<sub>3</sub>: 333.6 (0.499), 320.8 (0.485) and 251.6 (2.966). IR  $\nu_{\max}$  cm<sup>-1</sup> (KBr disc): 3118, 1767, 2943, 2839, 1619, 1496, 1368, 1264 and 1090. EIMS m/z (% intensity): 259 [M]<sup>+</sup> (46), 244 (100), 230 (59), 213 (38), 201 (36), 184 (10), 173 (19), 156 (9), 144 (10), 130 (17), 101 (9), 86 (13), 75 (12), 63 (9) and 50 (7). <sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>):  $\delta$  8.01 (1H, *d*, *J* = 9.5 Hz, H-5), 7.23 (1H, *d*, *J* = 9.5 Hz, H-6), 7.58 (1H, *d*, *J* = 2.9 Hz, H-2'), 7.03 (1H, *d*, *J* = 2.9 Hz, H-3'), 4.42 (3H, *s*, 4-OCH<sub>3</sub>), 4.12 (3H, *s*, 8-OCH<sub>3</sub>) and 4.03 (3H, *s*, 7-OCH<sub>3</sub>). <sup>13</sup>C-NMR (100 MHz, CDCl<sub>3</sub>):  $\delta$  164.4 (C-2), 102.0 (C-3), 157.2 (C-4), 114.9 (C-4a), 118.2 (C-5), 112.1 (C-6), 152.2 (C-7), 142.0 (C-8), 141.5 (C-8a), 143.0 (C-2'), 104.6 (C-3'), 58.9 (4-OCH<sub>3</sub>), 56.8 (7-OCH<sub>3</sub>) and 61.7 (8-OCH<sub>3</sub>). Lunamarine (2): colorless prism crystals, m.p. 245-247°C (Dictionary of Natural Products, 1991, m.p. 245-246°C). The compound showed  $R_f$  value of 0.38 (40% MeOH: 60% CHCl<sub>3</sub>). IR  $\nu_{\max}$  cm<sup>-1</sup> (KBr disc): 3020, 2951, 2902, 1640, 1567, 1434, 1223, 1032, 816 and 456. UV  $\lambda_{\max}$  nm (log  $\epsilon$ ), CHCl<sub>3</sub>: 320.5 (3.374) and 247.0 (3.763). EIMS m/z (% intensity): 309 [M]<sup>+</sup> (58), 281 (52), 266 (100), 265 (3), 238 (12), 236 (6), 223 (4), 208 (7), 193 (3), 180 (6), 164 (4), 140 (19), 132 (5), 103 (3), 89 (5) and 77 (7). <sup>1</sup>H-NMR and <sup>13</sup>C-NMR (CDCl<sub>3</sub>)

- see Table 1. Ribalinidine (3): colorless needle crystals, m.p. 257-259°C (Corral et al. 1973, m.p. 257-258°C). The compound showed  $R_f$  value of 0.66 (90% CHCl<sub>3</sub>: 10% MeOH). UV  $\lambda_{\max}$  nm (log  $\epsilon$ ), CHCl<sub>3</sub>: 334.2 (0.226), 238.4 (0.913) and 235.4 (0.925). IR  $\nu_{\max}$  (KBr disc) cm<sup>-1</sup>: 3506, 2976, 2889, 2779, 2224, 1666, 1602, 1508, 1471, 1415, 1363, 1238, 1122 and 1083. EIMS m/z (% intensity): 275 [M]<sup>+</sup> (78), 242 (16), 232 (14), 205 (58), 204 (100), 203 (68), 202 (44), 188 (4), 176 (12), 175 (8), 150 (20), 134 (2), 122 (10), 94 (2), 77 (2), 65 (2). <sup>1</sup>H-NMR and <sup>13</sup>C-NMR (CDCl<sub>3</sub>) - see Table 1 and Figure 1.

## RESULTS AND DISCUSSION

The dried ground aerial parts of *Haplophyllum laeviusculum* (920 g) were soaked sequentially with petroleum ether, ethyl acetate and methanol. The filtrates were concentrated under reduced pressure with rotary evaporator to yield 12.2, 11.5 and 30.0 g of viscous crude extracts, respectively. Each extracts were chromatographically separated and the pure compounds obtained were submitted for spectral analysis and identified as skimmianine (1), lunamarine (2) and ribalinidine (3),  $\gamma$ -sitosterol (5) and campesterol (6). Compound (1) was obtained as colourless prisms after recrystallization from chloroform with m.p. 178-179°C. A positive test with *Dragendorff's reagent* indicated it to be an alkaloid. The molecular formula of C<sub>14</sub>H<sub>13</sub>NO<sub>4</sub> was determined by EIMS with molecular ion peak at m/z 259 [M]<sup>+</sup>. The IR spectrum gave absorption at 3118 cm<sup>-1</sup> while indicated the presence of C-H stretching band and a strong band at 1090 cm<sup>-1</sup> showed the presence of C-O group. The UV spectrum absorptions at 333.6, 320.8 and 251.6 nm indicated the existence of a typical furanoquinoline moiety (Tsai et al. 1995).

The integration of the <sup>1</sup>H-NMR further supported the presence of 13 protons in which the four methine protons occurred as two set of doublets and the other nine protons representing three sharp methoxyl singlets in the high field region. A pair of coupled signals at  $\delta$  8.01 and 7.23 each with common coupling constants 9.5 Hz indicated ortho-coupling protons assigned to H-5 and H-6, respectively. Another pair of doublets due to the two methine protons of the furan ring occurred at  $\delta$  7.58 (1H, *d*, *J* = 2.9 Hz) and 7.03 (1H, *d*, *J* = 2.9 Hz). The rest of the proton resonances were due to the three methoxyl groups which appeared at  $\delta$  4.42, 4.12 and 4.03. The existence of 14 carbons in the molecule could be seen clearly in the DEPT spectrum with the presence of four methine, three methoxyl and seven quaternary carbons. The attachment of the protons to their respective carbon atoms are clearly shown by the HMQC spectrum. This was further supported by the two-bond and three-bond connectivities in the HMBC spectrum as shown by the proton H-5 exhibited correlations with C-4, C-7 and C-8a. Similarly, proton H-6 showed correlations with C-4a, C-7 and C-8. The observation of cross-correlations between the three methoxyl groups to respective carbon atoms on the aromatic rings confirmed their position. Based on these

TABLE 1. <sup>1</sup>H-NMR, <sup>13</sup>C-NMR, HMBC, COSY and NOESY spectral data of (2) and (3)

H/C	(2)		(4)		(3)	
	$\delta_{\text{H}}$	$\delta_{\text{C}}$	$\delta_{\text{H}}$ (CDCI <sub>3</sub> )*	HMBC	$\delta_{\text{H}}$	$\delta_{\text{C}}$
2	-	154.1	-	-	-	156.2
3	6.21 (s, 1H)	112.5	6.86	C-2, C-4a, C-1'	-	97.9
4	-	177.2	-	-	-	177.5
4a	-	129.6	-	-	-	125.5
5	8.41 (d, <i>J</i> = 8.8 Hz, 1H)	128.7	8.22	C-7, C-8a, C-4	7.62 (1H, <i>d</i> , <i>J</i> = 2.8 Hz)	109.5
6	7.01 (dd, <i>J</i> = 8.8, 1.8 Hz, 1H)	111.7	7.76	C-8	-	154.6
7	-	162.9	7.76	-	7.24 (1H, <i>dd</i> , <i>J</i> = 9.2, 2.8 Hz)	122.8
8	6.86 (d, <i>J</i> = 1.8 Hz, 1H)	99.3	7.52	C-7, C-6, C-4a	7.58 (1H, <i>d</i> , <i>J</i> = 9.2 Hz)	118.2
8a	-	143.7	-	-	-	133.9
1'	-	121.2	-	-	-	-
2'	6.88 (dd, <i>J</i> = 9.6, 1.8 Hz, 1H)	108.6	6.60-6.62	C-1'/C-6'/C-3'	-	84.0
3'	6.89 (d, <i>J</i> = 9.6, 1.8 Hz, 1H)	109.0	6.60-6.62	C-5', C-1'	3.89 (1H, <i>t</i> , <i>J</i> = 5.5 Hz)	69.0
4'	-	147.9	-	-	2.91 (1H, <i>dd</i> , <i>J</i> = 16.5, 5.5 Hz) 2.95 (1H, <i>dd</i> , <i>J</i> = 16.5, 5.5 Hz)	26.7
5'	-	148.6	-	-	-	-
6'	6.90 (d, <i>J</i> = 1.8 Hz, 1H)	122.7	6.60-6.62	C-2, C-1', C-5'	-	-
7-OCH <sub>3</sub>	3.95 (s, 3H)	55.6	-	C-7	-	-
OCH <sub>2</sub> O	6.07 (s, 2H)	101.6	5.67	C-5', C-4'	-	-
N-CH <sub>3</sub>	3.57 (s, 3H)	37.3	3.83	C-2, C-8a	3.75 (3H, <i>s</i> )	31.3
2'-CH <sub>3</sub>	-	-	-	-	1.46 (3H, <i>s</i> )	22.1
2''-CH <sub>3</sub>	-	-	-	-	1.44 (3H, <i>s</i> )	25.3

\*Shakirov, 1996.

spectral data evidence and comparison with literature reports, the compound is identified as skimmianine (1) previously identified from roots of *Glycosmis elongata* and *G. arborea* (Chakravarty et al. 1999; Rahmani et al. 2010).

Lanumarine (2) was crystallized from mixture of chloroform and methanol as colourless prism crystals, m.p. 245-247°C and gave positive result to *Dragendorff's*

*reagent* that indicated the alkaloidal structure. The IR spectrum revealed bands at  $\nu_{\max}$  1640  $\text{cm}^{-1}$  for carbonyl group and strong bands at 1223 and 1032  $\text{cm}^{-1}$  indicated the presence of ether group. The UV absorptions at 320.5 and 247.0 nm supported the existence of quinolinone alkaloid skeleton (Cordell 1981). The EIMS spectrum showed a molecular ion peak at  $m/z$  309 which corresponded to molecular formula of  $\text{C}_{18}\text{H}_{15}\text{NO}_4$ . Other prominent peaks

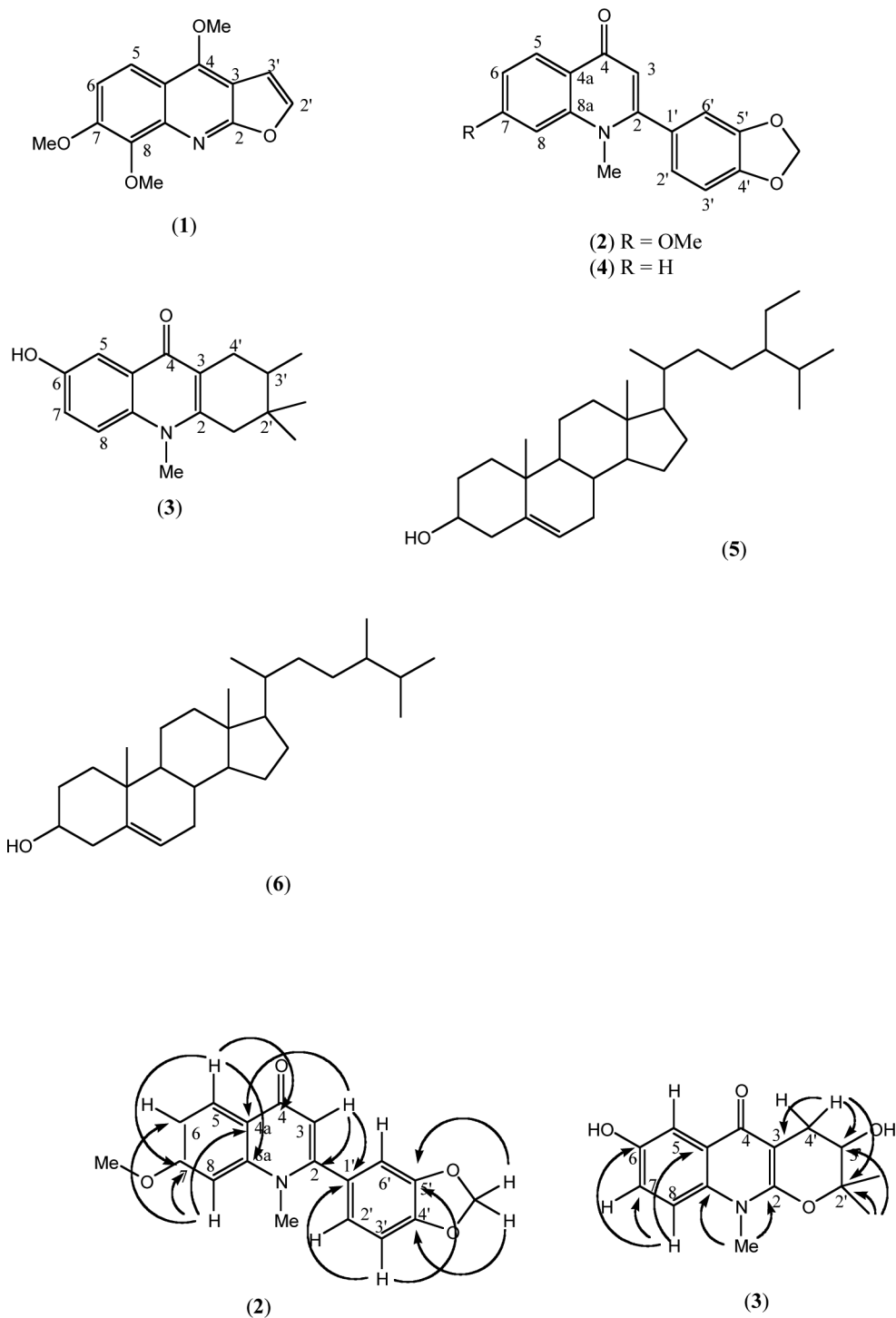


FIGURE 1. Selected HMBC correlations of (2) and (3)

observed are at  $m/z$  281 and 266 representing  $[M-CO]^+$  and  $[M-CO-CH_3]^+$  ions.

The aromatic region of the  $^1H$ -NMR of the compound exhibited two sets of 1,2,4-trisubstituted benzene ring systems as can be seen in the correlation analysis of the COSY spectrum. The first set showed resonances at  $\delta$  8.41 ( $d, J = 8.8$  Hz, H-5), 7.01 ( $dd, J = 8.8, 1.8$  Hz, H-6) and 6.86 ( $d, J = 1.8$  Hz, H-8). The other set of 1,2,4-trisubstituted benzene ring displayed signals at  $\delta$  6.90 ( $d, J = 1.8$  Hz, H-6'), 6.88 ( $dd, J = 9.6, 1.8$  Hz, H-2') and 6.89 ( $d, J = 9.6$  Hz, H-3'). Other signals observed are an isolated aromatic proton, a methylenedioxy, methoxyl and N-methyl protons at  $\delta$  6.21, 6.02, 3.95 and 3.57, respectively. The DEPT spectrum supported the presence of eighteen carbon atoms made up of a carbonyl ( $\delta$  177.2), methylenedioxy ( $\delta$  101.6), methoxyl ( $\delta$  55.6), N-methyl ( $\delta$  37.3), seven methines, three oxygen-substituted quaternary carbons and four quaternary carbons. These spectral data suggest that the compound belongs to quinolinone alkaloid and identified as lunamarine (2) previously isolated from *Lunasia amara* (Goodwin et al. 1959). Since the spectral data of the compound could not be obtained in literature, this has been compared to closely related graveoline (4) as can be seen in Table 1 (Shakirov et al. 1996). The two-bond and three-bond connectivities of the HMBC spectrum provide further evidence for the suggested structure and some selected correlations are given in Figure 1.

Compound (3) was obtained as colourless needle-shaped crystals with m.p. 257-259°C (Corral et al. 1973, m.p. 257-258°C) from the methanol extract of plant. The IR spectrum exhibited sharp peak at  $\delta$  3506  $cm^{-1}$  and two strong bands at 1238 and 1122  $cm^{-1}$  indicated the presence of free OH groups and C-O bands, respectively. The signal at 1666  $cm^{-1}$  was due to the presence of a carbonyl group. The typical characteristic for N-methyl-4-quinolone structure (Cordell 1981) is shown in the UV spectrum by absorption bands at 235.4, 238.4 and 334.2 nm. The EIMS of compound (3) showed a molecular ion peak at  $m/z$  275, which corresponded to molecular formula of  $C_{15}H_{17}NO_4$  with prominent base peak at  $m/z$  204 due to the  $[M-C_4H_7O]^+$  ion in the MS spectrum.

The occurrence of a pair coupled signal at  $\delta$  7.24 ( $dd, J = 9.2$  Hz, 2.8 Hz, 1H, H-7), 7.58 ( $d, J = 9.2$  Hz, 1H, H-8) and 7.62 ( $d, J = 2.8$  Hz, 1H, H-5) in the  $^1H$ -NMR spectrum showed the existence of 1,2,4-trisubstituted aromatic ring. A triplet, which is integrated for one proton at  $\delta$  3.89 was assigned to H-3' with the coupling constant value of 5.5 Hz. The splitting of this signal occurred due to the protons resonance effect of methylene protons of H-4'. These two protons appeared as two different chemical shifts at  $\delta$  2.91 and 2.95, rationally due to different configuration for axial and equatorial position. The presence of two singlets in the high field region at  $\delta$  1.46 and 1.44 ppm clearly indicated the two geminal methyl groups at position C-2'. Another three proton singlet at 3.75 is assigned to N-CH<sub>3</sub> group. The  $^{13}C$ -NMR spectrum further supported the existence of 15 carbon atoms which consists of four methine carbons

( $\delta$  122.8 (C-7), 118.2 (C-8), 109.5 (C-5) and 69.0 (C-3')), three methyl groups ( $\delta$  22.1, 25.3 and 31.3), one methylene group ( $\delta$  26.7) and seven quaternary carbons ( $\delta$  156.2 (C-2), 97.9 (C-3), 177.5 (C-4), 125.5 (C-4a), 154.6 (C-6), 133.9 (C-8a) and 84.0 (C-2')). The lowest field signal at  $\delta$  177.5 confirmed the presence of a carbonyl group at C-4. The proton-proton correlations displayed by COSY spectrum, showed the cross peak correlations between H-7 to H-8, H-3' to H-4'-axial and H-4'-equatorial, and H-4'-axial to H-4'-equatorial. The two-bond and three-bond correlations of H-8 to C-4a and C-7 can be seen clearly as exhibited by the HMBC spectrum. The N-methyl protons showed  $^3J$  correlations to C-8a and C-2. The two methylene protons at H-4' clearly showed two-bond and three-bond correlations to C-3', C-2', C-3 and C-4. Some of the selected HMBC correlations are given in Figure 1. All the assignments were in accordance to the structure of ribalinidine previously isolated from Balfourodendron rielianum (Corral et al. 1973). The two ubiquitous sterols,  $\gamma$ -sitosterol (5) and campesterol (6) were also isolated from the ethyl acetate extract as white crystals with m.p. 134-136°C and 155-157°C, respectively. The spectral data of the compounds were identical to those reported in literature (Hill et al. 1991)

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#### REFERENCES

- Bessonova, I.A., Kurbanov, D. & Yunusov, S.Y. 1989. Components of *Haplophyllum ramosissimum*. *Chemistry of Natural Compounds* 25: 39-40.
- Chakravarty, A.K., Sarkar, T., Masuda, K. & Masuda, K. 1999. Carbazole alkaloids from roots of *Glycosmis arborea*. *Phytochemistry* 50: 1263-1266.
- Cordell, G.A. 1981. *Introduction to Alkaloids - A Biogenetic Approach*. New York: John Wiley and Sons. 246pp.
- Corral, R.A., Orazi, O.O. & Benages, I.A. 1973. New balfourodendron 4-quinolone alkaloids. *Tetrahedron* 29: 205-211.
- Dictionary of Natural Products, 1991. 3: 3689. London: Chapman and Hall.
- Goodwin, S., Smith, A.F., Velasquez, A.A. & Horning, E.C. 1959. Alkaloids of *Lunasia amara* Blanco. Isolation studies. *Journal of American Chemical Society* 81: 6209-6213.
- Gozler, B., Gozler, T., Saglam, H. & Hess, M. 1996. Minor lignans from *Haplophyllum cappadocicum*. *Phytochemistry* 42: 689-693.
- Hill, R.A., Kirk, D.N., Makin, H.L.J. & Murphy, C.M. 1991. *Dictionary of Steroids*, Great Britain: Chapman and Hall.
- Komala, I., Rahmani, M., Sukari, M.A., Ismail, H.B.M., Ee G.C.L. & Rahmat, A. 2006. Furoquinoline alkaloids from *Melicope bonwickii* (F. Muell.) T. Hartley. *Natural Product Research* 20: 355-360.
- Mozaffarian, V. 1996. *A Dictionary of Iranian Plant Names, Farhang Moaser*, Tehran. 260pp.

- Parhoodeh, P., Rahmani, M., Hashim, N.M., Sukari, M.A. & Ee, G.C.L. 2011. Lignans and other constituents from aerial parts of *Haplophyllum villosum*. *Molecules* 16: 2268-2273.
- Rahmani, M., Rosmiati, M.S., Hashim, N.M., Sukari, M.A., Ee, G.C.L., Ali, A.M. & Ismail, H.B.M. 2010. Alkaloids and sulphur containing amides from *Glycosmis citrifolia* and *Glycosmis elongata*. *Sains Malaysiana* 39: 445-451.
- Rahmani, M., Susidarti, R.A., Ismail, H.B.M., Sukari, M.A., Tuafiq-Yap, Y.H., Ee G.C.L., Ali, A.M., Kulip, J. & Waterman, P.G. 2003. Coumarins from Malaysian *Micromelum minutum*. *Phytochemistry* 64: 873-877.
- Shakirov, R., Telezhenetskaya, M.V., Bessonova, I.A., Aripova, S.F., Israilov, I.A., Sultankhodzhaev, M.N., Vinogradova, V.I., Akhmedzhanova, V.I., Tulyaganov, T.S., Salimov, B.T., Tel'nov, V.A. 1996. Alkaloids, plants, structure, properties. *Chemistry of Natural Compounds* 32: 216-334.
- Sompheary, E., Giacometti, S., Ciccolini, J., Akhmedjanova, V., & Aubert, C. 2008. Cytotoxic Effects of Haplamine and its Major Metabolites on Human Cancer Cell Lines. *Planta Medica* 74: 1265-1268.
- Susidarti, R.A., Rahmani, M., Ismail, H.B.M., Sukari, M.A., Taufiq-Yap, Y.H., Ee, G.C.L. & Ali, A.M. 2009. Cytotoxic activity of coumarins from *Micromelum minutum*. *Pharmaceutical Biology* 47: 182-185.
- Staerk, D., Kesting, J.R., Sairafianpour, M., Witt, M., Asili, J., Emami, S.A. & Jaroszewski, J. W. 2009. Accelerated dereplication of crude extracts using HPLC-PDA-MS-SPE-NMR: Quinolinone alkaloids of *Haplophyllum acutifolium*. *Phytochemistry* 70: 1055.-1061.
- Townsend, C.C. 1986. Taxonomic revision of the genus *Haplophyllum* (Rutaceae). Kew, London. Royal Botanic Gardens, Tsai, I.L., Wu, S.J., Ishikawa, T., Seki, H., Yan, S.T. & Chen, I.S. 1995. Evomerrine from *Melicope semecarpifolia*. *Phytochemistry* 40: 1561-1562.
- Yuldashev, M.P. 2002. Flavonoids and coumarins of *Haplophyllum leptomerum* and *H. dubium*. *Chemistry of Natural Compounds* 38: 192-193.

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