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Triterpene Saponins from *Nigella sativa* L.

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Three known triterpene glycosides were isolated from the MeOH extract of dried and powdered seeds of *Nigella sativa*. The structures of the compounds were established as 3-*O*-[β-D-xylopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl]-28-*O*-[α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl]-hederagenin (**1**), 3-*O*-[α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl]-28-*O*-[α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl]-hederagenin (**2**), and 3-*O*-[β-D-xylopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl] -hederagenin (**3**) by means of chemical and spectral methods. Compound **2** was isolated for the first time from the genus *Nigella*.

Key Words: Ranunculaceae, *Nigella sativa*, Saponins, Triterpene glycoside, hederagenin.

Introduction

Nigella sativa L. (Ranunculaceae) is an annual herbaceous plant native to (and cultivated in) S. W. Asia, and cultivated and naturalized in Europe and N. Africa. The seeds and seed oil have been used as a diuretic, appetitizer, hemorrhagic and anti-dandruff therapy in folk medicine[1]. The seeds have also been used traditionally for centuries in the Middle East, Far East, and some Mediterranean and European countries for the treatment of different ailments such as diabetes, hypertension, cardiac diseases, hemorrhoids, and sexual diseases and as an abortifacient[2−7]. Recent pharmacological investigations on the seed extract revealed a wide spectrum of activities such as anti-tumor[8], anti-inflammatory, analgesic, anti-pyretic[9] and gastroprotective[10]. Other notable pharmacological properties are anticestode and antinematode[11], anti-diabetic[12], cytotoxic and immunopotentiating activities[13], as well as protection against cytotoxic damage

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from chemotherapeutic drugs and oxidative stress[14−16]. In a previous paper, Al-Gharably et al. stated that the seeds and the major active constituent, thymoquinone, showed a hepatoprotective effect against liver damage induced by carbontetrachloride[17]. Moreover, fixed oil of *N. sativa* and derived thymoquinone have inhibited eicosanoid generation in leukocytes and membrane lipid peroxidation[18]. Anti-viral[19] and anti-bacterial[20,21] properties of different fractions of *N. sativa* seeds have also been reported.

Several classes of compounds have been isolated from the seeds of *N. sativa*, such as alkaloids[22−27], flavonol triglycosides[28], saponins[29,30] and an isobenzofuranone derivative[31]. Moreover, the contents of total polyphenols and tocopherols of the fixed oil were determined (polyphenols: 1744 μg/g; α, β, and γ-tocopherols: 40, 50 and 250 μg/g, respectively) together with water-soluble vitamins and minerals in the seeds[32].

In the present study, we report the isolation and structure elucidation of 3 hederagenin-type triterpene saponins (**1**-**3**).

Experimental

General Experimental Procedures: The NMR spectra were recorded on a Bruker Avance DRX-500 instrument at 500 MHz (^{1}H) and 125 MHz (^{13}C) in C_5D_5N, using TMS as internal standard. Multiplicity determinations (DEPT) and 2-D NMR spectra (COSY, HMQC, HMBC) were run using a standard Bruker pulse program. The delay for long-range coupling in the HMBC (1/2 J) was set to 63 ms. LC-MS data were obtained using a Finnigan AQA ThermoQuest instrument in the ESI mode. Column chromatography was carried out on Kieselgel 60 (Merck 7734), Sephadex LH-20 (Pharmacia 17-0090-02) and Li Chroprep RP (C-18) (Merck 9303). Analytical TLC was conducted on pre-coated Kieselgel 60 F_{254} aluminum sheets (Merck 5554 and Merck 5569). Compounds were detected by 1% vanillin/H_2SO_4 followed by heating at 105 °C for 1-2 min.

Plant Material: Dried seeds of *N. sativa* were purchased from a local market, KİPA-İzmir, and authenticated by botanist Serdar Gökhan Şenol (Ege University, Faculty of Science, Dept. of Biology).

Extraction and Isolation: Powdered seeds of the plant (400 g) were defatted with *n*-hexane (2 x 3 L). Further extraction was performed with 80% MeOH (2 x 6 L). After evaporation of the MeOH under vacuum, the crude residue was obtained as a syrup (49.35 g). An aliquot of this residue (10 g) was subjected to VLC using reversed-phase material (Lichroprep RP-18, 25-40 μm, 200 g), employing H_2O/MeOH mixtures (0-100%) and 8 fractions (Fr. A-H) were obtained. Fraction A (3.7 g), which was eluted with MeOH, was chromatographed over Sephadex LH-20 (150 g). Eluates obtained with 90% MeOH afforded 190 fractions. Fractions 62-64 were combined and gave a pure compound (**1**; 46.6 mg). Fractions 80-90 (102.9 mg) were further fractionated over a Si gel (Merck 7734, 40 g) column and eluted with $CHCl_3$-MeOH-H_2O (70:30:3, 400 mL; 61:32:7, 600 mL) to yield 27 fractions. Fractions 5-7 were combined and afforded compound **2** (10.4 mg). An aliquot of the MeOH extract (25 g) was resubjected to VLC (RP-18, 388 g). Nine fractions were collected. Fractions 4-5, which obtained with 75% MeOH, were combined and fractionated over a Si gel column. Six hundred fifty fractions were collected. Fractions 95-147 (115 mg) were purified on a Si gel column (30 g) to afford a pure compound (**3**, 23.9 mg).

Alkaline Hydrolysis: Ten milligrams of compound **1** was dissolved in 5 mL of MeOH and then 1 mL 33% KOH was added to the solution. It was hydrolyzed under reflux at 90 °C for 1 h. After the hydrolysis

the reaction mixture was adjusted to pH 6 with dilute HCl and then evaporated to dryness.

Results

3-*O*-[*β*-D-xylopyranosyl-(1→3)-*α*-L-rhamnopyranosyl-(1→2)-*α*-L-arabinopyranosyl]-28-*O*-[*α*-L-rhamnopyranosyl-(1→4)-*β*-D-glucopyranosyl-(1→6)-*β*-D-glucopyranosyl]-hederagenin (**1**)**:** Amorphous white powder; negative-ion LC-ESIMS m/z: 1389.2 $[M+Cl]^-$ calcd. for $C_{64}H_{104}O_{30}$; IRυ_{max} (KBr, cm^{-1}) 3387 (OH), 1738 (C=O, ester group), 1250 (C-O); ^{1}H NMR (C_5D_5N, 500 MHz) and ^{13}C NMR (C_5D_5N, 125 MHz) data (Tables 1 and 2).

3-*O*-[*α*-L-rhamnopyranosyl-(1→2)-*α*-L-arabinopyranosyl]-28-*O*-[*α*-L-rhamnopyranosyl-(1→4)-*β*-D-glucopyranosyl-(1→6)-*β*-D-glucopyranosyl]-hederagenin (**2**): Amorphous white powder; negative-ion LC-ESIMS m/z: 1221.1. $[M-H]^-$ calcd. for $C_{59}H_{96}O_{26}$; IRυ_{max} (KBr, cm^{-1}) 3387 (OH), 1738 (C=O, ester group), 1250 (C-O); ^{1}H NMR (C_5D_5N, 500 MHz) and ^{13}C NMR (C_5D_5N, 125 MHz) data (Tables 1 and 2).

3-*O*-[*β*-D-xylopyranosyl-(1→3)-*α*-L-rhamnopyranosyl-(1→2)-*α*-L-arabinopyranosyl]-hederagenin (**3**)**:** Amorphous white powder; negative-ion LC-ESIMS m/z: 881.5 $[M-H]^-$ calcd. for $C_{46}H_{74}O_{16}$; IRυ_{max} (KBr, cm^{-1}) 3387 (OH), 1738 (C=O, ester group), 1250 (C-O); ^{13}C NMR (C_5D_5N, 125 MHz) data (Table 2).

Discussion

The LC-ESIMS spectrum of **1** exhibited a pseudomolecular ion $[M+Cl]^-$ at m/z 1389.2, compatible with the molecular formula $C_{64}H_{104}O_{30}$.

The ^{1}H NMR spectrum (Table 1) of **1** contained signals typical for a saponin, with an olefinic proton at 5.40 ppm and 6 methyl singlets in the upfield region.

In addition, the ^{1}H NMR spectrum displayed signals for 6 anomeric protons at δ 6.32 (d, J = 8.5 Hz), 4.99 (m, J= 8.5 Hz), 5.07 (d, J = 6 Hz), 6.35 (brs), 5.84 (s) and 5.34 (d, J= 7.5 Hz), which correlated with the carbon signals at 95.4, 104.5, 104.3, 101.0, 102.5 and 107.2, respectively, in the HMQC spectrum (Table 2). In the ^{1}H NMR spectrum 2 methyl doublets at δ 1.55 and 1.70 suggested the presence of 2 deoxy-sugars in compound **1**. The ring protons of the monosaccharide residues were assigned starting from the readily identifiable anomeric protons by means of the COSY, HMQC and HMBC. Evaluation of spin-spin couplings and chemical shifts allowed the identification of 2 *β*-glucopyranosyl, 2 *α*-rhamnopyranosyl, 1 *α*-arabinopyranosyl and 2 *β*-xylopyranosyl units[33]. The common D-configuration for xylose and glucose and the L-configuration for rhamnose and arabinose were assumed, according to those most often encountered among the plant glycosides in each case. The ^{13}C spectrum of compound **1** revealed 64 carbon signals, 34 of which were assigned to 2 pentose and for hexose units and the remaining 30 signals to a triterpenoid aglycon. A detailed analysis of the NMR spectral data of the aglycon part displayed the features of a hederagenin-type triterpene skeleton[34].

Assignments for all proton and carbon resonances (Tables 1 and 2) were achieved by COSY, HMQC and HMBC experiments. The ^{13}C NMR spectrum of **1** showed marked glycosylation shifts for C-3 (δ 80.9) and C-28 (δ 178.0), suggesting the bidesmosidic nature of the triterpene skeleton. An HMBC experiment clarified all interglycosidic connectivities showing correlations between C-3 of aglycone (δ 80.9) and the anomeric proton of arabinose (δ 5.07), C-2 of arabinose (δ 75.0) and H-1 of rhamnose (δ 6.35), C-3 of

Table 1. ^{1}H Assignments of **1** and **2** (in C_5D_5N)[a].

Position (aglycone)	**1**(δ_H, J Hz)	**2** (δ_H, J Hz)	Position (sugar)	**1**(δ_H, J Hz)	**2** (δ_H, J Hz)
1	1.03 m, 1.53 m	1.03 m, 1.53 m	**at C-3**		
2	1.95 m	1.95 m	Ara-1′	5.07 d (6.0)	5.12 d (6.0)
3	4.31[b]	4.31[b]	2′	4.60 t (7.0)	4.60 t (7.0)
4			3′	4.13[b]	4.17[b]
5	1.29[b]	1.29[b]	4′	4.14[b]	4.23[b]
6	1.32 m, 1.52 m	1.32 m, 1.52 m	5′	4.28[b], 3.67[b]	4.27[b], 3.72[b]
7	1.32 m, 1.61 m	1.32 m, 1.61 m	Rha-1″	6.35 brs	6.19 brs
8			2″	4.90[b]	4.72[b]
9	1.75 m	1.68 m	3″	4.77[b]	4.64[b]
10			4″	4.49 t (9.5)	4.28 t (9.5)
11	1.88 m	1.88 m	5″	4.73[b]	4.68[b]
12	5.40 brs	5.40 brs	6″	1.55 d (6.0)	1.65 d (6.0)
13			Xyl-1‴	5.34 d (7.5)	
14			2‴	4.07[b]	
15	1.08 m, 2.23 m	1.08 m, 2.23 m	3‴	4.16[b]	
16	1.91 m, 1.97 m	1.85 m, 1.96 m	4‴	4.17[b]	
17			5‴	4.23[b], 3.67[b]	
18	3.17 m	3.18 m	**at C-28**		
19	1.19 m, 1.70 m	1.19 m, 1.69 m	Glu-1⁗	6.23 d (8.5)	6.22 d (8.0)
20			2⁗	4.15[b]	4.12[b]
21	1.09 m, 1.29 m	1.09 m, 1.29 m	3⁗	4.23[b]	4.22[b]
22	1.74 m, 1.83 m	1.72 m, 1.83 m	4⁗	4.33[b]	4.32[b]
23	3.94[b], 4.32[b]	3.94[b], 4.32[b]	5⁗	4.12[b]	4.13[b]
24	1.12 s	1.09 s	6⁗	4.36[b], 4.68[b]	4.18[b], 4.64[b]
25	0.98 s	0.99 s	Glu-1⁗	4.99 d (8.5)	5.01 d (8.0)
26	1.16 s	1.14 s	2⁗	3.95[b]	3.95 t (8.0)
27	1.20 s	1.18 s	3⁗	4.14[b]	4.13[b]
28			4⁗	4.44 t (9.5)	4.38 t (9.5)
29	0.86 s	0.87 s	5⁗	3.66[b]	3.75[b]
30	0.89 s	0.89 s	6””	4.08[b], 4.22[b]	4.06[b], 4.17[b]
			Rha-1⁗′	5.84 brs	5.87 brs
			2⁗′	4.68[b]	4.68[b]
			3⁗′	4.56[b]	4.53[b]
			4⁗′	4.35 t (9.5)	4.32 t (9.5)
			5⁗′	4.93[b]	4.92[b]
			6⁗′	1.70 d (6.0)	1.72 d (6.0)

[a]Assignments confirmed by COSY and HMQC experiments.
[b]Signal pattern was unclear due to overlapping.

Table 2. ^{13}C Assignments of **1-3** (125 MHz, δ ppm, in C_5D_5N).

Position (aglycon)	**1**	**2**	**3**	Position (sugar)	**1**	**2**	**3**
				at C-3			
1	39.1 t	39.0 t	39.3 t	Ara-1′	104.3 d	104.0 d	104.7 d
2	26.4 t	26.2 t	26.5 t	2′	75.0 d	75.7 d	74.9 d
3	80.9 d	80.9 d	81.4 d	3′	73.7 d	73.8 d	73.0 d
4	43.6 s	43.5 s	43.8 s	4′	69.4 d	69.1 d	69.7 d
5	48.1 d	47.7 d	48.4 d	5′	66.1 t	65.3 t	66.1 t
6	18.2 t	18.3 t	18.4 t	Rha-1″	101.0 d	101.4 d	101.5 d
7	32.8 t	32.8 t	33.5 t	2″	71.8 d	72.2 d	71.2 d
8	40.0 s	40.0 s	40.0 s	3″	82.8 d	72.7 d	82.8 d
9	47.6 d	48.2 d	47.8 d	4″	72.8 d	74.1 d	72.1 d
10	37.1 s	36.9 s	37.1 s	5″	69.2 d	69.7 d	69.7 d
11	23.9 t	23.9 t	23.9 t	6″	18.2 q	18.6 q	18.7 q
12	122.6 d	122.6 d	122.8 d	Xyl-1‴	107.2 d		107.3 d
13	144.0 s	144.0 s	145.1 s	2‴	75.4 d		75.6 d
14	42.2 s	42.3 s	42.2 s	3‴	78.2 d		78.4 d
15	28.3 t	28.4 t	28.6 t	4‴	70.9 d		69.9 d
16	23.4 t	23.4 t	23.4 t	5‴	67.2 t		67.4 t
				at C-28			
17	47.2 s	47.1 s	47.0 s	Glu-1⁗	95.4 d	95.5 d	
18	41.6 t	41.7 t	40.4 t	2⁗	76.4 d	76.4 d	
19	46.1 t	46.2 t	46.7 t	3⁗	78.6 d	78.6 d	
20	30.7 s	30.8 s	30.9 s	4⁗	70.8 d	70.9 d	
21	34.0 t	34.0 t	34.4 t	5⁗	77.9 d	78.3 d	
22	32.6 t	32.6 t	33.0 t	6⁗	69.1 t	69.4 t	
23	63.9 t	64.1 t	64.2 t	Glu-1′′′′′	104.5 d	104.6 d	
24	14.2 q	14.0 q	14.3 q	2′′′′′	75.1 d	75.3 d	
25	16.2 q	16.3 q	16.3 q	3′′′′′	76.4 d	78.0 d	
26	17.6 q	17.7 q	17.7 q	4′′′′′	78.2 d	78.3 d	
27	26.1 q	26.1 q	26.4 q	5′′′′′	77.0 d	77.1 d	
28	178.0 s	178.0 s	180.7 s	6′′′′′	61.2 t	61.4 t	
29	33.1 q	33.1 q	31.2 q	Rha-1′′′′′′	102.5 d	102.6 d	
30	23.7 q	23.7 q	24.1 q	2′′′′′′	72.3 d	72.5 d	
				3′′′′′′	72.6 d	72.5 d	
				4′′′′′′	73.8 d	73.9 d	
				5′′′′′′	70.1 d	70.3 d	
				6′′′′′′	18.2 q	18.6 q	

rhamnose (δ 82.8) and the anomeric proton of the terminal xylose unit (δ 5.34), C-28 of the aglycone (δ 178.0) and H-1 (δ 6.23) of the inner glucose, C-6 of the inner glucose (δ 69.1) and the anomeric proton of the second glucose moiety (δ 4.99), and C-4 of the second glucose (δ 78.2) and H-1 of the terminal rhamnose (δ 5.84) (Figure 2).

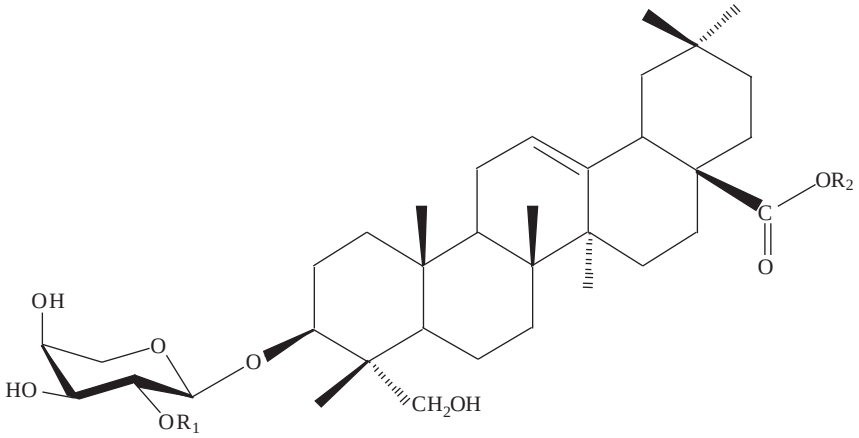

Figure 1. Structure of compounds **1-3**.

Compound	R_1	R_2
1	β-D-xyl(1→3)-α-L-rha	α-L-rha(1→4)−β-D-glu-(1→6)- β-D-glu
2	α-L-rha	α-L-rha(1→4)−β-D-glu(1→6)- β-D-glu
3	β-D-xyl(1→3)-α-L-rha	H

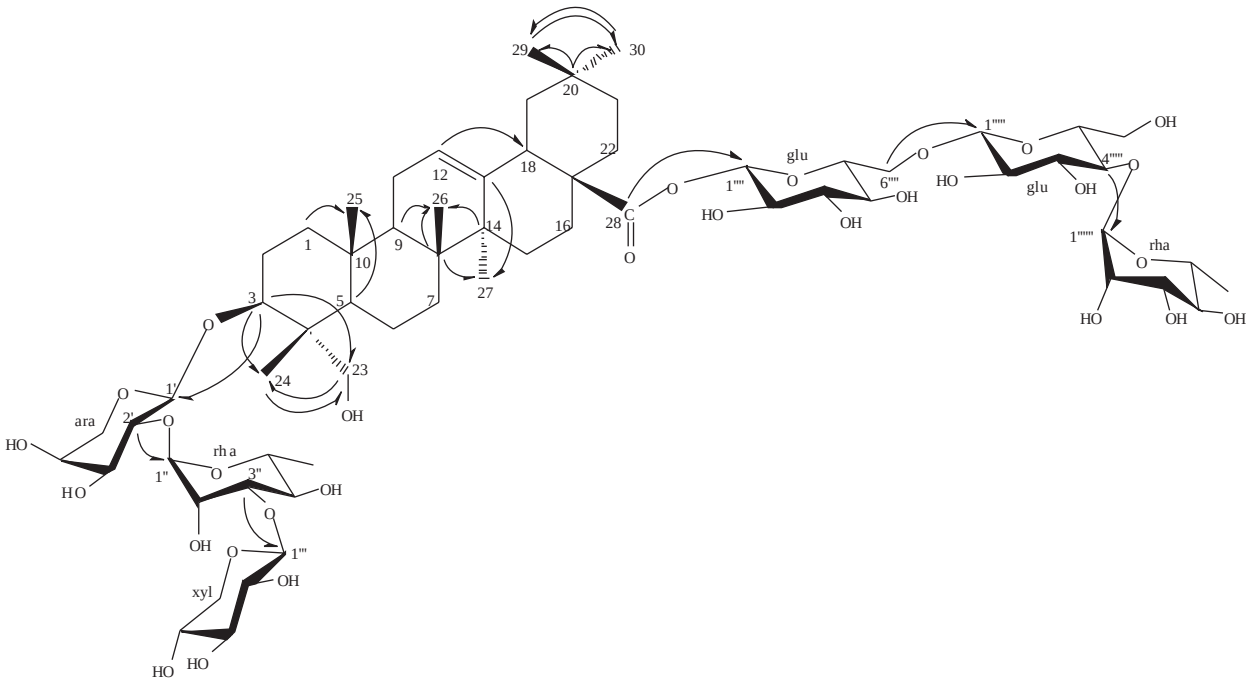

Figure 2. Key long-range correlations of compound **1** (deduced from HMBC).

Based on the above data compound **1** was established as 3-*O*-[β-D-xylopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl]-28-*O*-[α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl]-hederagenin, previously isolated from *N. sativa* seeds [29].

The HR-ESI mass spectrum of **2** displayed a quasimolecular ion peak corresponding to a molecular formula of $C_{59}H_{96}O_{26}$, at m/z 1257.0 $[M+Cl]^-$, indicating a molecular weight 132 mass unit less than **1**.

The ^{1}H-NMR spectrum of **2** showed signals due to 6 tertiary methyl signals [δ 0.87, 0.89, 0.99, 1.09, 1.14, 1.18], and an olefinic proton (δ 5.40, brs). A detailed inspection of the ^{1}H and ^{13}C NMR spectra of **2** and comparison with those of **1** indicated that the data were generally similar, except for the sugar chain attached at C-3 of the sapogenol moiety. The difference was only in the absence of the terminal sugar, β-D-xylopyranosyl moiety attached at C-3 of the inner rhamnopyranosyl of **1**, suggesting a disaccharide unit [(α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl)] at the C-3 position of compound **2**.

The assignment of the sugar moieties and aglycone part was achieved by a combination of COSY, HMQC and HMBC experiments.

Consequently, the structure of **2** was established as 3-*O*-[α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl]-28-*O*-[α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl]-hederagenin[35].

The ^{1}H and ^{13}C NMR spectra of **3** showed resonances typical of a monodesmoside hederagenin-type sapogenol glycosylated at C-3[36]. The ^{1}H NMR spectrum clearly showed 3 anomeric proton signals (δ 6.32, 5.34 and 5.08) in the downfield region, indicative of a triglycosidic structure. On comparison of the ^{1}H and ^{13}C NMR spectra of **3** with those of **1**, the signals due to the sugar moieties at C-3 were superimposable, indicating that they have the same sugar chain at C-3, while the signals arising from the C-28-trisaccharide portion in **1** were not present in **3**.

Upon alkaline hydrolysis of **1** followed by TLC analysis, direct evidence for the structure of **3** was derived.

On the basis of the spectral data as well as the same Rf values observed on the TLC plate for the alkaline hydrolysis product of **1** and compound **3**, the structure of **3** was elucidated as 3-*O*-[β-D-xylopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl]- hederagenin[37,38].

Conclusion

Compounds **1** and **3** were previously reported from *N. sativa* by Ansari et al. and Singab et al. However, in these studies the structures of **1** and **3** were determined mainly based on acid and alkaline hydrolyses followed by GC-MS analysis, and MS data. In the present study, the structures of **1** and **3** were characterized by means of 1D- and 2D-NMR, and LC-ESIMS experiments, and detailed NMR data were reported for the first time. As far as could be ascertained, this is the first report of compound **2** from the genus *Nigella*.

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