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PHYTOCHEMICAL STUDIES OF *Berberis vulgaris*

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This study presents a phytochemical investigation of the fruits of Berberis vulgaris Linn. *The isolation and structure elucidation of four compounds are discussed. The terpenoids lupeol (**1**) and oleanolic acid (**2**) and the steroids stigmasterol (**3**) and stigmasterol glucoside (**4**) are isolated for the first time from this plant. The structure and stereochemistry at various asymmetric centers were established by different spectroscopic techniques.*

Key words: *Berberis vulgaris*, Berberidaceae, lupeol, oleanolic acid, stigmasterol, stigmasterol glucoside.

Berberis vulgaris Linn belongs to the family *Berberidaceae*. Berberis is the genus of spiny deciduous evergreen shrubs, with yellow wood and yellow flowers, and comprises 190 species. *Berberis vulgaris* is commonly known as Barberry. The bark of the stem and root is a cathartic, diuretic, febrifuge, antibilious, and antiseptic. The decoction of leaves is used as antiscorbutic in dysentery, scurvy angina, and sore throat [1]. The Berries are used as bitter tonic and administered in the form of a tincture [2]. During phytochemical investigations of *Berberis vulgaris*, four known compounds have been isolated for the first time.

Lupeol (**1**) was isolated from the fruits and was purified by eluting the fraction with 55% chloroform followed by repeated crystallizations. The molecular ion peak was established by HRMS at *m/z* 426.3858, corresponding to the molecular formula $C_{30}H_{50}O$. Besides the molecular ion peak at *m/z* 426, the EI mass spectrum also showed other fragment ion peaks at *m/z* 411 $[M\text{-}CH_3]^+$, 218 $[M\text{-}C_{14}H_{28}]^+$, 207 $[M\text{-}C_{16}H_{27}]^+$, which are characteristic for pentacyclic triterpene with an isopropenyl group. The IR spectrum of **1** showed absorption bands for hydroxyl group at 3400 cm^{-1} and for terminal methylene group at 3070, 1040, and 880 cm^{-1}. The PMR spectrum of **1** showed the presence of seven tertiary methyl groups at δ 0.98, 0.77, 0.84, 1.04, 0.97, 0.79 and 1.69 (3H each). All of them appeared as singlets except the signal appearing at δ 1.69, which showed allylic coupling (J = 1.3 Hz) with H-29. A pair of multiplets at δ 4.56 and 4.69 (1H each) was indicative of terminal isopropenyl group. This revealed that 1 belong to the lupane group of terpenoids. In the PMR spectrum of **1**, the double-doublet at δ 3.18 (dd, $J_{ax,ax}$ = 10.8 Hz, $J_{ax,eq}$ = 5.4 Hz) was due to the proton attached to the carbon-bearing hydroxyl group. These chemical shifts and biogenetic consideration lead to the assumption of β-orientation of the hydroxyl group at C-3. A one-proton doublet of triplet at δ 2.39 (J = 10.6, 10.6, 5.3 Hz) was assigned to 19β-H on comparison with literature values [3, 4].

The ^{13}C-NMR assignments of various carbon atoms of **1** were substantiated by DEPT experiments, which revealed the presence of seven methyl, eleven methylene, and six methine carbons. The chemical shifts of these signals and other physical data were identical to that of lupeol (Table 1) [3, 4].

Oleanolic acid (**2**) was separated as an amorphous powder from the ethyl acetate-soluble part of the ethanolic extract of *Berberis vulgaris*. The electron impact mass spectrum of **2** showed the molecular ion peak at *m/z* 456 and HREI MS demonstrated the exact mass of this peak at *m/z* 456.3601, in agreement with the molecular formula $C_{30}H_{48}O_3$. The base peak occurred at *m/z* 248, which is characteristic for a pentacyclic triterpene of the β-amyrin series with a 12–13 double bond [5]. The IR spectrum of **2** showed absorptions at 3450 cm^{-1} and 1705 cm^{-1} due to the presence of hydroxyl and a carbonyl function of carboxylic acid, respectively.

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TABLE 1. ^{13}C NMR Chemical Shifts of the Four Compounds Isolated from *Berberis vulgaris*

C atom	Compound, δ				C atom	Compound, δ			
	1	**2**	**3**	**4**		**1**	**2**	**3**	**4**
1	38.6	38.8	37.3	39.2	19	47.9	41.4	19.4	19.3
2	27.3	27.9	28.2	29.3	20	150.8	30.9	36.1	36.7
3	78.9	79.0	71.8	79.6	21	29.8	34.1	19.0	19.1
4	38.4	38.4	40.3	40.4	22	39.9	33.2	138.3	138.8
5	55.2	55.6	140.8	141.1	23	27.9	30.8	129.3	129.9
6	18.2	18.5	121.7	122.4	24	15.3	15.6	50.2	46.6
7	34.2	39.5	31.7	32.4	25	16.1	15.4	29.2	29.8
8	40.7	37.2	31.9	32.5	26	15.9	20.9	21.2	20.0
9	50.3	47.8	51.2	51.9	27	14.5	27.9	21.14	19.6
10	37.1	37.2	36.5	37.3	28	17.9	180.9	23.1	23.6
11	20.9	17.0	21.12	21.6	29	109.3	33.1	12.2	12.4
12	25.0	122.5	39.7	40.3	30	19.2	23.3		
13	38.0	144.1	42.2	42.8	1′				101.7
14	42.7	42.0	56.9	57.5	2′				74.3
15	27.4	28.1	24.4	25.8	3′				76.6
16	35.5	23.6	26.1	26.8	4′				71.0
17	42.9	23.3	56.0	56.7	5′				77.7
18	48.2	41.5	12.1	12.2	6′				62.5

The PMR spectrum of 2 showed the presence of an olefinic proton resonating at δ 5.51 (1H, t, J = 3.5 Hz), corresponding to H-12. Another downfield signal at δ 3.45 (1H, t, J = 6.8 Hz) was assigned to H-3α on the basis of comparison with literature values [6]. The ^{13}C-NMR spectrum of **2** showed a total of thirty signals. With the help of DEPT experiment, these signals were resolved into seven methyls, ten methylenes, and five methines. The eight quaternary carbons were identified from broad band and decoupled ^{13}C-NMR spectrum of **2**. The chemical shift assignments of all carbon atoms were made with the help of HMQC and HMBC experiments (Table 1).

Stigmasterol (**3**) was isolated from the hexane-soluble part of *Berberis vulgaris*. The EI mass spectrum showed the molecular ion peak as well as base peak at *m/z* 412 associated to the molecular formula as $C_{29}H_{48}O$ (412.3709 a.m.u.) and consistent with six degrees of unsaturation, established with the help of HREI mass spectrum. The ^{13}C-NMR spectrum of **3** indicated 29 signals, which were resolved through DEPT experiment into six methyl, nine methylene, eleven methine, and three quaternary carbons. The six methyls appeared in the PMR spectrum (500 MHz, $CDCl_3$) of **3** at δ 0.68 (s, H-18), 0.98 (s, H-19), 1.00 (d, J = 6.5 Hz, H-21), 0.82 (d, J = 6.0 Hz, H-26), 0.77 (d, J = 6.0 Hz, H-27) and 0.78 (t, J = 7.5 Hz, H-29). The olefinic signals, each of one proton integration, at δ 4.98 (dd, J = 15.5, 8.5 Hz, H-23), 5.11 (dd, J = 15.5, 8.5 Hz, H-22) 5.32 (br.s, H-6), and their associated carbons resonating at δ 129.3 (C-23), 138.3 (C-22), and 121.7 (C-6) respectively, showed the presence of two double bonds in the molecule. The methine resonating in the ^{13}C-NMR spectrum at δ 71.8 (C-3) and in the ^{1}H-NMR spectrum at δ 3.46 (m, H-3) revealed that the hydroxyl function is attached to it [7].

Stigmasterol glucoside (**4**) was isolated from the ethyl acetate soluble portion of *Berberis vulgaris*. The molecular mass of 4 was confirmed as *m/z* 574 Daltons with the help of a peak observed in the negative FAB mass spectrum at *m/z* 573 $[M-H]^-$. The ^{13}C-NMR spectrum showed the presence of 35 signals which were resolved through DEPT experiments as six methyl, ten methylene, sixteen methine, and three quaternary carbons. The six signals in the ^{13}C-NMR spectrum of 4 at δ 60–80 were associated with the protons at δ 3.15–3.84, and a methine at δ 101.8 indicated the presence of sugar moiety in the molecule. The sugar was confirmed as β-D-glucose through the magnitude of the coupling constant of the anomeric signal at δ 4.35 (J = 7.7 Hz) and co-TLC of hydrolyzed sugar with an authentic sample. The PMR spectrum showed three olefinic signals at δ 4.96 (dd, J = 15.2, 8.2 Hz, H-23), 5.09 (dd, J = 15.2, 8.4 Hz, H-22) and 5.32 (br. s, H-6). The six methyls indicated by the ^{13}C-NMR spectrum appeared in the PMR spectrum at δ 0.68 (s, H-18), 1.00 (s, H-19), 0.88 (d, J = 6.2 Hz, H-21), 0.81 (d, J = 6.3 Hz, H-26), 0.76 (d, J = 6.4 Hz, H-27) and 0.78 (t, J = 7.0 Hz, H-29) (Table 1).

Compound **4** was identified as 3-O-β-D-glucopyranosyl stigmasterol by matching the NMR spectral data with that reported in the literature [7].

EXPERIMENTAL

Extraction and Isolation. The fruits of *Berberis vulgaris* (7 kg) were collected from Kaghan and soaked in ethanol for one week. The ethanolic extract was then evaporated to a gum. The gummy residue obtained was then partitioned with *n*-hexane, chloroform, and *n*-butanol. The hexane soluble part was subjected to column chromatography on silica gel grade 60 (70–230 mesh) and eluted with hexane-chloroform (30:70). A gummy residue was obtained, which was purified by repeated crystallizations with a mixture of acetone-methanol to yield 0.07 g of lupeol (**1**). Oleanolic acid (2.1 g) was isolated as an amorphous powder from the ethyl acetate soluble part of the plant. Purification of compound **2** was carried out by recrystallization with methanol. Stigmasterol (**3**) was isolated from the hexane soluble part of the plant extract. The fraction eluted with 60% chloroform in hexane yielded compound **3**. This was purified as a white solid (0.062 g) with 80% chloroform in hexane. 3-O-β-D-glucopyranosyl stigmasterol (**4**) was isolated from the ethyl acetate soluble part. The fraction containing **4** was eluted from the column using $CHCl_3$–CH_3OH (92.5:7.5). Compound **4** was purified as a white solid (0.038 g) by column chromatography over a silica gel with $CHCl_3$–CH_3OH (95:5).

Lupeol (1). EI MS *m/z* (rel. int. %): 426 $[M^+]$ (18), 411 $[M\text{-}Me]^+$ (26), 408 $[M\text{-}H_2O]^+$ (31), 393 $[M\text{-}Me\text{-}H_2O]^+$ (38), 220 $[M\text{-}C_{15}H_{26}]^+$ (75), 218 $[M\text{-}C_{14}H_{24}O]^+$ (50) and 207 $[M\text{-}C_{16}H_{27}]^+$ (20). HREIMS *m/z* (formula, calcd. value): 426.3855 ($C_{30}H_{50}O$, 426.3861), 238.2295 ($C_{16}H_{30}O$, 238.2297) and 207.1748 ($C_{14}H_{23}O$, 207.1749).

PMR (400 MHz,$CDCl_3$, δ, ppm, J/Hz): 4.69 and 4.56 (each 1H, m, H-29), 3.18 (1H, dd, H-3), 1.69 (3H, s, H-30), 1.04 (3H, s, H-26), 0.98 (3H, s, H-23), 0.97 (3H, s, H-27),0.84, 0.79, and 0.77 (each 3H, s, H-25, 28, 24).

^{13}C-NMR (100 MHz, $CDCl_3$): Table 1.

Oleanolic Acid (2). IR (KBr, ν_{max}, cm^{-1}): 3450 (OH), 1705 (CO), 2930, 1450, 1250 and 820.EI MS *m/z* (rel. int. %): 456-$[M]^+$ (6), 248 (100), 207 (30), 204 (72) and 189 (30).

HREI MS *m/z* (formula, calcd. value): 456.3601 ($C_{30}H_{48}O_3$, 456.3603).

PMR (500 MHz, $CDOD_3$, δ, ppm, J/Hz): 0.74 (3H, s), 0.76 (3H, s), 0.89 (3H, s), 0.90 (3H, s), 0.91 (3H, s), 0.96 (3H, s), 1.12 (3H, s), 0.76 (3H, s), 3.32 (1H, dd, J = 4.0, 14.0, H-18), 3.45 (1H, t, J = 6.8, H-3), 5.51 (1H, t, J= 3.5, H-12).

^{13}C-NMR (75.43 MHz, CD_3OD): Table 1.

Stigmasterol (3). EIMS *m/z* (rel. int. %): 412 (100), 397 (16), 369 (7), 368 (21), 340 (6), 301 (13), 274 (11), 273 (22), 220 (35), 164 (9), 139 (9). HREIMS *m/z* (formula, calcd. value): 412.3709 ($C_{29}H_{48}O$, 412.3704), 397.3467 ($C_{28}H_{45}O$, 397.3470), 369.3160 ($C_{26}H_{41}O$, 369.3157), 368.3446 ($C_{27}H_{44}$, 368.3442), 340.3201 ($C_{25}H_{40}$, 340.3129), 301.2529 ($C_{21}H_{33}O$, 301.2531), 274.2658 ($C_{20}H_{34}$, 274.2660), 273.2221 ($C_{19}H_{29}O$, 273.2218), 220.2187 ($C_{16}H_{28}$, 220.2190), 164.1205 ($C_{11}H_{16}O$, 164.1201), 139.1490 ($C_{10}H_{19}$, 139.1486), 138.1040 ($C_9H_{14}O$, 138.1044), 111.1170 (C_8H_{15}, 111.1173) and 43.0541 (C_3H_7, 43.0547).

PMR (500 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.68 (3H, s, H-18), 0.98 (3H, s, H-19), 1.00 (3H, d, J = 6.5, H-21), 0.82 (3H, d, J = 6.0, H-26), 0.77 (3H, d, J = 6.0, H-27), 0.78 (3H, t, J = 7.5, H-29), 4.98 (1H, dd, J = 15.5, 8.5, H-23), 5.11 (1H, dd, J = 15.5, 8.5, H-22), 5.32 (1H, br. s, H-6).

^{13}C-NMR (75 MHz, $CDCl_3$): Table 1.

3-O-β-D-Glucopyranosyl Stigmasterol (4). IR (KBr, ν_{max}, cm^{-1}): 3600–3400 (O-H), 1590, 1460 (C=C). FABMS (-ve) *m/z*: 573, 411. Peak matching *m/z* (formula): 573.4151 ($C_{35}H_{57}O_6$).

PMR (300 MHz, $CDCl_3$+CD_3OD, δ, ppm, J/Hz): 0.68 (3H, s, H-18), 0.76 (3H, d, J = 6.4, H-27), 0.78 (3H, t, J = 7.0, H-29), 0.81 (3H, d, J = 6.3, H-26), 0.88 (3H, d, J = 6.2, H-21), 1.00 (1H, s, H-19), 4.96 (1H, dd, J = 15.2, 8.2, H-23), 5.09 (1H,dd, J = 15.2, 8.4, H-22), 5.32 (1H, br. s, H-5), 4.35 (1H, d, J = 7.7, H-1′).

^{13}C-NMR ($CDCl_3$+CD_3OD, 75.43 MHz): Table 1.

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