DOI 10.1007/s10600-019-02660-4
Chemistry of Natural Compounds, Vol. 55, No. 2, March, 2019

ANTIMICROBIAL AND ANTIOXIDANT POTENTIAL OF BERBERISINOL, A NEW FLAVONE FROM *Berberis baluchistanica*

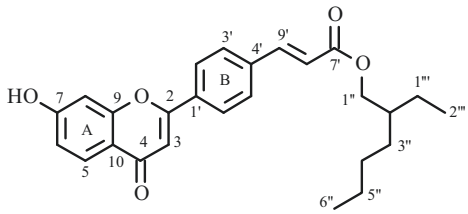
Samreen Pervez,[1*] Muhammad Saeed,[1] Muhammad Shaiq Ali,[2] Itrat Fatima,[2] Haroon Khan,[3] and Irfan Ullah[4]

*A new flavone, berberisinol (**1**), has been isolated from the EtOAc fraction of the MeOH extract of* Berberis baluchistanica*, along with known compounds, palmatine (**2**), berberine (**3**), 8-oxoberberine (**4**),* β*-sitosterol (**5**), oleanolic acid (**6**), and gallic acid (7), isolated for the first time from this species. Spectroscopic techniques including two-dimensional NMR were used for structural elucidation. Berberisinol (**1**) showed significant antibacterial and antioxidant potential.*

Keywords: antimicrobial, antioxidant, *Berberis baluchistanica*, berberisinol.

The genus *Berberis* (Berberidaceae) consists of 650 species [1], mostly evergreen or deciduous shrubs or small trees with typical yellow or yellowish red wood and flowers [2]. It is commonly found in Europe, Asia, East Africa, North America, and South America [3]. In Pakistan, this genus is represented by 20 species [4], which are found in Kalat, Quetta, Ziarat, Loralai, and Sibi districts of Balochistan [5]. One of the indigenous species of this genus is *Berberis baluchistanica*, which is valued for its bark and roots [5]. It is used as tonic, antibacterial [6], antileishmanial, antioxidant [7], and antidiabetic [8]. There are few reports on phytochemical and pharmacological investigations on this plant [7, 9]. The ethnopharmacological and chemotaxanomic significance of the genus *Berberis* prompted us to carry out extensive phytochemical studies on *B. baluchistanica.* Herein we report the isolation and structural elucidation of a new flavone named berberisinol (**1**), together with palmatine (**2**) [10], berberine (**3**) [11], 8-oxoberberine (**4**) [10], *β*-sitosterol (**5**) [12], oleanolic acid (**6**) [13], and gallic acid (**7**) [14], isolated for the first time from this species.

The medicinal plants with compounds possessing antifungal, antibacterial, antiparasitic, and antioxidant properties are used for the treatment of a number of infections [15, 16]. These agents are of great therapeutic importance because of emerging resistance to the usual drugs [17]. In the present study we determined the antifungal, antibacterial, and antioxidant potential of berberisinol (**1**).

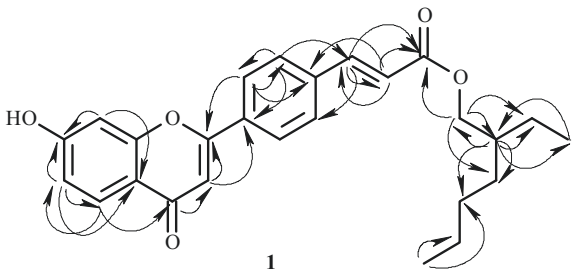


1

1) Department of Pharmacy, University of Peshawar, 25120, Peshawar, Pakistan, e-mail: samreengcp@gmail.com; 2) H.E.J. Research Institute of Chemistry, International Center for Chemical and Biological Sciences, University of Karachi, 75270, Karachi, Pakistan; 3) Department of Pharmacy Abdul Wali Khan University Mardan, 23200, Pakistan; 4) Department of Pharmacy, Sarhad University of Science and Information Technology, Peshawar, Pakistan. Published in *Khimiya Prirodnykh Soedinenii*, No. 2, March–April, 2019, pp. 214–216. Original article submitted November 26, 2017.

TABLE 1. ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR Data of Compound **1** ($CDCl_3$, δ, ppm, J/Hz)

C atom	δ_H	δ_C	C atom	δ_H	δ_C
2	–	161.4	4′	–	139.6
3	7.04 (1H, s)	108.3	7′	–	166.2
4	–	177.6	8′	6.39 (1H, d, J = 16.0)	116.4
5	7.38 (1H, d, J = 8.4)	131.5	9′	7.77 (1H, d, J = 16.0)	144.9
6	6.84 (1H, dd, J = 8.4, 2.7)	113.4	1″	4.20 (2H, m)	68.1
7	–	165.5	2″	1.28 (1H, m)	40.3
8	6.88 (1H, d, J = 2.7)	103.5	3″	1.65 (2H, m)	30.7
9	–	159.2	4″	1.37 (2H, m)	30.1
10	–	119.4	5″	1.28 (2H, m)	22.6
1′	–	129.1	6″	0.91 (3H, t, J = 7.0)	14.3
2′/6′	7.58 (2H, d, J = 8.2)	130.0	1‴	1.43 (2H, m)	24.0
3′/5′	7.10 (2H, d, J = 8.2)	127.2	2‴	0.89 (3H, t, J = 6.4)	11.2



1

Fig. 1. Important HMBC correlations of **1**.

Berberisinol (**1**) was obtained as a yellow gummy solid which gave a violet coloration with $FeCl_3$ for a phenol. The molecular formula was established as $C_{26}H_{28}O_5$ through HR-EI-MS showing an $[M]^+$ peak at *m/z* 420.1937 (calcd 420.1929). The IR spectrum showed the presence of a hydroxyl group (3380 cm^{-1}), α,β-unsaturated ester (1690 cm^{-1}), α,β-unsaturated ketone (1660 cm^{-1}), and an olefinic bond (1615 cm^{-1}). The ^{13}C NMR (Table 1) and DEPT spectra of **1** showed 26 carbon signals including two methyl, five methylene, eleven methine, and eight quaternary carbons. The signals at δ 161.4, 108.3, 177.6, 159.2, and 119.4 were typical of C-2, C-3, C-4, C-9, and C-10 carbons of the flavone skeleton [15]. The EI-MS showed the daughter fragments at *m/z* 136 and 284 due to retro Diels-Alder fragmentation revealing the presence of one hydroxyl group in ring A. The UV spectrum showed the characteristic absorption maxima at 215, 274, and 340 nm for a flavone skeleton [16]. On addition of NaOAc, a bathochromic shift of 12 nm was observed, suggesting the presence of hydroxyl group at C-7. This was confirmed by ^{1}H NMR, which showed doublets of H-5 and H-8 at δ 7.38 (1H, d, J = 8.4 Hz) and 6.88 (1H, d, J = 2.7 Hz) besides the signal of H-6 at δ 6.84 (1H, dd, J = 2.7, 8.4 Hz). The two signals of ring B with the AA′BB′ pattern at δ 7.58 (2H, d, J = 8.2 Hz) and 7.10 (2H, d, J = 8.2 Hz) indicated a 4′-substituted ring B. The involvement of ring B in the formation of a *para*-substituted cinnamic ester moiety was revealed by signals of the olefinic protons at δ 7.77 (1H, d, J = 16.0 Hz) and 6.39 (1H, d, J = 16.0 Hz). The larger value of the coupling constant allowed us to assign the *E*-configuration to the double bond. The ^{13}C NMR showed the signals of olefinic carbons at δ 144.9 and 116.4, along with the downfield signal of the ester carbonyl at δ 166.2. The alkyl group bound to the ester moiety could be identified as 2-ethylhexyl based on the NMR data illustrated in Table 1.

This was confirmed through acid hydrolysis, which furnished, besides other products, 2-ethylhexanol, which could be identified through the boiling point and comparison of the spectral data with literature. The structure of **1** could further be authenticated through HMBC experiments (Fig. 1).

The signal of H-5 showed J^3 correlations with C-7 (δ 165.5) and C-9 (δ 159.2). The H-8 showed J^2 correlations with C-7 and C-10 (δ 119.4) and J^3 correlations with C-6 (δ 113.4) and C-10. The more downfield olefinic proton at δ 7.77 showed J^3 correlations with C-3′/C-5′ (δ 127.2) as well as the carbonyl carbon of the ester moiety (δ 166.2). The oxymethylene protons at δ 4.20 showed J^3 correlations with the carbonyl carbon C-7; C-1‴ (δ 24.0), and C-3″ (δ 30.7). On the basis of these cumulative evidences the structure of berberisinol (**1**) could be assigned as (2″-ethylhexyl (*E*)-9′-[1′-(7-hydroxy-4-oxo-4*H*-chromen-2-yl)-phenyl]-8′-propenoate).

TABLE 2. Antifungal Activity of Compound **1**

Microorganism	**1** (zone of inhibition, mm)	Miconazole*	% Inhibition
M. canis	15 ± 0.9	37 ± 0.5	40.5
C. glabrata	14 ± 0.7	42 ± 0.4	33.3
F. solani	12 ± 0.3	33 ± 0.5	36.4
A. niger	15 ± 0.9	42 ± 0.9	35.7
A. flavus	15 ± 0.5	34 ± 0.9	44.1
C. albicans	14 ± 0.8	45 ± 0.4	31.1

Values are expressed in mean ± SD of three assays. *Positive control.

TABLE 3. Antibacterial Activity of Compound **1**

Microorganism	**1** (zone of inhibition, mm)	Imipenem (mm)*	% Inhibition
E. coli	29 ± 0.9	37 ± 0.5	79.7
B. subtilis	22 ± 0.7	39 ± 0.4	57.6
K. pneumoniae	25 ± 0.3	46 ± 0.5	54.4
S. aureus	30 ± 0.9	42 ± 0.9	71.4
S. pyogenes	33 ± 0.5	41 ± 0.9	80.4
P. aeroginosa	19 ± 0.8	45 ± 0.4	42.22

Legends are the same as in Table 2.

The antifungal potential of compound **1** was also tested by the agar well diffusion method [18] against different fungal strains, namely *C. glabrata*, *A. flavus*, *M. canis*, *F. solani*, *C. albicans*, and *A. niger*. However, no significant antifungal activity was observed against all the fungal strains (Table 2).

Compound **1** was evaluated for antibacterial activity by the agar well diffusion method [19]. It exhibited significant activity against *E. coli*, *S. aureus*, and *S. pyogenes*, moderate activity against *B. subtilis* and *K. pneumoniae*, and weak activity against *P. aeroginosa* (Table 3).

Compound **1** was evaluated for its antioxidant potential by the 1,1-diphenyl-2-picryl-hydrazyl (DPPH) free radical scavenging protocol [20].

EXPERIMENTAL

General Procedure. For column chromatography (CC), silica gel 230–400 mesh (Merck, Darmstadt, Germany) was used. Precoated silica gel F_{254} plates (Merck, 0.25 and 0.50 mm thickness) were used for thin-layer chromatography (TLC) detected at 254 nm and by spraying with ceric sulfate. A JASCO-320-A spectrophotometer was used for IR spectra. A Bruker AM-400 spectrometer (500 MHz for ^{1}H and 125 MHz for ^{13}C NMR) was used for recording of ^{1}H and ^{13}C NMR spectra in $CDCl_3$ with TMS as internal standard. A JEOL JMS-HX 110 mass spectrometer was used for recording of HR-EI-MS. A JASCO DIP-360 digital polarimeter (l = 10 cm) was used for the measurement of optical rotation.

Plant Material. The roots of the plant (35 kg) of *B. baluchistanica* were collected from Kalat District of Balochistan Province in April 2012. It was identified by Prof. Dr. Muhmmad Ibrar, the Plant Taxonomist, Department of Botany, University of Peshawar, where a voucher specimen (No. Bot. 20105 (PUP) has been deposited in the Herbarium of the Department.

Extraction and Isolation. The freshly collected shade-dried bark of roots (35 kg) was powdered and extracted by maceration in 90% methanol for 10 days (3 × 50 L). The combined methanolic extract was evaporated on a rotary evaporator at 45°C. The viscous extract (2.2 kg) was divided into subfractions soluble in *n*-hexane (85 g), ethyl acetate (250 g), chloroform (260 g), butanol (165 g), and water (55 g).

The EtOAc soluble subfraction was chromatographed over silica gel and eluted with mixtures of *n*-hexane–$CHCl_3$ and $CHCl_3$–MeOH in increasing order of polarity to obtain eight major fractions EtA_1–EtA_8. Fraction EtA_1 (467 mg) eluted

with *n*-hexane–$CHCl_3$ (6.0:4.0) was rechromatographed and eluted with *n*-hexane–$CHCl_3$ (7.0:3.0) to furnish *β*-sitosterol (**5**) (15 mg) and oleanolic acid (**6**) (10 mg). On increasing the polarity with *n*-hexane–$CHCl_3$ (5.0:5.0), gallic acid (**7**) (12 mg) was isolated. Fraction EtA_2 eluted with *n*-hexane–$CHCl_3$ (5.0:5.0) was again chromatographed and eluted with mixtures of *n*-hexane and $CHCl_3$ in increasing order of polarity. The fraction obtained with *n*-hexane–$CHCl_3$ (3.0:7.0) provided berberisinol (**1**) (35 mg). Fraction EA_3 obtained from *n*-hexane–$CHCl_3$ (2.0:8.0) was rechromatographed and eluted with $CHCl_3$–MeOH (4.5:5.5, 4.0:6.0, and 2.0:8.0) to afford berberine (**3**) (12 mg), 8-oxoberberine (**4**) (10 mg), and palmatine (**2**) (14 mg), respectively.

Berberisinol (1) was obtained as a yellow gummy solid, which gave a violet coloration with $FeCl_3$ for a phenol. UV (MeOH, λ_{max}, nm) (log ε): 220 (2.28), 288 (3.31). The molecular formula was established as $C_{26}H_{28}O_5$ through HR-EI-MS showing an $[M]^+$ peak at *m/z* 420.1937 (calcd 420.1929). IR (KBr, ν_{max}, cm^{-1}): 3380, 1690, 1660, and 1615. ^1H, ^{13}C NMR, see Table 1.

Acid Hydrolysis. A solution of berberisinol (4 mg) in MeOH (2 mL) containing 1 N HCl (2 mL) was refluxed for 4 h, concentrated under reduced pressure, and diluted with H_2O (4 mL). It was extracted with CH_2Cl_2, and the organic phase was extracted with dilute KOH, washed with water, dried, and freed of solvent under reduced pressure to afford a colorless liquid (bp 187°C), characterized as 2-ethylhexanol (M^+ peak at *m/z* 130, superimposable IR). The aqueous layer was acidified with dilute HCl and extracted with EtOAc. The residue recovered from the organic phase was found to be an inseparable mixture of products.

Bioassays. The antifungal activity was performed on different pathogens. The growth inhibitory potential of compound **1** and standard drug (both at the concentration of 100 μg/mL of Sabouraud glucose agar) was tested against different strains, *A. niger* (ATCC-10549), *F. solani* (ATCC-11712), *C. glabrata* (ATCC-90030), *A. flavus* (ATCC-32611), *C. albicans* (ATCC-2091), and *M. canis* (ATCC-11622) according to the previously reported protocol [21], and the results are illustrated in Table 2.

Similarly, compound **1** and standard drugs (each at a concentration of 100 μg/mL of nutrient agar) were tested for antibacterial potential. Antibacterial bioassay was carried out on six strains, including Gram-positive bacteria *S. pyogenes* (ATCC-29213), *S. aureus* (ATCC-25923), and *B. subtilis* (ATCC-6051), Gram-negative bacteria *P. aeruginosa* (ATCC-27853), *E. coli* (ATCC-25922), and *K. pneumoniae* (ATCC-700603) according to the literature [19]. The results are presented in Table 3.

The antioxidant activity of compound **1** (IC_{50} 52.5 ± 0.4 μg/10 μL) was determined by the DPPH free radical scavenging activity method [22]. Butylhydroxyanisole (IC_{50} 42.5 ± 0.6 μg/10 μL) was employed as standard.

ACKNOWLEDGMENT

We are grateful to Prof. Dr. Abdul Malik of HEJ Research Institute of Chemistry, University of Karachi for his help in structural elucidation of isolated compounds.

REFERENCES

1. T. Khan, I. A. Khan, and A. Rehman, *J. Med. Plants*, **2** (6), 16 (2014).
2. I. Khan, S. Najeebullah, M. Ali, and Z. K. Shinwari, *Trop. J. Pharm. Res.*, **15**, 2047 (2016).
3. R. Rao, T. Husain, B. Dutt, and A. Garg, *Rheedea-Kerala*, **8**, 1 (1998).
4. A. Perveen and M. Qaiser, *Pak. J. Bot*., **42**, 1 (2010).
5. F. Aqil and I. Ahmad, *Meth. Find Exp. Clin. Pharmacol*., **29**, 79 (2007).
6. R. Tareen and S. Qadir, *Pak. J. Bot.*, **23**, 90 (1991).
7. N. Baloch, S. Nabi, M. Yasser, and A. Kahraman, *J. Phytopharmacol.*, **4**, 282 (2013).
8. T. Bibi, M. Ahmad, R. B. Tareen, N. M. Jabeen, R. Rehman, S. U. Sultana, S. Zafar, and M. Yaseen, *J. Ethnopharmacol.*, **157**, 79 (2014).
9. M. Bi, Iqbal, J. Khan, T. Shahwani, N. A. Razzaque, G. Baloch, and M. Y. Baloch, *J. Sci. Techlol.*, **2306**, 185 (2016).
10. A. Karimov, M. Levkovich, N. Abdullaev, and R. Shakirov, *Chem. Nat. Compd*., **29**, 361 (1993).
11. I. M. Tadzhibaev, I. Zatorskaya, K. Lutfullin, and T. Shakirov, *Chem. Nat. Compd*., **10**, 42 (1974).
12. N. Kovganko and Z. N. Kashkan, *Chem. Nat. Compd.*, **30**, 533 (1994).

13. G. Genkina, L. Mzhelskaya, T. Shakirov, and N. Abubakirov, *Chem. Nat. Compd.*, **13**, 188 (1977).
14. Z. Tunalier, M. Kosar, N. Ozturk, K. H. C. Baser, H. Duman, and N. Kirimer, *Chem. Nat. Compd*., **40**, 206 (2004).
15. A. Stochmal, A. M. Simonet, F. A. Macias, M. A. Oliveira, J. M. Abreu, R. Nash, and W. Oleszek, *Phytochemistry*, **57** (8), 1223 (2001).
16. G. Cheng, Y. Bai, Y. Zhao, J. Tao, Y. Liu, G. Tu, L. Ma, N. Liao, and X. Xu, *Tetrahedron*, **56**, 8915 (2000).
17. T. C. White, K. A. Marr, and R. A. Bowden, *Clin. Microbiol. Rev.*, **11**, 382 (1998).
18. E. N. Quiroga, A. R. Sampietro, and M. A. Vattuone, *J. Ethnopharmacol.*, **74** (1), 89 (2001).
19. M. S. Alam, S.M. Rahman, and D.-U. Lee, *Chem. Papers*, **69** (8), 1118 (2015).
20. A. Shirwaikar, K. Rajendran, and I. S. R. Punitha, *Biol. Pharm. Bull.*, **29**, 1906 (2006).
21. M. I. Choudhary, Z. Parveen, A. Jabbar, and I. Ali, *Phytochemistry*, **40** (4), 1243 (1995).
22. I. Gulcin, D. Berashvili, and A. Gepdiremen, *J. Ethnopharmacol.*, **101** (1), 287 (2005).