ejpmr, 2020,7(10), 578-584

SJIF Impact Factor 6.222

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

CHEMICAL CONSTITUENTS FROM THE AERIAL PARTS OF *BARLERIA PRIONITIS* L.

Imran Khan[1], Hinna Hamid[1], Mohammed Ali[2]*, Mohammad Sarwar Alam[1], and Showkat Rassol Mir[2]

[1]Department of Chemistry, School of Chemical and Life Sciences, Jamia Hamdard (Hamdard University), New Delhi – 110 062, India.

[2]Phytochemistry Research Laboratory, School of Pharmaceutical Education and Research, Jamia Hamdard (Hamdard University), New Delhi - 110 062, India.

***Corresponding Author: Mohammed Ali**
Phytochemistry Research Laboratory, School of Pharmaceutical Education and Research,Jamia Hamdard Hamdard University), New Delhi - 110 062, India.

Article Received on 05/08/2020 **Article Revised on 25/08/2020** **Article Accepted on 15/09/2020**

ABSTRACT

Barleria prionitis L. (family Acanthaceae) is an erect, perennial, prickly, much branched shrub, used to treat catarrh, dropsy, dysuria, gastrointestinal disorders, jaundice, hepatic, nervine, paralytic and urinary disorders, rheumatism and sinusitis. This research work was proposed to isolate chemical constituents from the plant aerial parts and to characterize their structures. An ethanol extract of the aerial parts was adsorbed with silica gel for column, dried and chromatographed over a silica gel column packed in *n*-hexane. Various solvent mixtures of increasing polarity, viz., *n*-hexane, ethyl acetate and methanol were used to elute the column. The isolated chemical constituents were characterized as oleic acid (**1**), herniarin (7-methoxycoumarin, **2**), 1-hydroxyanthraquinonyl (3→7′)-coumarin (**3**), 8-dehydroxyemodin-(5→5′)- 8′-dehydroxyemodin (**4**), *n*-tetracosane (**5**), (Z)-*n*-heptatriacont-8-ene (**6**), β- sitosterol *n*-octadec-9′-enoate (β- sitosterol oleate, **7**), stigmast-5,22–dien-3β –ol 3-O-α-D-glucopyranoside (**8**) and [1,1'-biphenyl]- 2,3,4,5,6, 2', 3', 4', 5',6'-decaol (**9**). Their structures were established on the basis of spectral data analysis and chemical reactions.

KEYWORDS: *Barleria prionitis* L., aerial parts, extraction, phytoconstituents, isolation, spectral data, characterization.

INTRODUCTION

Barleria prionitis L., syn. *B. coriacea* Oberm., *B. echinata* St.-Lag., *B. quadrispinosa* Stokes, *B. spicata* Roxb. (family Acanthaceae), known as vjradanti and porcupine flower, is found in India, Sri Lanka and eastern, southern and central Africa. It is an erect, perennial, prickly, much branched shrub, 0.6 to 1.7 m high; spines sharp, axillary; bark whitish; stems and branches terete, glabrous; leaves elliptic, acuminate, opposite, entire, bristle-tipped, lineolate, glabrous, base tapering into the petiole; flowers axillary or terminal spikes, sessile, yellow; fruits brown, glabrescent, beaked capsule; seeds 2, orbicular, compressed, hairy.[1] The leaves are antiseptic, diuretic and tonic, used to treat febrile catarrh, fever, gastric ulcer, indigestion with constipation, jaundice, liver diseases, premature ejaculation, rheumatism, toothache and urinary infections. A leaf paste or juice is applied to heal feet cracking, laceration, joint pains, pimples, toothache and wounds; the juice is installed into the ear to cure otitis.[2-5] The plant aerial parts are beneficial as a febrifuge, to reduce dropsy, jaundice, gastrointestinal, hepatic, paralytic and urinary disorders, rheumatism, stiffness of limbs, enlargement of scrotum and sciatica. The bitter plant juice is given to children to cure catarrh. The plant extracts are antiseptic, diuretic, tonic and incorporated into herbal cosmetics and hair products to promote skin and scalp health and to treat dysuria, rheumatic affections, internal abscesses, nervine disorders and chronic sinusitis. The plant ash is given with honey to relieve bronchial asthma. The stem bark is diaphoretic and expectorant, used against anasarca and whooping cough. The flowers are useful to comfort internal abscesses, viral fever, haemoptysis, migraine, obesity, oedema, urethral discharges, seminal disorders and painful menstruation.[2-6] The roots are abortifacient and febrifuge. An infusion of the roots and leaves is applied to subside boils and sores, to reduce glandular swellings, and to calm down earache and headache. A mouthwash made from the roots is effective to relieve toothache and to arrest gum bleeding.[2-5]

The *B. prionitis* plant contained balarenone, pipataline, lupeol and prionisides A-C, 6-O-trans-p-coumaroyl-8-O-acetylshanzhiside methyl ester and its cis isomer, phenylethanoid glycoside, barlerinoside, shanzhiside methyl ester, 6-O-trans-p-coumaroyl-8-O-acetylshanzhiside methyl ester, barlerin, acetylbarlerin, 7-methoxydiderroside and lupulinoside, 1,8-dihydroxy -2,7-dimethyl 3,6-dimethoxy anthraquinone and 1,3,6,8-

tetramethoxy-2,7-dimethyl anthraquinone.[7-11] The leaves and other plant parts afforded scutellarein, melilotic, syringic, vanillic and p-hydroxybenzoic acids, 6-hydroxyflavones, apigenin and luteolin-7-O-glucosides, β-sitosterol, scutellarein 7-neohesperidoside, 13, 14-seco-stigmasta-5, 14-diene-3-alpha-ol, 6-O-acetylshanzhiside methyl ester, α-amyrin, verbascoside verbascoside and stigmasterol-3-O-D-glucoside. [6,12–14] Keeping in view the various therapeutic values of the plants and the development of ecofriendly, biodegradable and safer herbal preparations, the aerial parts of *Barleria prionitis* were procured from Delhi to isolate and characterize their chemical constituents.

MATERIALS AND METHODS

The protocols of all methodologies (procedures, experimental designs and analysis assays) were adopted from the earlier published work.[15-17]

General procedures: The melting points were determined in one end open capillary tubes on a melting point M-560 apparatus (Perfit, India) heated thermoelectrically. UV spectra were determined with Lambda Bio 20 spectrophotometer (Perkin Elmer, Schwerzenbach, Switzerland) in methanol. The IR spectra were recorded by using KBr pellets with Jasco FT/IR-5000 Spectrometer (FTS 135, Hong Kong). The ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR spectra were recorded on Bruker DRX Spectrometer (Rheinstetten, 2 Germany) using $CDCl_3$ or DMSO-d_6 as a solvent and TMS (Fluka analytical, Sigma-Aldrich, Netherland) as an internal standard. Mass spectra were recorded on a Jeol JMS-D 300 instrument using Argon/Xenon gas as the ESI. *n*-Hexane, ethyl acetate, chloroform, methanol and other solvents of analytical grade were purchased from E. Merck (India) Ltd, New Delhi. Silica gel with 60-120 mesh particle size was procured from Qualigens, Mumbai, India and used for column chromatography. The purity of the isolated compounds was checked on precoated TLC plates with Silica gel 60 F_{254} (Merck, 0.25 mm) and the spots were visualized by exposure to iodine vapors or under UV radiations and spraying with ceric sulfate solution.

Plant material: The aerial parts of *Barleria prionitis* were procured from the Khari Baoli market and authenticated by Prof. M. P. Sharma, Taxonomist, Department of Botany, Jamia Hamdard, New Delhi. A voucher specimen of the plant material is preserved in the herbarium of the Department of Pharmacognosy and Phytochemistry, Jamia Hamdard, New Delhi.

Extraction and isolation: The aerial parts of *Barleria prionitis* were shade dried for three days, coarsely powdered (2.0 kg) and extracted with ethanol (95%) in a Soxhlet apparatus. The solvent was evaporated under reduced pressure to yield a dark brown viscous mass (426 g). The dried residue (400 g each) was dissolved in minimum amount of methanol and adsorbed on silica gel column grade (60-120 mesh) to obtain a slurry. It was air-dried and chromatographed over a silica gel column (1.6 m x 16 mm x 2 mm) packed in *n*-hexane. Various solvent mixtures of increasing polarity, viz., *n*-hexane, *n*-hexane-ethyl acetate (9:1, 3:1, 1:1, 1:3, *v/v*), ethyl acetate and ethyl acetate - methanol (99: 1; 97: 3; 19: 1; 93: 7, v/v) were used to elute the column. The fractions were collected separately and matched by TLC to check homogeneity. Similar fractions having the same R_f values were combined and crystallized. The isolated compounds were recrystallized to get the pure compounds.

Oleic acid (**1**)

Elution of the column with *n*-hexane produced a pale yellow oily mass of **1**, recrystallized from chloroform-methanol (1:1), yield 89 mg, m. p. 38 - 39 ^{0}C, R_f 0.85 (*n*-hexane); IR υ_{max} (KBr): 3405, 2953, 2841, 1677, 1605, 1376, 1295, 935, 1131, 1097, 942, 766 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 5.28 (1H, m, H-9), 5.25 (1H, m, H-10), 2.27 (2H, t, J = 7.2 Hz, H_2-2), 1.98 (2H, m, H_2-11), 1.93 (2H, m, H_2-8), 1.57 (2H, m, H_2-7), 1.52 (2H, m, H_2-12), 1.23 (4H, m, 2 x CH_2), 1.18 (14H, brs, 7 x CH_2), 0.80 (3H, t, J = 6.4 Hz, Me-18); ^{13}C NMR ($CDCl_3$): δ 180.21 (C-1), 34.12 (C-2), 29.72 (C-3), 29.68 (C-4), 29.61 (C-5), 29.54 (C-6), 29.45 (C-7), 31.34 (C-8), 130.02 (C-9), 129.72 (C-10), 29.78 (C-11), 29.38 (C-12), 29.34 (C-13), 29.04 (C-14), 27.16 (C-15), 24.67 (C-16), 22.68 (C-17), 14.06 (C-18); ESI MS *m/z* (rel. int.): 282 $[M]^+$ ($C_{18}H_{34}O_2$) (100).

Herniarin (**2**)

Elution of the column with *n*-hexane- ethyl acetate (3:2) mixture afforded colourless needles of **2**; recrystallized from acetone; 104 mg, m. p. 117 - 119 ºC; UV λmax (MeOH): 253, 296, 363 nm (log ε 2.6, 5.4, 1.8); IR υ_{max} (KBr): 2937, 2832, 1680, 1528, 1431, 1299, 1204, 1119, 1029, 917 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 7.79 (1H, d, J = 9.2 Hz, H-4), 7.46 (1H, d, J = 8.1 Hz, H-5), 7.41 (1H, m, H-6), 7.38 (1H, d, J = 2.0 Hz, H-8), 6.32 (1H, d, J = 9.2 Hz, H-3), 3.83 (3H, brs, OMe); ^{13}C NMR ($CDCl_3$): δ 167.21 (C-2), 114.80 (C-3), 147.01 (C-4), 123.39 (C-5), 131.32 (C-6), 150.94 (C-7), 112.68 (C-8), 150.94 (C-9), 121.63 (C-10), 55.43 (OMe); ESI MS *m/z* (rel. int.): 176 $[M]^+$ ($C_{10}H_8O_3$) (2.3).

1-Hydroxyanthraquinonyl (3→7′)-coumarin (**3**)

Elution of the column with *n*-hexane-ethyl acetate (3:17) mixture afforded red crystals of **3**; recrystallized from chloroform-methanol (1:1); 113 mg, m. p. 226 -228 ºC; R_f 0.49 (*n*-hexane -ethyl acetate, 3:17); UV λmax (MeOH): 251, 295, 352 nm (log ε 2.2, 5.4, 4.7); IR υ_{max} (KBr): 3443, 2918, 2872, 1678, 1660, 1634, 1545, 1450, 1310, 1265, 885 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 8.71 (1H, d, J = 2.9 Hz, H-4), 8.68 (1H, d, J = 2.9 Hz, H-2), 8.37 (1H, d, J = 7.4 Hz, H-8), 8.33 (1H, d, J = 7.5 Hz, H-5), 8.27 (1H, m, H-6), 8.13 (1H, m, H-7), 8.11 (1H, d, J = 9.6 Hz, H-4′), 7.55 (1H, m, H-6′), 7.53 (1H, d, J = 2.8 Hz, H-8′), 7.25 (1H, d, J = 7.5 Hz, H-5′), 7.21 (1H, d, J = 9.6 Hz, H-3′); ^{13}C NMR ($CDCl_3$): δ 165.90 (C-1), 127.48 (C-2), 140.09 (C-3), 126.95 (C-4), 135.17 (C-5),

135.37 (C-6), 129.82 (C-7), 133.74 (C-8), 182.11 (C-9), 180.51 (C-10), 133.12 (C-11), 121.61 (C-12), 112.39 (C-13), 134.26 (C-14), 167.20 (C-2′), 113.23 (C-3′), 147.22 (C-4′), 121.61 (C-5′), 135.74 (C-6′), 135.70 (C-7′), 106.62 (C-8′), 163.36 (C-9′), 120.34 (C-10′); ESI MS *m/z* (rel. int.): 368 $[M]^+$ ($C_{23}H_{12}O_5$) (2.8).

8-Dehydroxyemodin-(5→5′)- 8′-dehydroxyemodin (4)

Elution of the column with *n*-hexane-ethyl acetate (4:1) mixture gave red crystals of **4**; recrystallized from chloroform-methanol (1:1); 97 mg, m. p. 172 -173 ºC; R_f 0.7 (*n*-hexane -ethyl acetate, 4:1); UV λmax (MeOH): 293, 343 nm (log ε 5.1, 4.2); IR υ_{max} (KBr): 3321, 2814, 1671, 1664, 1651, 1576, 1451, 1305, 1241, 880 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 8.11 (1H, d, J = 2.8 Hz, H-2), 8.05 (1H, d, J = 2.8 Hz, H-4), 7.98 (1H, d, J = 7.8 Hz, H-8), 7.54 (1H, m, H-7), 7.19 (1H, d, J = 7.0 Hz, H-6), 2.24 (3H, brs, Me-15), 8.08 (1H, d, J = 3.0 Hz, H-2′), 8.02 (1H, d, J = 3.0 Hz, H-4′), 7.95 (1H, d, J = 7.8 Hz, H-8′), 7.48 (1H, m, H-7′), 7.17 (1H, d, J = 7.0 Hz, H-6′), 2.21 (3H, brs, Me-15′); ^{13}C NMR ($CDCl_3$): δ 162.83 (C-1), 126.61 (C-2), 144.60 (C-3), 125.19 (C-4), 135.09 (C-5), 133.81 (C-6), 129.27 (C-7), 126.60 (C-8), 182.86 (C-9), 181.49 (C-10), 130.76 (C-11), 120.86 (C-12), 112.18 (C-13), 132.87 (C-14), 21.36 (C-15), 162.72 (C-1′), 126.67 (C-2′), 143.96 (C-3′), 126.56 (C-4′), 135.03 (C-5′), 134.34 (C-6′), 129.34 (C-7′), 129.27 (C-8′), 181.45 (C-9′), 181.17 (C-10′), 130.88 (C-11′), 120.97 (C-12′), 112.37 (C-13′), 133.03 (C-14′), 21.30 (C-15′); ESI MS *m/z* (rel. int.): 474 $[M]^+$ ($C_{30}H_{18}O_6$) (2.5), 237 (31.2).

***n*-Tetracosane (5)**

Elution of the column with hexane - ethyl acetate (1:3) afforded colourless amorphous powder of **5**, m. p. 104 – 106 ^{o}C; R_f 0.5 (chloroform-methanol, 1:1); UV λmax (MeOH): 205 nm (log ε 2.9). IR υ_{max} (KBr): 2927, 2838, 1454, 1380, 1261, 1057, 951, 721 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 1.55 (2H, m, CH_2), 1.34 (2H, m, CH_2), 1.29 (40H, m, 20 x CH_2), 0.87 (3H, t, J = 6.5 Hz, Me-1), 0.84 (3H, t, J = 6.5 Hz, Me-24); ^{13}C NMR ($CDCl_3$): δ 14.16 (C-1), 22.68 (C-2), 27.61 (C-3), 29.24 (C-4), 29.49 (C-5), 29.65 (C-6), 31.48 (C-7), 29.73 (C-8 to C-18), 34.27 (C-19), 29.58 (C-20), 29.37 (C-21), 28.46 (C-22), 25.34 (C-23), 14.09 (C-24); ESI MS *m/z* (rel. int.): 338 $[M]^+$ ($C_{26}H_{54}$) (48.5).

(Z)-*n*-Heptatriacont-8-ene (6)

Further elution of the column with hexane - ethyl acetate (1:3) produced a colourless amorphous powder of **6**, yield 67 mg, m. p. 109 – 111^{o}C, R_f 0.8 (*n*-hexane); UV λmax (MeOH): 213 nm (log ε 4.7); IR υ_{max} (KBr): 2937, 2825, 1635, 1454, 1335, 1261, 1125, 925, 721 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 5.30 (1H, m, $w_{1/2}$ = 6.9 Hz, H-8), 5.25 (1H, m, $w_{1/2}$ = 6.6 Hz, H-9), 2.27 (2H, m, H_2-7), 1.98 (2H, m, H_2-10), 1.91 (2 H, m, H_2-6), 1.74 (2H, m, H_2-11), 1.59 (6 H, brs, 3 x CH_2), 1.52 (30 H, br s, 15 x CH_2), 1.23 (4H, m, H_2-2, H_2-36), 1.18 (18H, brs, 9 x CH_2), 0.82 (3 H, t, J = 6.5 Hz, Me-1), 0.77 (3 H, t, J = 6.6 Hz, Me-37); ^{13}C NMR ($CDCl_3$): δ 16.09 (C-1), 25.71 (C-2), 26.68 (C-3), 29.78 (C-4), 31.91 (C-5), 37.18 (C-6), 39.74 (C-7), 124.28 (C-8), 124.21 (C-9), 39.71 (C-10), 33.72 (C-11), 29.87 (C-12), 29.74 (C-13), 29.72 (C-14), 29.67 (C-15, C-16), 29.55 (C-17 to C-24), 29.37 (C-25 to C-31), 29.32 (C-32), 29.27 (C-33), 28.30 (C-34), 26.77 (C-35), 22.72 (C-36), 14.13 (C-37); ESI MS *m/z* (rel. int.): 518 $[M]^+$ ($C_{37}H_{74}$) (42.5), 419 (33.9), 393 (52.1), 349 (11.8), 125 (21.3), 99 (16.1).

β- Sitosterol oleate (7)

Elution of the column with ethyl acetate furnished colourless amorphous powder of **7**, recrystallized from chloroform – methanol (1:1), yield 63 mg, R_f 0.61 (*n*-hexane – ethyl acetate, 9:1), m. p. 232 – 234 °C; UV λmax (MeOH): 212 nm (log ε 4.8); IR υ_{max} (KBr): 2927, 2841, 1725, 1643, 1454, 1370, 1262, 1151, 1033, 835, 721 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 5.30 (1H, m, H-6), 5.27 (1H, m, H-9′), 5.04 (1H, m, H-10′), 4.50 (1H, brm, $w_{1/2}$ = 18.5 Hz, H-3α), 2.23 (2H, t, J = 8.8 Hz, H_2-2′), 0.98 (3H, brs, Me-19), 0.88 (3H, d, J= 7.3 Hz Me-21), 0.78 (3H, d, J = 6.0 Hz, Me-26), 0.75 (3H, d, J= 6.3 Hz, Me-27), 0.72 (3H, t, J= 6.5 Hz, Me-18′), 0.70 (3H, d, J = 6.2 Hz Me-29), 0.63 (3H, brs, Me-18), 2.19-1.02 (57H, m, 25 x CH_2, 7 x CH); ^{13}C NMR ($CDCl_3$): δ 37.14 (C-1), 30.93 (C-2), 72.64 (C-3), 41.96 (C-4), 138.65 (C-5), 120.56 (C-6), 30.89 (C-7), 33.36 (C-8), 49.01 (C-9), 36.05 (C-10), 21.70 (C-11), 38.71 (C-12), 41.17 (C-13), 55.67 (C-14), 27.24 (C-15), 28.27 (C-16), 55.01 (C-17), 10.83 (C-18), 20.09 (C-19), 35.99 (C-20), 18.81 (C-21), 35.56 (C-22), 26.80 (C-23), 44.80 (C-24), 30.84 (C-25), 18.30 (C-26), 18.02 (C-27), 24.16 (C-28), 10.96 (C-29), 172.25 (C-1′), 59.10 (C-2′), 35.14 (C-3′), 28.11 (C-4′), 28.16 (C-5′), 28.38 (C-6′), 29.47 (C-7′), 28.72 (C-8′), 128.94 (C-9′), 128.71 (C-10′), 28.60 (C-11′), 28.33 (C-12′), 28.27 (C-13′), 28.09 (C-14′), 26.15 (C-15′), 25.03 (C-16′), 22.04 (C-17′), 13.24 (C-18′); ESI MS *m/z* (rel. int.): 678 $[M]^+$ ($C_{47}H_{82}O_2$) (2.3), 413 (6.8), 281 (11.2), 265 (8.5).

Stigmasterol 3 β -O-α-D-glucopyranoside (8)

Elution of the column with ethyl acetate - methanol (19:1) furnished colourless amorphous powder of **8**, recrystallized from chloroform – methanol (1:1), yield 106 mg, R_f 0.4 (chloroform – methanol, 4:1), m. p. 249 – 250 °C; UV λ_{max} (MeOH): 213 nm (log ε 5.1); IR υ_{max} (KBr): 3510, 3425, 3341, 2927, 2839, 1635, 1464, 1378, 1166, 1073, 1023 cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 5.30 (1H, m, H-6), 5.22 (1H, m, H-22), 5.03 (1H, m, H-23), 3.47 (1H, brm, $w_{1/2}$ = 18.3 Hz, H-3α), 1.01 (3H, brs, Me-19), 0.92 (3H, d, J = 6.4 Hz, Me-21), 0.83 (3H, d, J = 6.1 Hz, Me-26), 0.79 (3H, d, J = 6.3 Hz, Me-27), 0.76 (3H, t, J = 7.2 Hz, Me-29), 0.64 (3H, brs, Me-18), 2.51 to 1.12 (25 H, m, 9 x CH_2, 7 x CH), 4.86 (1H, d, J = 4.0 Hz, H-1′), 4.78 (1H, m, H-5′), 4.31 (1H, dd, J = 4.0, 7.1 Hz, H-2′), 4.23 (1H, m, H-3′), 3.67 (1H, m, H-4′), 3.15 (2H, d, J = 6.5 Hz, H_2-6′); ^{13}C NMR (DMSO-d_6): δ 36.83 (C-1), 29.23 (C-2), 73.36 (C-3), 41.81 (C-4), 140.25 (C-5), 121.12 (C-6), 27.75 (C-7), 31.38 (C-8), 50.61 (C-9), 36.18 (C-10), 23.82 (C-11), 38.31 (C-12), 41.69 (C-13),

56.16 (C-14), 24.88 (C-15), 25.43 (C-16), 55.40 (C-17), 11.70 (C-18), 19.03 (C-19), 35.49 (C-20), 20.55 (C-21), 137.93 (C-22), 128.68 (C-23), 49.58 (C-24), 29.62 (C-25), 18.51 (C-26), 18.73 (C-27), 22.55 (C-28), 12.01 (C-29), 100.81 (C-1′), 76.71 (C-2′), 76.52 (C-3′), 69.99 (C-4′), 77.12 (C-5′), 61.10 (C-6′); ESI MS *m/z* (rel. int.): 574 $[M]^+$ ($C_{35}H_{58}O_6$) (2.1), 411 (13.3), 395 (5.6), 179 (8.5), 163 (18.1).

Decahydroxydibenzene (**9**)

Further elution of the column with ethyl acetate - methanol (19:1) mixture yielded yellow mass of **9**; recrystallized from acetone; 104 mg, m. p. 252 - 254 ºC; R_f 0.45 (chloroform – methanol, 1:1); UV λmax (MeOH): 251, 296 nm (log ε 2.8, 5.7); IR υ_{max} (KBr): 3350, 3227, 2972, 2815, 1635, 1547, 1465, 1378, 1276, 930 cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 8.16 (1H, s, OH, D_2O exchangeable), 6.64 (9H, s, 9 x OH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6): δ 165.92 (10 x C-OH), 133.88 (C-C); ESI MS *m/z* (rel. int.): 314 $[M]^+$ ($C_{12}H_{10}O_{10}$) (2.8).

RESULTS AND DISCUSSION

Compound **1** was a familiar fatty acid characterized as oleic acid.[18,19] Compound **2** was a known coumarin characterized as herniarin (7-methoxycoumarin).[20,21]

Compound **3** responded positive tests for anthraquinones, showed UV absorption maxima at 251, 295, 352 nm for anthraquinones and had IR absorption bands for a hydroxyl group (3443 cm^{-1}), carbonyl functions (1678, 1660, 1634 cm^{-1}) and aromaticity (1545 cm^{-1}). Its molecular ion peak was established at *m/z* 368 on the basis of mass and ^{13}C NMR spectra consistent to a molecular formula of an anthraquinone linked with a coumarin, $C_{23}H_{12}O_5$. The ^{1}H NMR spectrum of **3** exhibited four one-proton doublets at δ 8.71 (J = 2.9 Hz), 8.68 (J = 2.9 Hz), 8.37 (J = 7.4 Hz) and 8.33 (J = 7.5 Hz) assigned to aromatic meta-coupled H-4 and H-2 protons, and ortho-coupled H-8 and H-5 protons, respectively, two one-proton multiplets at δ 8.27 (H-6) and 8.13 (H-7), the coumarin protons as one-proton doublets at δ 8.11 (J = 9.6 Hz) and 7.21 (J = 9.6 Hz) ascribed to vinylic H-4′ and H-3′ protons, as one-proton doublets at δ 7.53 (J = 2.8 Hz) and 7.25 (J = 7.5 Hz) accounted to aromatic H-8′ and H-5′ protons, respectively, and as a one-proton multiplet at δ 7.55 attributed to H-6′ proton. The ^{13}C NMR spectrum of **3** showed signals for carbonyl carbons of anthraquinone unit at δ 182.11 (C-9) and 180.51 (C-10), for coumarin carbonyl carbon at δ 167.20 (C-2′), and for vinylic and aromatic carbons between δ 165.90 - 106.62. On the basis of these evidences, the structure of **3** has been characterized as 1-hydroxyanthraquinonyl (3→7′)-coumarin, a new anthraquinone linked with a coumarin (Fig. 1).

Compound **4** exhibited UV absorption maxima at 293 and 343 nm for anthraquinones and IR absorption bands for hydroxyl groups (3321 cm^{-1}), conjugated carbonyl functions (1671, 1664, 1651 cm^{-1}) and aromaticity (1576 cm^{-1}). On the basis of mass and ^{13}C NMR spectra the molecular ion peak of **4** was determined at *m/z* 474 consistent to a molecular formula of a dianthraquinone, $C_{30}H_{18}O_6$. An ion fragment produced at *m/z* 237 [C_5 – $C_{5'}$ fission, $C_{15}H_9O_3$]$^+$ indicated that two anthraquinone units were linked to each other. The ^{1}H NMR spectrum of **4** exhibited four deshielded one-proton meta-coupled doublets at δ 8.11 (J = 2.8 Hz), 8.05 (J = 2.8 Hz), 8.08 (J = 3.0 Hz) and 8.02 (J = 3.0 Hz) assigned to aromatic H-2, H-4, H-2′ and H-4′ protons, respectively, four ortho-coupled doublets at δ 7.98 (J = 7.8 Hz), 7.19 (J = 7.0 Hz), 7.95 (J = 7.8 Hz) and 7.17 (J = 7.0 Hz) ascribed correspondingly to H-8, H-6, H-8′ and H-6′ protons, two one-proton multiplets at δ 7.54 (H-7) and 7.48 (H-7′) and two three-proton singlets at δ 2.24 and 2.21 associated with C-15 and C-15′ methyl protons linked to aromatic carbons. The ^{13}C NMR spectrum of **4** displayed signals for carbonyl carbons of anthraquinone units at δ 182.86 (C-9), 181.49 (C-10,), 181.45 (C-9′) and 181.17 (C-10′), aromatic carbons between δ 162.83 – 112.18, and methyl carbons at δ 21.36 (C-15) and 21.30 (C-15′). The presence of C-5 and C-5′ carbon signals in the downfield region at δ 135.09 and 135.03, respectively, suggested C-5→ C-5′ linkage of the anthraquinone units. These data led to establish the structure of **4** as 8-dehydroxyemodin-(5→5′)- 8′-dehydroxyemodin, a new anthraquinone derivative (Fig. 1).

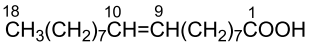
Compounds **5** was a long chain aliphatic hydrocarbon identified as *n*-tetracosane.[22, 23]

Compound **6** showed IR absorption bands for unsaturation (1635 cm^{-1}) and long aliphatic chain (721 cm^{-1}). Its mass spectrum displayed a molecular ion peak at *m/z* 518 consistent with the molecular formula of a long chain alkene, $C_{37}H_{74}$. The generation of the ion peaks at *m/z* 419 [C_7-C_8 fission, $CH_3(CH_2)_{27}CH{=}CH]^+$, 99 $[M - 419]^+$, 393 [C_9-C_{10} fission, $CH_3(CH_2)_{27}]^+$ and 125 $[M - 393]^+$ indicated the presence of the vinylic linkage at C-8 position in the alkene chain. The ^{1}H NMR spectrum of **6** exhibited two one-proton multiplets at δ 5.30 and 5.25 with half-widths of 6.9 and 6.6 Hz assigned correspondingly to cis-oriented vinylic H-8 and H-9 protons. Two triplets integrating each for three protons at δ 0.82 (J = 6.5 Hz) and 0.77 (J = 6.6 Hz) were due to C-1 and C-37 primary methyl protons, respectively. The remaining methylene protons appeared between δ 2.27 - 1.18. The ^{13}C NMR spectrum of **6** showed signals for vinylic carbons at δ 124.28 (C-8) and 124.21 (C-9), methylene carbons between δ 39.74 - 22.72 and methyl carbons at δ 16.09 (C-1) and 14.13 (C-37). The absence of any signal from δ 5.01 to 2.08 in the ^{1}H NMR spectrum and between δ 124.28 - 39.74 in the ^{13}C NMR spectrum ruled out the existence of any carbinol proton in the molecule. On the basis of the foregoing account, the structure of **6** was formulated as (Z)-*n*-heptatriacont-8-ene, a new alkene (Fig. 1).

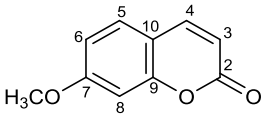
Compound **7** was a known phytosterol ester identified as β-sitosterol *n*-octadec-9′-enoate (β-sitosterol oleate).[24,25]

Compound **8**, $[M]^+$ at *m/z* 574 ($C_{35}H_{58}O_6$), responded positively to steroidal glycoside tests and showed IR characteristic absorption bands for hydroxyl groups (3510, 3425, 3341 cm^{-1}) and unsaturation (1635 cm^{-1}). The mass ion peaks generated at *m/z* 411 [M – $C_6H_{11}O_5$, 163]$^+$, 395 [411- Me]$^+$ and 179 $[C_6H_{11}O_6]^+$ suggesting that stigmasterol was linked with a hexoside unit. The ^{1}H NMR spectrum of **8** displayed three one-proton multiplets at δ 5.30, 5.22 and 5.03 assigned to vinylic H-6, H-22 and H-23 protons, respectively. A one-proton doublet at δ 4.86 (J = 4.0 Hz) was ascribed to α-oriented anomeric H-1′ proton. The other sugar proton appeared as one-proton multiplets at δ 4.78 (H-5′), 4.23 (H-3′) and 3.67 (H-4′), as a one-proton double doublet at δ 4.31 (J = 4.0, 7.1 Hz, H-2′) and as a two-proton doublet at δ 3.15 (J = 6.5 Hz, H_2-6′). A one-proton broad multiplet at δ 3.47 with half width of 18.3 Hz was attributed to *α*-oriented oxymethine H-3 proton. Two three-proton broad singlets at δ 0.64 and 1.01 were assigned to tertiary C-18 and C-19 methyl protons, respectively. Three doublets at δ 0.92 (J = 6.4 Hz), 0.83 (J = 6.1 Hz) and 0.79 (J = 6.3 Hz) and a triplet at δ 0.76 (J = 7.2 Hz), all integrating for three protons each, were accounted to secondary C-21, C-26 and C-27 methyl and primary C-29 methyl protons, respectively, all attached to the saturated carbons. The remaining methylene and methine protons resonated between δ 2.51 - 1.12. The ^{13}C NMR spectrum of **8** showed signals for vinylic carbons at δ 140.25 (C-5), 121.12 (C-6), 137.93 (C-22) and 128.68 (C-23), oxymethine carbon at δ 73.36 (C-3), anomeric carbon at δ 100.81 (C-1′), other sugar carbons from δ 77.12 to 61.10 and the remaining methyl, methylene and methine carbons between δ 56.16 -11.70. The ^{1}H NMR and ^{13}C NMR spectral data of the steroidal nucleus were compared with other stigmasterol-type molecules.[26,27] Acid hydrolysis of **8** yielded stigmasterol, m. p. 166-168 °C; R_f 0.43 (petroleum ether - chloroform - methanol, 7:1:2); and D-glucose, R_f 0.26 (*n*-butanol- acetic acid – water, 4 : 1 : 5). On the basis of spectral data analysis and chemical reactions, the structure of **8** has been established stigmast-5,22–dien-3β –ol 3-O-α-D-glucopyranoside, a new steroidal glucoside.

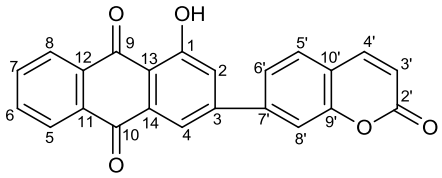
Compound **9**, $[M]^+$ at *m/z* 314 ($C_{12}H_{10}O_{10}$), gave positive tests for phenols, showed UV absorption maxima at 251, 296 nm for aromatic compounds and IR absorption bands for hydroxyl groups (3350, 3227 cm^{-1}) and aromaticity (1547 cm^{-1}). The ^{1}H NMR spectrum of **9** exhibited two D_2O exchangeable signals at δ 8.16 (1H) and 6.64 (9H) assigned to protons to the phenolic group protons. The ^{13}C NMR spectrum of **9** displayed signals for phenolic carbons at δ 165.92 (10 x C-OH) and aromatic carbons at δ 133.88 (C-C). These spectral data led to establish the structure of **9** as [1,1'-biphenyl]-2,3,4,5,6, 2', 3', 4', 5',6'-decaol.



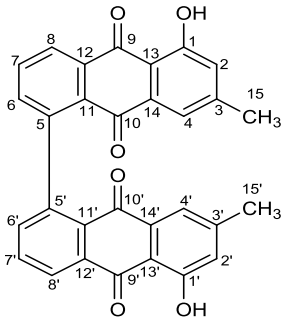
Oleic acid **(1)**



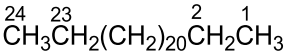
Herniarin **(2)**



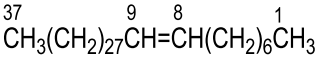
1-Hydroxyanthraquinonyl-(3→7′)-coumarin **(3)**



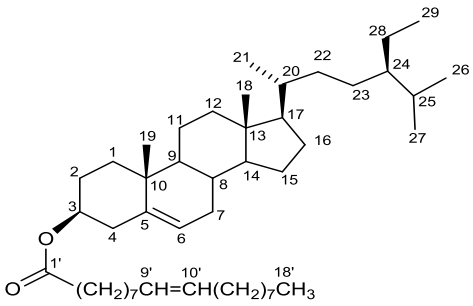
8-Dehydroxyemodin (5→5′)-8′-dehydroxyemodin **(4)**



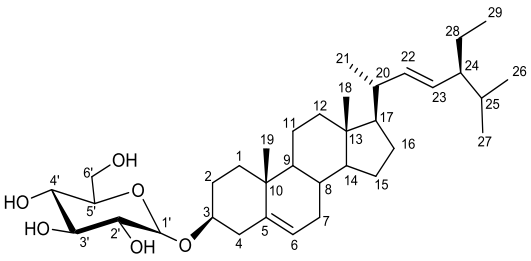
n-Tetracosane **(5)**



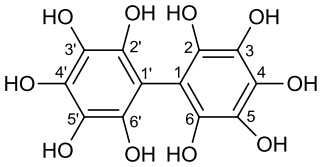
(Z)-*n*-Heptatriacont-8-ene **(6)**



β- Sitosterol oleate (**7**)



Stigmasterol-3β-*O*-α-D-glucopyranoside **(8)**



[1, 1′-Biphenyl]-2, 3, 4, 5, 6, 2′, 3′, 4′, 5′, 6′-decaol **(9)**

Fig.1. Chemical constituents 1 - 9 isolated from the aerial parts of *Barleria prionitis*.

CONCLUSION

Phytochemical investigation of the aerial parts of *Barleria prionitis* afforded oleic acid (**1**), herniarin (**2**), 1-hydroxyanthraquinonyl (3→7′)-coumarin (**3**), 8-dehydroxyemodin-(5→5′)- 8′-dehydroxyemodin (**4**), *n*-tetracosane (**5**), (Z)-*n*-heptatriacont-8-ene (**6**), β-sitosterol oleate (**7**), stigmast-5,22–dien-3β –ol 3-O-α-D-glucopyranoside (**8**) and [1,1'-biphenyl]- 2,3,4,5,6, 2', 3', 4', 5',6'-decaol (**9**). This work has enhanced understanding about the phytoconstituents of the undertaken plant. These secondary metabolites can be used as analytical markers for quality control of the aerial parts of *B. prionitis*.

ACKNOWLEDGEMENTS

The authors are thankful to the Heads, Advanced Instrumentation Research Facility, Jawaharlal Nehru University, New Delhi and Sophisticated Instrumentation Analytical Facility, Central Drug Research Institute, Lucknow for recording spectral data of the compounds.

REFERENCES

1. Talukdar SN, Rahman MB, Paul S. A review on *Barleria prionitis*: its pharmacognosy, phytochemicals and traditional use. Journal of Advances in Medical and Pharmaceutical Sciences, 2015; 4(4): 1-13.
2. Nadkarni AK. Indian Materia Medica, 3rd ed., Popular Book Depot. Bombay, 1994.
3. Khare CP. Indian Herbal Remedies: Rational Western Therapy, Ayurvedic and Other Traditional Usage, Botany. 1st Edn., Springer, New York, 2004; 93-94.
4. Singh B, Bani S, Gupta DK, Chandan BK, Kaul A. Anti-inflammatory activity of 'TAF' an active fraction from the plant *Barleria prionitis* Linn. Journal of Ethnopharmacology, 2003; 85: 187–193. doi: 10.1016/s0378-8741(02)00358-6.
5. Quattrocchi, U. CRC World Dictionary of Medicinal and Poisonous Plants: Common Names, scientific names, eponyms, synonyms, and etymology. CRC Press, Boca Raton, Florida, 2012; 538.
6. Banerjee D, Maji AK, Mahapatra S, Banerji P. *Barleria prionitis* Linn.: A review of its traditional uses, phytochemistry, pharmacology and toxicity. Research Journal of Phytochemistry, 2012; 6: 31-41. DOI: 10.3923/rjphyto.2012.31.41.
7. Ata SA, Van Den Bosch, Harwanik DJ, Pidwinski GE. Glutathione S-transferase and acetyl cholinesterase-inhibiting natural products from medicinally important plants. Pure and Applied Chemistry, 2007; 79: 2269-76.
8. Chen JL, Blanc P, Stoddart CA, Bogan M, Rozhon EJ, Parkinson N, Ye Z, Cooper R, Balick M, Nanakorn W, Kernan MR. New iridoids from the medicinal plant *Barleria prionitis* with potent activity against respiratory syncytial virus. Journal of Natural Products, 1998; 61(10): 1295-1297. doi: 10.1021/np980086y.
9. Kosmulalage KS, Zahid CS, Udenigwe S, Ahktar A, Ata R, Samarasekera. Glutathione S-transferase, acetyl cholineseterase inhibitory and antibacterial activities of chemical constituents of *Barleria prionitis*. Zeitschrift für Naturforschung, 2007; 62: 580-586.
10. Ata A, Kalhari KS, Samarasekera R. Chemical constituents of *Barleria prionitis* and their enzyme inhibitory and free radical scavenging activities. Phytochemistry Letters, 2009; 2(1): 37-40. DOI: 10.1016/j.phytol.2008.11.005.
11. Ganga Raju SV, Naidu KC, Chakradhar V, Prasad RY. Anthraquinones from *Barleria prionitis*, Indian Drugs, 2002; 39(7): 400-401.
12. Damtoft S, Jensen SR and Nielsen BJ. Structural revision of barlerin and acetyl barlerin. Tetrahedron Letters, 1982; 23: 4155-4156.
13. Gupta HM, Saxena VK. A new acylated luteolin-7-O-β-D-glucoside from the roots of *Barleria prionitis* (Linn.). National Academy Science Letters, 1984; 7: 187-189.
14. El-Emary NA, Makboul MA, Abdel-Hafiz MA and Ahmed AS. Phytochemical study of *Barleria cristata* L. and *Barleria prionitis* L. cultivated in Egypt. Bulletin of Pharmaceutical Science, Assiut University, 1990; 13: 65-72.
15. Haider S, Kharbanda C, Alam MS, Hamid H, Ali M, Alam M, Nazreen S, Ali Y. Anti- inflammatory and anti-nociceptive activities of two new triterpenoids from *Adiantum capillus-veneris* Linn. Natural Product Research, 2013; 27(24): 2304-2310. DOI: 10.1080/14786419.2013.828292.
16. Shahnaz Sultana, Mohammed Ali, Showkat Rassol Mir. Chemical constituents from the leaves of *Callistemon lanceolatus* (Sm.) Sweet. European Journal of Pharmaceutical and Medical Research, 2020; 7(6): 692-701.
17. Ejiofor IMIC, Das A, Mir SR, Ali M, Zaman K. Novel phytocompounds from *Vernonia amygdalina* with antimalarial potentials. Pharmacognosy Research, 2020; 12: 53-59; DOI: 10.4103/pr.pr_81_19.
18. Dilika F, Bremner PD, MMeyer JJ. Antibacterial activity of linoleic and oleic acids isolated from *Helichrysum pedunculatum*: a plant used during circumcision rites, Fitoterapia, 2000; 71(4): 450-452. https://doi.org/10.1016/S0367-326X(00)00150-7.
19. Malarvizhi D, Anusooriya P, Meenakshi P, Sundaram S, Oirere E, Gopalakrishnan VK.

Isolation, structural characterization of oleic acid from *Zaleya decandra* root extract. Analytical Chemistry Letters, 2016; 6 (5): 699 – 677. DOI: 10.1080/22297928.2016.1238319.

20. Ahmad A, Misra LN. Isolation of herniarin and other constituents from *Matricaria chamomilla* flowers. International Journal of Pharmacognosy, 1997; 35(2): 121-125. DOI: 10.1076/phbi.35.2.121.13280.
21. Cheriyan BV Sr, Kadhirvelu P Sr, Nadipelly J Jr, Shanmugasundaram J, Sayeli V Sr, Subramanian V Sr. Anti-nociceptive effect of 7-methoxy coumarin from *Eupatorium Triplinerve* Vahl (Asteraceae). Pharmacognosy Magazine, 2017; 13(49): 81-84. doi: 10.4103/0973-1296.197650.
22. Uddin SJ, Grice D, Tiralongo E. Evaluation of cytotoxic activity of patriscabratine, tetracosane and various flavonoids isolated from the Bangladeshi medicinal plant *Acrostichum aureum.* Pharmaceutical Biology, 2012; 50(10): 1276–1280. DOI: 10.3109/13880209.2012.673628.
23. Da Silva Sa FA, De Paula JAM, Santos PAD, Oliveira LDAR, Oliveira GDAR, Liao LM, De Paula JR, Silva MDRR. Phytochemical analysis and antimicrobial activity of *Myrcia tomentosa* (Aubl.) DC. leaves. Molecules, 2017; 22(7): 1100. https://doi.org/10.3390/molecules22071100.
24. Ali A, Jameel M, Ali M. New naphthyl esters from the bark of *Ficus religiosa* Linn. The Natural Products Journal, 2014; 4: 248-253.
25. Ali A, Jameel M, Ali M. New fatty acid and acyl glycoside from the aerial parts of *Phyllanthus fraternus* Webster. Journal of Pharmacy & Bioallied sciences, 2016; 8(1): 43-46.
26. Ali M, Alam P., Singh V, Jameel M, Sultana S. Phytochemical investigations of the leaves and flowers of *Hibiscus rosa-sinensis* L. Indian Drugs, 2017; 54(10): 30 – 37.
27. Sultana S, Ali M, Jameel M. Aliphatic Constituents from the leaves of *Dillenia indica* L., *Halothamus bottae* Jaub. and *Xylosma longifolium* Clos. Chemistry Research Journal, 2018; 3(3): 109-117.