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Research Paper

PHYTOCHEMICAL STUDIES ON *HALOTHAMNUS AURICULUS*

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Abstract

Eleven compounds (1-11) namely quercetin 3-glucoside (1), 8-C-glucopyranosylapigenin (2), 5, 6-dihydroxy-7, 3′, 4′-trimethoxy flavone (3), 4′, 5, 7-trihydroxy-3′, 6-dimethoxy flavones (4), Quercetin 3′,4′-dimethyl ether (5), allantoic acid (6), allantoin (7), oleanolic acid (8), *β*-sitosterol (9) *β*-sitosterol 3-O-*β*-D-glucopyranoside (10) and lupeol (11) were isolated from the methanolic extract of the whole plant of *Halothamnus auriculus*. The structures of these compounds were confirmed by UV, IR, NMR (1D and 2D) spectroscopy and mass spectrometry (EIMS, HREIMS, FABMS, HRFABMS) and in comparison with reported data in literature.

Key words: Amaranthaceae; *Halothamnus auriculus,* Secondary metabolites;, Methanolic Extract.

INTRODUCTION

The genus *Halothamnus* of the plant family Amaranthaceae was formerly placed in the family Chenopodiaceae. The word *Halothamnus* comes from the Greek word, Halo, means “salt” and thamnus means “bush”, which explains the habitat to be salty and also the accumulation of salt in the plants. Nearly 21 specie of the genus are known so far, which are listed as: *H. afghanicus*, *H. auriculus*, *H.beckettii*, *H.bamianicus*, *H. bottae*, *H. cinerascens*, *H. ferganensis*, *H. glaucus*, *H. hierochunticus*, *H. iranicus*, *H. iliensis*, *H. iraqensis*, *H. kermanensis*, *H. lancifolius*, *H. somalensis*, *H. schurobi*, *H. sistanicus*, *H. seravschanicus*, *H. subaphyllus*, *H. turcomanicus* and *H. oxianus*. Mostly the species are distributed in Saudi Arabia and United Arab Emirates, Yemen and Oman [1]. Some species covers the area of eastern Turkey, Armenia, Turkamanistan, Azerbaijan, China, Pakistan and Afghanistan. Generally the species are grown as fodder plant for grazing animals [2-3]. But traditionally these are reported to be used for hair strengthening and women's diseases. Also it has been used for sheep to treat scabies, anthrax and wound healing [4]. It has been using as tissue dying agent and in soap formation [5-6]. Parts of *H. somalensis* have been used for the remedy against parasitic worm disease [7].

H. auriculus is a good fodder plant and is grown for recultivation of grazing land. The specie is distributed in Northern Iran, Afghanistan, Turkmenistan, Pakistan (baluchistan), Tajikistan, Kirghistan and usbekistan. Literature survey revealed that no detailed phytochemical studies

are carried out on species of this genus. For the first time we have explored *H. auriculus* for its biological potential and secondary metabolites. In recent study 11 secondary metabolites have been isolated and characterized for the first time from this specie including quercetin 3-glucoside **(1)** [8], 8-C-glucopyranosylapigenin **(2)** [9], 5, 6-dihydroxy-7, 3′, 4′-trimethoxy flavone **(3)** [10], 4′, 5, 7-trihydroxy-3′, 6-dimethoxy flavones **(4)** [11], Quercetin 3′,4′-dimethyl ether **(5)** [12], allantoic acid **(6)** [13], allantoin **(7)** [14], oleanolic acid **(8)** [15], *β*-sitosterol **(9)** [16], *β*-sitosterol 3-O-*β*-D-glucopyranoside **(10)** [16] and lupeol **(11)** [17].

MATERIALS AND METHODS

EI-MS, HR-EI-MS, FAB-MS and HR-FAB-MS were recorded on Finnigan (Varian MAT, Waldbronn, Germany) JMS H×110 with a data system and JMSA 500 mass spectrometers, respectively. For IR spectra KBr pellets were prepared and the instruments used were Jasco 320-A infrared spectrometer. NMR spectra were recorded on Bruker AM-400 and 500 MHz in duterated solvents. Column chromatography was performed using silica gel (MERCK, 70-230 mesh) and silica gel (230-400 mesh, Darmstadt, Germany) as stationary phase packed in glass columns, eluted by using gradient of organic solvents. Chromatographic separations were monitored using aluminium sheets pre-coated with silica gel 60 F_{254} (20×20 cm, 0.2 mm thick; E-Merck; Darmstadt, Germany) for thin layer chromatography (TLC).UV light (254 and366) was used to see fluorescence of chromatogram. Ceric sulphate solution followed by heating was used to locate the spot on chromatogram.

Collection and Identification of Plant

The whole plant of *Halothamnus auriculus* (4 kg) was collected in September 2010 from Ziarat, Baluchistan and was identified by Prof. Dr. Rasool Bakhsh Tareen, Department of Botany, University of Baluchistan, Quetta, where the voucher specimen has been deposited in the herbarium.

Extraction and Isolation

The shade dried whole plant of *H. auriculus* (4 kg) was extracted thrice with MeOH (15 L). The methanolic extract was evaporated to dryness under vacuum, and the residue (50 g) was divided into *n*-hexane (4 g), ethylacetate (22 g), butanol (12 g) and water (12 g) layer. Column chromatography of the ethylacetate fraction over silica gel eluting with *n*-hexane, *n*-hexane-EtOAC, EtOAC and EtOAC-CH_3OH in increasing order of polarity yielded eight fractions (HA_1-HA_8).

HA_7 yielded compound **2, 3** and **7** at *n*-hexane: ethyl acetate (3:7). HA_5 (2.5g) obtained at *n*-hexane:EtOAc (2.0:8.0) yielded **5** when eluted with *n*-hexane:ethyl acetate (6:4) and 1, **4** and **6** with *n*-hexane: EtOAc (5.5:4.5). HA_4 *n*-hexane:ethyl acetate (4.0:6.0), on further silica gel column chromatography with an isocratic of *n*-hexane:ethyl acetate (3:7) yielded **10** (150 mg) as major component of *halothamnus auriculus***.** HA_2 collected from the main column with *n*-hexane:ethyl acetate (8.0:2.0), was rechromatographed to get **8** and **9** using *n*-hexane:ethylacetate (9.0:1.0). HA_1 obtained with *n*-hexane was further purified on silica gel column when eluted with a gradient of *n*-hexane and ethyl acetate yielding three sub-fractions ($HA_{1a\text{-}c}$). The sub-fraction HA_{1c} on further silica gel column chromatography afforded **11** when eluted with *n*-hexane:ethyl acetate (7.5:2.5).

RESULTS

Quercetin-3-glucoside (1): Yellow amorphous powder (20 mg); UV λ_{max}: 255, 278, 315 and 332 nm; IR (KBr): 3240, 1665, 2925, 1606, 1520; ^{1}H-NMR (CD_3OD, 400 MHz): δ 7.70 (1H, d, *J* = 2.0 Hz, H-2′), 7.58 (1H, dd, *J* = 8.4, 2.0 Hz, H-6′), 6.86 (1H, d, *J* = 8.4 Hz, H-5′), 6.36 (1H, d, *J* = 1.6 Hz, H-8), 6.18 (1H, d, *J* = 1.6 Hz, H-6), 5.23 (1H, d, *J* = 7.2 Hz, H-1″), 3.72 (1H, dd, *J* =12.0, 2.4 Hz, H-6″), 3.59 (1H, dd, *J* = 12.0, 5.2 Hz, H-6″), 3.52 (1H, m, H-3″), 3.48 (1H, t, *J* = 8.4 Hz, H-2″), 3.34 (1H, t, *J* =7.2 Hz, H-4″), 3.22 (1H, m, H-5″); ^{13}C-NMR (CD_3OD, 100 MHz): δ 179.4 (C-4), 165.9 (C-7), 163.0 (C-5), 159.0 (C-2), 158.4 (C-8a), 149.8 (C-4′), 145.8 (C-3′), 135.6 (C-3), 123.2 (C-6′), 123.0 (C-1′), 117.6 (C-2′), 116.0 (C-5′), 105.7 (C-4a), 104.4 (C-1″), 99.9 (C-6), 94.7 (C-8), 78.3 (C-

5″), 78.1 (C-2″), 75.7 (C-3″), 71.2 (C-4″), 62.6 (C-6″); FABMS: m/z 465.0 $[M+H]^+$; HRFABMS: 465.3839 $[M+H]^+$ (465.3842 calcd. for $C_{21}H_{21}O_{12}$).

8-C-glucopyranosylapigenin (2): Yellow powder (20 mg); UV λ_{max}: 372, 345, 281, 278 nm; IR (KBr): 3450, 1665, 1650, 1505, 1467 cm^{-1}; ^{1}H-NMR (DMSO-d6, 400 MHz): δ 13.1 (1H, s, OH-5), 8.02 (2H, d, *J* = 8.8 Hz, H-2′, 6′), 6.89 (2H, d, *J* = 8.8 Hz, H-3′, 5′), 6.76 (1H, s, H-3), 6.26 (1H, s, H-6), 4.90 (1H, d, *J* = 5.2 Hz, OH-2″), 4.88 (1H, d, *J* = 4.0 Hz, OH-3″), 4.68 (1H, d, *J* = 7.6 Hz, H-1″), 4.59 (1H, t, *J* = 5.6 Hz, OH-6″), 3.82 (1H, m, H-5″), 3.70 (1H, dd, *J* = 6.0, 11.2 Hz, H-6″), 3.58 (1H, dd, *J* = 5.6, 11.0 Hz, H-6″), 3.37 (1H, m, H-4″), 3.28 (1H, t, *J* = 8.4 Hz, H-3″), 3.23 (1H, m, H-2″); ^{13}C-NMR (DMSO-d6, 100 MHz): δ 182.1 (C-4), 163.9 (C-2), 162.5 (C-7), 161.1 (C-4'), 160.3 (C-8a), 155.9 (C-5), 128.9 (C-2′,6'), 121.6 (C-1'), 115.8 (C-3', 5'), 104.5 (C-8), 104.0 (C-4a), 102.4 (C-3), 98.1(C-6), 81.8 (C-2''), 78.6 (C-3''), 73.3 (C-1''), 70.8 (C-5''), 70.5 (C-4''), 61.2 (C-6''); FABMS: m/z 433 $[M+H]^+$; HRFABMS: m/z 433.3849 $[M+H]^+$ (433.3854 calcd. for $C_{21}H_{21}O_{10}$).

5, 6-dihydroxy-3′, 4′,7-trimethoxy flavone (3); Yellow powder (22 mg); UV λ_{max}: 278, 280, 345, 370 nm; IR: 3455, 1670, 1655, 1505, 1465, 1310 cm^{-1}; ^{1}H-NMR (CD_3OD, 400 MHz): δ 7.38 (1H, dd, *J* = 8.4, 1.6 Hz, H-6′), 7.23 (1H, d, *J* = 1.6 Hz, H-2′), 6.86 (1H, d, *J* = 8.4 Hz, H-5′), 6.49 (1H, s, H-8), 6.46 (1H, s, H-3), 3.88 (3H, s, 3′-OMe), 3.86 (3H, s, 7-OMe), 3.85 (3H, s, 4′-OMe); ^{13}C-NMR (CD_3OD, 100 MHz): δ 182.0 (C-4), 163.9 (C-2), 158.0 (C-7), 153.3 (C-8a), 152.9 (C-5), 149.0 (C-4′), 146.5 (C-3′), 132.0 (C-6), 123.0 (C-1′), 121.5 (C-6′), 116.0 (C-5′), 108.3 (C-2′), 105.2 (C-4a), 104.1 (C-3), 93.9 (C-8), 60.3 (7-OCH_3), 56.8 (3′-OCH_3), 56.3 (4′-OCH_3); EIMS: m/z 344 $[M]^+$; HREIMS: m/z 344.0891 (344.0896 Calcd. for $C_{18}H_{16}O_7$).

4′, 5, 7-trihydroxy-3′, 6-dimethoxy flavone (4): Yellow powder (21 mg); UV λ_{max}: 215, 271, 278, 345, 351 nm; IR (KBr): 3452, 1671, 1656, 1502, 1462, 1314 cm^{-1}; ^{1}H-NMR (CD_3OD, 400 MHz): δ 7.45 (1H, dd, *J* = 1.6, 8.0 Hz, H-6′), 7.36 (1H, d, *J* = 1.6 Hz, H-2′), 7.12 (1H, d, *J* = 8.0 Hz, H-5′), 6.65 (1H, s, H-3), 6.55 (1H, s, H-8), 3.84 (3H, s, 6-OMe), 3.82 (3H, s, 3′-OMe); ^{13}C-NMR (CD_3OD, 100 MHz): δ 182.4 (C-4), 164.5 (C-2), 159.0 (C-7), 154.2 (C-8a), 153.5 (C-5), 149.1 (C-4′), 147.1 (C-3′), 133.2 (C-6), 128.3 (C-6′), 123.5 (C-1′), 114.6 (C-5′), 110.3 (C-2′), 105.5 (C-4a), 105.3 (C-3), 90.4 (C-8), 64.6 (6-OCH_3), 55.6 (3′-OCH_3). EIMS: m/z 330 $[M]^+$; HREIMS: m/z 330.0732 $[M]^+$ (330.0740 calcd. for $C_{17}H_{14}O_7$).

Quercetin 3′,4′-dimethyl ether: (5): Yellow crystals (35 mg)**;** UV λ_{max}. 286, 352, 255 nm; IR (KBr): 3415, 1748, 1655, 1610, 1310 cm^{-1}; ^{1}H-NMR (CD_3OD, 400 MHz): δ 7.61 (1H, dd, *J* = 8.4, 2 Hz, H-6′), 7.30 (1H, d, *J* = 2 Hz, H-2′), 6.90 (1H, d, *J* = 8.4 Hz, H-5′), 6.38 (1H, d, *J* = 2 Hz, H-8), 6.17 (1H, d, *J* = 2 Hz, H-6), 3.83 (3H, s, 3′-OMe), 3.81 (3H, s, 4′-OMe); ^{13}C-NMR (CD_3OD, 100 MHz): δ 182.5 (C-4), 166.0 (C-7), 163.0 (C-5), 159.0 (C-2), 158.0 (C-8a), 148.5 (C-3′), 149.0 (C-4′), 133.6 (C-3), 123.8 (C-1′), 121.7 (C-6′), 116.0 (C-5′), 108.0 (C- 2′), 104.5 (C-4a), 99.2 (C-6), 94.4 (C-8), 56.0 (3′-OMe), 55.5 (4′-OMe). EIMS: m/z 330 $[M]^+$. HREIMS: 330.0735 (330.0740 calcd. for $C_{17}H_{14}O_7$).

Allantoic acid (6): Needle like crystals (12 mg); IR (KBr): 3435, 1710, 1645cm^{-1}; ^{1}H-NMR (D_2O, 500 MHz): δ 5.30 (1H, s); ^{13}C-NMR (D_2O, 125 MHz): δ 178.0 (C-6, 7), 161.1 (C-2), 65.7 (C-4, 5). EIMS: m/z 176.0 $[M]^+$; HREIMS: m/z 176.0539 $[M]^+$ (176.0546 calcd. for $C_4H_8N_4O_4$).

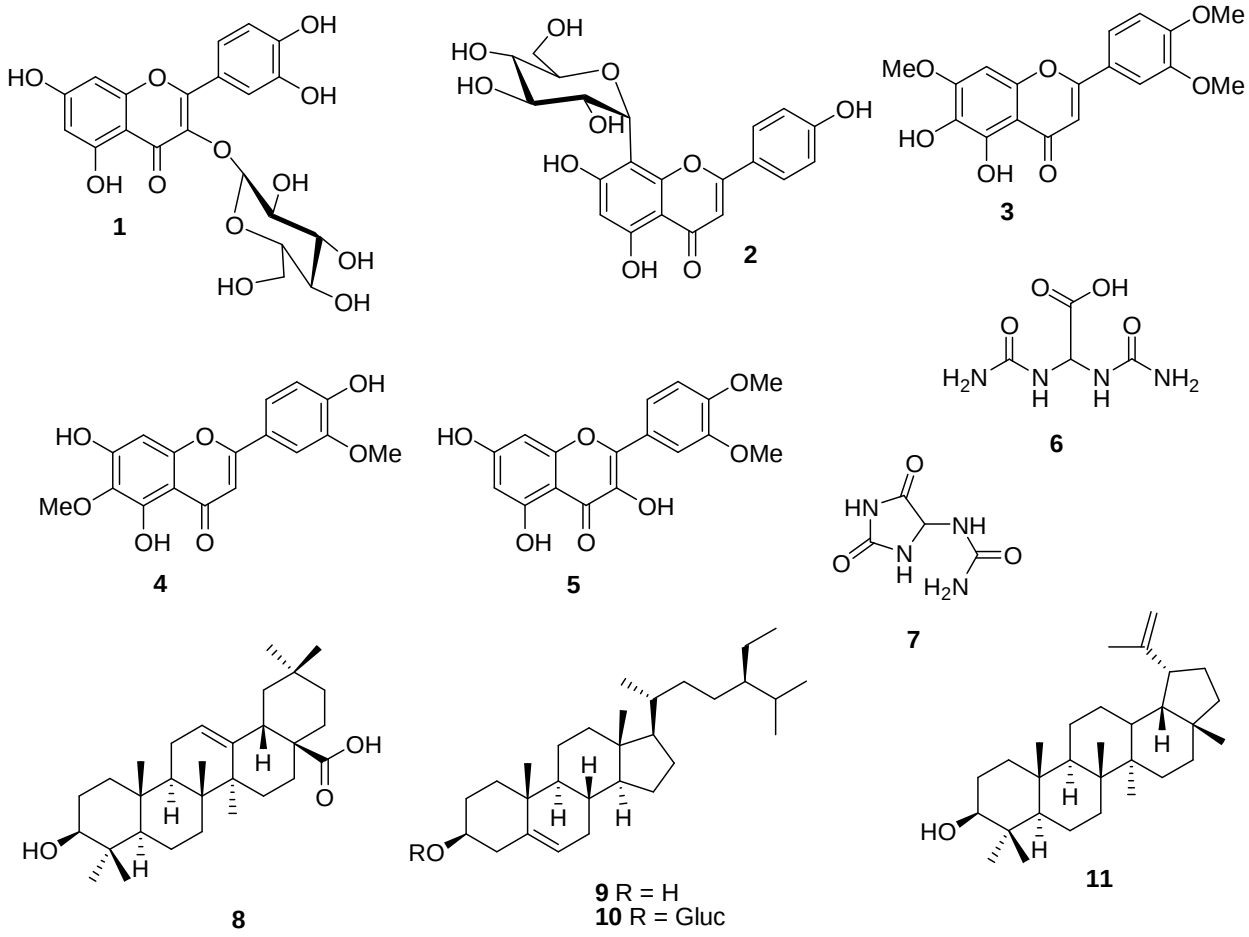
Allantoin (7): Needle like crystals (35 mg); IR (KBr): 3435, 1778 cm^{-1}; ^{1}H-NMR (Pyr, 500 MHz): δ 10.04 (1H, s, NH-1), 8.70 (1H, s, NH-3), 8.60 (1H, d, 8.5 Hz, NH-6), 6.88 (2H, s, NH_2-8), 6.38 (1H, dd, *J* = 8.8, 1.5 Hz, H-5); ^{13}C-NMR (Pyr, 125 MHz): δ 159.5 (C-2), 174.9 (C-4), 64.5 (C-5), 158.6 (C-7); EIMS: m/z 158.0 $[M]^+$; HREIMS: m/z 158.0436 $[M]^+$ (158.0440 calcd. for $C_4H_6N_4O_3$).

Oleanolic acid (8): White amorphous solid (45 mg); IR (KBr): 3403, 1716, 1664, and 822 cm^{-1}; ^{1}H-NMR: ($CDCl_3$, 400 MHz): δ 5.28 (1H, t, *J* = 3.8 Hz, H-12), 3.49 (1H, dd, *J* = 4.4, 11.9 Hz ,H-3), 2.84 (1H, dd, *J* = 3.6, 13.2 Hz, H-18), 1.13, 1.06, 0.98 , 0.95, 0.92, 0.88 and 0.82 (3H, each s, Me); ^{13}C-NMR: ($CDCl_3$, 100 MHz); δ 179.8 (C-28), 144.3 (C-13), 122.2 (C-12), 79.0 (C-3), 53.9 (C-5), 48.9 (C-9), 47.1 (C-17), 46.6 (C19), 41.4 (C-14), 41.0 (C-18), 40.4 (C-8), 39.2 (C-4), 37.6 (C-1), 37.3 (C-10), 33.1 (C-7), 33.0 (C-21), 32.8 (C-29), 32.5 (C-22), 31.0 (C-20), 29.6 (C-23), 26.9 (C-2), 26.7 (C-15), 25.7 (C-27), 23.8 (C-11), 22.6 (C-30), 22.3 (C-16), 19.6 (C-26), 18.7 (C-6), 14.8 (C-24), 14.6 (C-25); EIMS: m/z 456.0 $[M]^+$; HREIMS: m/z 456.3603 $[M]^+$ (456.3613 calcd. for $C_{30}H_{48}O_3$).

β-Sitosterol (9): White needle like crystals (55 mg); IR (KBr): 3457, 3052, 1648, and 817 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 5.23 (1H, m, H-6), 3.49 (1H, m ,H-3), 1.03 (3H, s, Me-19), 0.89 (3H, d, *J* = 6.4 Hz, Me-21), 0.85 (3H, t, *J* = 6.8 Hz, Me- 29), 0.82 (3H, d, *J* = 6.5 Hz, Me-27), 0.73 (3H, d, *J* = 6.5 Hz, Me-26), and 0.71 (3H, s, Me-18); ^{13}C-NMR ($CDCl_3$, 100 MHz): δ 140.9 (C-5), 123.7 (C-6), 76.2 (C-3), 56.7 (C-17), 55.4 (C-14), 51.3 (C-9), 49.3 (C-24), 46.1 (C-13), 42.5 (C-4), 39.2 (C-12), 39.0 (C-10), 38.6 (C-20), 36.9 (C-1), 36.8 (C-8), 35.2 (C-7), 32.9 (C-22), 30.0 (C-23), 29.1 (C-2), 26.8 (C-15), 25.3 (C-16), 22.2 (C-28), 22.1 (C-25), 20.1 (C-26), 20.0 (C-11), 19.9 (C-27), 19.6 (C19), 17.8 (C-21), 17.6 (C-18), 12.8 (C-29). EIMS: m/z 414 $[M]^+$; HREIMS: m/z 414.3857 $[M]^+$ (414.3861 calcd. for $C_{29}H_{50}O$).

β-Sitosterol 3-O-β-D-glucopyranoside (10): White solid (150 mg); IR (KBr): 3440, 1640, 1615, 1585-1540 cm^{-1}: ^{1}H-NMR ($CDCl_3$+CD_3OD, 400MHz): δ 5.29 (1H, d, *J* = 7.8 Hz, H-1'), 5.17 (1H, br d, *J* = 5.6 Hz, H-6), 3.76 (1H, m, H-3), 3.90-4.57 (6H, m, Glc-H), 0.97 (3H, s, Me-19), 0.88 (3H, d, *J* = 6.4 Hz, Me-21), 0.78 (3H, t, *J* = 6.8 Hz, Me-29), 0.76 (3H, d, *J* = 6.4 Hz, Me-26), 0.74 (3H, d, *J* = 7.0 Hz, Me-27), 0.67 (3H, s, Me-18): ^{13}C-NMR ($CDCl_3$+CD_3OD, 100MHz): δ 140.3 (C-5), 120.4 (C-6), 105.3 (C-1'), 80.9 (C-3), 77.3 (C-5'), 75.8 (C-3'), 72.1 (C-2'), 71.2 (C-4'), 62.1 (C-6'), 57.2 (C-17), 53.2 (C-14), 49.9 (C-9), 47.3 (C-24), 41.9 (C-13), 41.5 (C-12), 40.7 (C-4), 37.5 (C-20), 37.1 (C-10), 36.6 (C-22), 36.3 (C-1), 33.9 (C-7), 33.2 (C-8), 29.8 (C-25), 28.0 (C-23), 27.7 (C-2), 25.9 (C-16), 25.7 (C-15), 23.9 (C-28), 22.8 (C-11), 18.7 (C-27), 18.1 (C-19), 17.5 (C-21), 14.7 (C-26), 13.8 (C-18) and 12.5 (C-29): FABMS: m/z 576.0 $[M]^+$; HRFABMS: m/z 576.4376 $[M]^+$ (576.4389 calcd. for $C_{35}H_{60}O_6$).

Lupeol (11): White solid (13 mg); IR (KBr): 3458, 3063, 1645, and 840 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 4.73 (1H, br s, H-29), 4.59 (1H, br s, H-29), 3.41 (1H, dd, *J* = 4.4, 9.8 Hz, H-3), 1.58, 1.03, 0.93 , 0.91, 0.89, 0.84 and 0.79 (3H, each s, Me); ^{13}C-NMR ($CDCl_3$, 100 MHz): δ 153.0 (C-20), 108.1 (C-29), 79.0 (C-3), 53.4 (C-5), 49.4 (C19), 49.3 (C-18), 47.2 (C-9), 44.8 (C-13), 43.7 (C-14), 40.4 (C-17), 40.1 (C-4), 39.0 (C-8), 38.2 (C-22), 37.0 (C-10), 36.0 (C-1), 32.5 (C-7), 30.0 (C-16), 29.7 (C-21), 27.9 (C-2), 27.3 (C-12), 27.0 (C-23), 25.9 (C-15), 21.7 (C-11), 21.5 (C-30). 19.4 (C-25), 18.9 (C-28), 18.1 (C-6), 17.8 (C-27), 17.6 (C-24), 17.1 (C-26). EIMS: m/z 426.0 $[M]^+$; HREIMS: 426.3858 $[M]^+$ (426.3862 calcd. for $C_{30}H_{50}O$).



DISCUSSION

The compound **1** was obtained as yellow amorphous powder, which exhibited the *pseudo*-molecular ion at *m/z* 465 $[M+H]^+$ in positive FABMS. The high resolution analysis (*m/z* 465.3839) of the same ion revealed the molecular formula as $C_{21}H_{21}O_{12}$. The IR spectrum showed prominent bands at 3240 for hydroxyl, 1665 for conjugated ketone, 1606 and 1520 cm^{-1} for phenyl groups. The UV spectrum showed absorption maxima at 255, 278, 315 and 332 nm as characteristic feature of quercetin glycoside [18]. The aromatic region of the ^{1}H-NMR spectrum of **1** displayed five signals at δ7.70 (1H, d, *J* = 2.0 Hz), 7.58 (1H, dd, *J* = 2.0, 8.4), 6.86 (1H, d, *J* = 8.4 Hz), 6.36 (1H, d, *J* = 1.6 Hz, H-8) and 6.18 (1H, d, *J* = 1.6 Hz, H-6). The first three signals splitted at ABX pattern were attributed to ring B of quercetin nucleus, whereas, remaining two were attested for ring A. A sugar moiety was also found in **1** due to the resonance of anomeric proton at δ5.23 (1H, d, *J* = 7.2 Hz), and other sugar protons in the range of δ 3.72-3.22. The amount of coupling constant of anomeric proton suggested the sugar to be a β hexose and was identified as glucose due to acidic hydrolysis followed by comparative TLC of the hydrolyzed sugar with the authentic sample of glucose and its optical rotation values $[\alpha]_D^{20}$ +50.0˚(c, 10 in H_2O) [19]. The ^{13}C NMR spectrum of **1** supported proton data, as it displayed signals for 21 carbon atoms including fifteen carbons for flavonoid nucleus and six for sugar unit. The sugar connectivity was found at C-3 due to the HMBC correlation of anomeric proton (δ5.23) with a quaternary carbon at δ135.6 (C-3). The whole discussed data resembled with the data of quercetin 3-O-β-D-glucoside [8]. The compound **2** was isolated as yellow amorphous powder, whose molecular formula $C_{21}H_{21}O_{10}$ was established through positive HRFABMS (*m/z* 433.3849 $[M+H]^+$). The IR and UV data was similar to the data of **1** to support a flavonoid nucleus. In ^{1}H-NMR two *ortho* coupled doublets were observed at δ8.02 (2H, d, *J* = 8.8 Hz) and 6.89 (2H, d, *J* = 8.8 Hz), attested for *p*-substituted aromatic ring B of flavonoid nucleus. The other

two singlets observed at δ 6.76 and 6.26 were attested for H-3 of ring C and H-6 of ring A respectively. This data revealed that ring A must be penta-substituted. Besides, the same spectrum displayed signals in the range of δ 4.68-3.23 due to a sugar moiety. The ^{13}C-NMR spectrum of **2** was in accordance with other data suggesting a flavone glycoside. A sugar proton at δ 4.68 (1H, d, *J* = 7.6 Hz) was correlated through HSQC spectrum with the carbon resonating at δ 73.3. This hydrogen (δ 4.68) showed HMBC correlation in aromatic region with the carbons at δ 162.5 (C-7), 155.9 (C-8a) and 104.5 (C-8), This observation led to the idea of a C-linked sugar in **2**. Further careful analysis of HMBC data established the sugar linkage at C-8. Comparison with literature data confirmed the compound **2** to be 8-C-glucopyranosylapigenin [9]. Compound **3** isolated as yellow amorphous powder was also found to be flavonoid. The EIMS spectrum displayed molecular ion at *m/z* 344 while in HREIMS the molecular ion peak was observed at *m/z* 344.0891 corresponding to the molecular formula $C_{18}H_{16}O_7$. In the ^{1}H-NMR spectrum an ABX system of protons resonated at δ 7.38 (1H, dd, *J* = 8.4, 1.6 Hz), 6.86 (1H, d, *J* = 8.4 Hz) and 7.23 (1H, d, *J* = 1.6 Hz) were attested for ring B of the flavonoid. In addition the spectrum displayed two singlets at δ 6.49 and 6.46, which were assigned to H-8 and H-3 respectively. Three methoxyl function displayed their position at δ 3.88, 3.86 and 3.85. The ^{13}C-NMR spectrum was in full agreement with ^{1}H NMR spectrum and formula as it displaying 18 carbon signals. The three methoxyl functions were fixed at C-7, C-3′ and C-4′ due to HMBC spectral analysis. The whole data of compound **3** was further compared to the literature values and were found identical the data of 5, 6-dihydroxy- 3′, 4′, 7-trimethoxy flavone [10]. The molecular formula $C_{17}H_{14}O_7$ of **4** showed one carbon less than that of **3**, whereas, the aromatic region of the ^{1}H-NMR spectrum was also identical to that of **3**. However, the same spectrum afforded two methoxyl groups at δ 3.82 and 3.84 instead of three as were found in **3**. Based on this information, it was concluded that **4** must has an extra hydroxyl group when compared to that of **3**. The position of the two methoxyl groups at C-6 and C-3' could be identified due to HMBC interaction of the two methoxyl groups i.e at δ 3.84 and 3.82 with C-6 (δ 133.2) and C-3' (δ 147.1) respectively. Combination of the whole data led to the structure of **4** as 4′, 5, 7-trihydroxy-3′, 6-dimethoxy flavone [11].

Compound **5** was found to be dimethyl ether of quercetin as the aromatic region showed five signals at δ 7.61 (1H, dd, *J* = 8.4, 2 Hz), 7.30 (1H, d, *J* = 2 Hz), 6.90 (1H, d, *J* = 8.4 Hz), 6.38 (1H, d, *J* = 2 Hz) and 6.17 (1H, d, *J* = 2 Hz). The first three signals splitted at ABX pattern were attributed to ring B, whereas, the rest two were attested for ring A. This data indicated that C-3 position in **5** must be substituted, which was substantiated due to the resonance of C-3 at δ 133.6 in ^{13}C-NMR spectrum. Further the comparison of these data with the reported values in literature helped to deduce the structure of **5** as quercetin3′,4′-dimethyl ether [12]. The HREIMS of **6** depicted the molecular formula $C_4H_8N_4O_4$ with 3 DBE. The IR spectrum showed absorption bands at 1710, 1645 and 3435 cm^{-1} for carbonyl and amine functions respectively. In the ^{1}H NMR spectrum of **6**, only one signal was observed at δ 5.30, correlated through HSQC experiment with a carbon resonating at δ 65.7. In addition to this carbon, the ^{13}C NMR spectrum displayed two more signals at δ 161.1 and 178.0. In HMBC spectrum the methine hydrogen at δ 5.30 exhibited long-range correlation with both the quaternary carbons (δ 161.1 and 178.0). The above discussed data could only be fitted for allantoic acid [13]. The HREIMS of **7** showed molecular formula $C_4H_6N_4O_3$ due to peak observed at *m/z* 158.1148. The ^{1}H-NMR of **7** showed resonance for only one methine at δ 5.24 (1H, d, *J* = 8.0), which was correlated in HSQC spectrum with the carbon at δ 62.4. Other proton signals observed at δ 8.03 (1H, s), 6.87 (1H, d, *J* = 8.0) and 5.75 (2H, s) were attributed to amine and amide functions. In ^{13}C-NMR spectrum three downfield resonances were observed at δ 156.7, 157.3, and 173.5, in addition, a methine carbon showed its position at δ 62.4. A strong COSY correlation was found between the methine proton (δ 5.24) and an amide proton (δ 6.87), In the HMBC experiment the methine proton showed correlations with the carbon signals at δ 156.7, 157.3 and 173.5. Comparison of available data with literature revealed compound **7** as allantoin [14]. Spectrpscopic data of **8** resembled with data published for oleanolic acid [15]. (**9**) was found to be sitosterol due to EIMS fragmentation pattern, in which the molecular ion peak was observed at *m/z* 414 with

other characteristic ions at *m/z* 399, 396, 381, 329, 275, 273 and 255 for sitosterol nucleus [20]. The comparative study of its NMR spectroscopic data with that of reported one confirmed its identity as *β*-sitosterol [16]. The structure of the compound **(10)** was established as *β*-sitosterol 3-*O*-*β*-D-glucoside due to comparison of available data with reported one [16]. It was found as major component of *halothamnus auriculus.* The EIMS spectrum of compound (**11**) exhibited molecular ion peak at m/z 426, along with characteristic fragment ions at *m/z* 385, 220, 218 and 207 attested for a lupane series [21]. Other spectroscopic data when compared to literature was found identical to the data reported for lupeol [17].

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