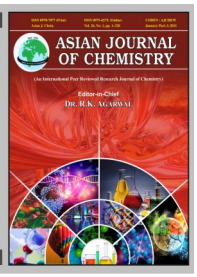
Asian Journal of Chemistry; Vol. 26, No. 4 (2014), 1086-1090

ASIAN JOURNAL OF CHEMISTRY

http://dx.doi.org/10.14233/ajchem.2014.15927



Phytochemical Investigations on *Tribulus longipetalus*

MUHAMMAD IMRAN ANJUM[1,2], EJAZ AHMED[3,*], AHSAN SHARIF[3], ABDUL JABBAR[1], ABDUL MALIK[4] and NAYAB BATOOL[3]

[1]Department of Chemistry, Baghdad-ul-Jadid Campus, The Islamia University Bahawalpur, Bahawalpur, Pakistan
[2]Post Graduate Department of Chemistry, Government SadiqEgerton College Bahawalpur, Bahawalpur, Pakistan
[3]Institute of Chemistry, University of the Punjab, Quaid-e-Azam Campus, Lahore-54590, Pakistan
[4]International Centre for Chemical and Biological Sciences, HEJ Research Institute of Chemistry, University of Karachi, Karachi-75270, Pakistan

*Corresponding author: E-mail: dr.ejaz.ahmed@gmail.com

Received: 17 June 2013; *Accepted*: 12 September 2013; *Published online*: 15 February 2014; AJC-14704

The bioassay directed phytochemical investigations of *Tribulus longipetalus* have resulted the isolation of eighteen compounds and their structures havebeen elucidated by extensive use of modern spectroscopic (UV, IR, EIMS, HREIMS, ^{1}H NMR, ^{13}C NMR, FAB +ve, FAB -ve) techniques. All the compounds have been isolated for the first time from this species.

Keywords: Phytochemical, *Tribulus longipetalus*, Spectroscopy.

INTRODUCTION

Tribulus is very important genus of the family zygophyllaceae and it grows as shrubs in tropical and subtropical regions throughout the world. The genus *Tribulus* comprises about 20 species and represented by 4 species in Pakistan[1]. *Tribulus longipetalus*, locally known as bhakri, is a prostrate, green or grayish-white in colour. Its leaves are 1-4 cm long and flowers are mostly yellow in colour. The fruit of this plant is broad and somewhat pointed. The plant possesses cooling, demulcent, diuretic, tonic aphrodisiacs aperients properties and its fruit is used in urinary disorders, impotence, heart diseases. The seeds of *T. longipetalus* are known to be used in diseases of kidney stone, gout and hemorrhages[2]. *Tribulus longipetalus* has a syn. name *Tribulus alatus*. No literature is available with the name *Tribulus longipetalus*, but a little bit work has been reported for the *Tribulus alatus*. Literature survey revealed that only three steroidal saponins from the MeOH extract of the aerial parts of the *Tribulusalatus* Del. have so far been reported[3]. In the present study eighteen compounds have been isolated for the first time from the methanolic extract of the whole plant of *T. longipetalus*. They were identified as 1-triacontanol[4] (**1**), hexacosanoic acid[5] (**2**), tetracosanoic acid[6] (**3**), benzoic acid[7] (**4**), 2-hydroxybenzoic acid[8] (**5**), 4-ethoxy benzoic acid[9] (**6**), 3,5-dimethoxybenzoic acid[10] (**7**), β-sitosterol[4] (**8**), β-amyrin[11] (**9**), stigmasterol[12] (**10**), 5α,8α-epidioxyergosta-6,9(11),22-trien-3β-ol[13] (**11**), ursolic acid[4,10] (**12**), cyclostenol[14] (**13**), oleanolic acid[15] (**15**), 5,6-dihydroxy-4,7-dimethoxy-2-methylanthracene-9,10-dione[16] (**15**), 1, 3-dihydroxycyclolanosterol[17] (**16**), stigmasterol 3-*O*-β-D-glucopyranoside[15] (**17**) and β-sitosterol 3-*O*-β-D-glucopyranoside[18] (**18**), (Fig. 1).

EXPERIMENTAL

UV and IR spectra were recorded on Hitachi-UV-3200 and Jasco-320-A spectrometers, respectively. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AM-400 spectrometer with tetramethylsilane (TMS) as an internal standard. The 2D-NMR spectra were recorded on a Bruker AMX 500 NMR spectrometer. Optical rotations were measured on a Jasco DIP-360 digital polarimeter using a 10 cm tube. Mass spectra (EIMS and HR-EIMS, FAB-MS) were measured in an electron impact mode on finnigan MAT 12 and MAT 312 spectrometers and ions were given in *m/z* (%). TLC was performed on precoated silica gel F254 plates, the detection was done at 254 nm and by spraying with ceric sulphate reagent. Silica gel (E. Merck, 230-400 mesh) was used for column chromatography. Melting points were determined on a gallenkemp apparatus and are uncorrected.

The whole plant of *Tribulus longipetalus Viv*. was collected from Cholistan Desert near Bahawalpur (Pakistan) in April 2008 and identified by Dr. Muhammad Arshad, Plant Taxonomist Cholistan Institute for Desert Studies (CIDS), Islamia University Bahawalpur, Bahawalpur, Pakistan, where a voucher specimen has been deposited.

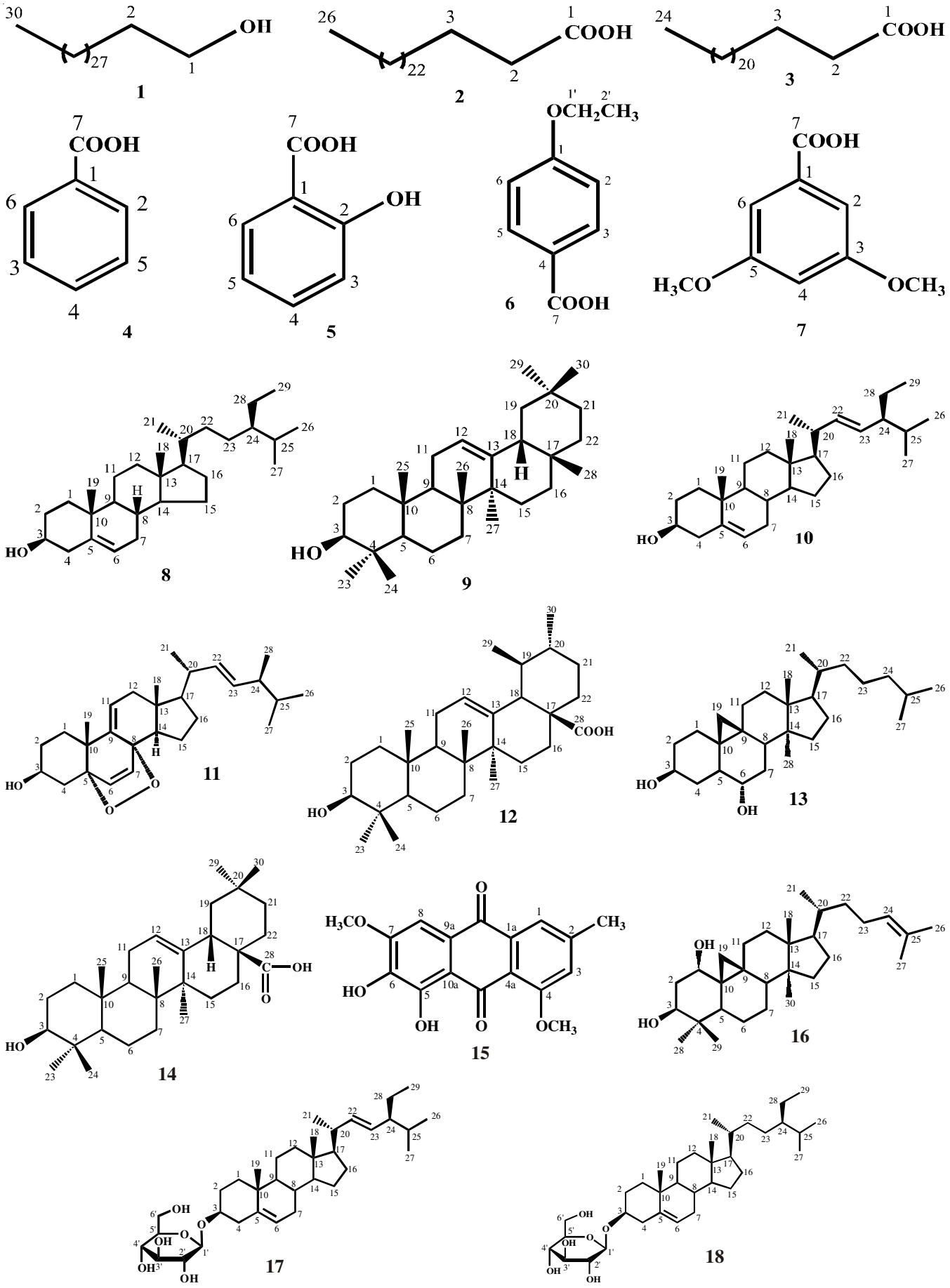


Fig. 1. Compounds isolated from T. *longipetalus*

Extraction and isolation: The air dried whole plant (10 Kg) of *Tribulus longipetalus* were powdered and extracted at room temperature with methanol (3 × 15 L). The extract was concentrated under reduced pressure to yield residue 800 g, partitioned with H_2O and *n*-hexane. The *n*-hexane soluble portion was concentrated with rotary evaporator and obtained greenish material 150 g amount. The crude extract was again partitioned between $CHCl_3$ (180 g), ethyl acetate (50 g), *n*-butanol (140 g) and H_2O (50 g) soluble fractions. The $CHCl_3$ soluble fraction (180 g) was subjected to column chromatography over silica gel eluting with *n*-hexane-$CHCl_3$, $CHCl_3$ and $CHCl_3$-MeOH in increasing order of polarity, to obtain 15 fractions (T1-T15). The fraction obtained from *n*-hexane-$CHCl_3$ (8.0: 2.0) labeled as T2 was again chromatographed over silica gel eluting with same solvent system gave three fractions A1-A3. The fraction A2 obtained from *n*-hexane-$CHCl_3$ (8.5: 1.5) produced three spots on TLC which were again purified by PTLC using the solvent system *n*-hexane-EtOAC (8.8:1.2), provided benzoic acid (**4**), 2-hydroxy benzoic acid (**5**) and 4-ethoxy benzoic acid (**6**). The fraction A3 was purified over silica gel column chromatography by using solvent system *n*-hexane-EtOAC (9.0: 1.0) gave 3,5-dimethoxy benzoic acid (**7**). The fractions obtained from *n*-hexane-$CHCl_3$ (7.0: 3.0) gave five spots on TLC, were combined and rechromatographed over silica gel column chromatography using the solvent system *n*-hexane-EtOAC (8.5: 1.5) produced three spots on TLC which were combined and purified on PTLC by using the same solvent system gave β-sitosterol (**8**), β-amyrine (**9**) and cyclostenol (**13**), respectively. The portion got from *n*-hexane-EtOAC (8.3:1.7) were combined and then rechromatographed over the silica gel column chromatography by using solvent system *n*-hexane-EtOAC (8.5:1.5) gave two pure compounds stigmasterol (**10**) and 1-triacontanol (**1**). The portions which were obtained from *n*-hexane-$CHCl_3$ (5.0: 5.0) showed many spots on TLC, again purified by repeated silica gel column chromatography to give four fractions (A-D). The fraction A (*n*-hexane-$CHCl_3$, 6.6: 3.4) was again purified by the silica gel column chromatography using same solvent system to provide tetracosanoic acid (**3**) and 5α,8α-epidioxy-ergosta 6,9(11), 22-triene-3-β-ol (**11**). The fractions B and C were separately treated with the silica gel column chromatography by using the same solvent system *n*-hexane-$CHCl_3$ (6.5: 3.5) and (6.4: 3.6) gave ursolic acid (**12**) and hexacosanoic acid (**2**), respectively. The fraction D was treated with PTLC using the solvent system *n*-hexane-$CHCl_3$ in the ratio (6.0:4.0) gave 1,3-dihydroxycyclolanosterol (**16**) and oleanolic acid (**14**). The fraction obtained from *n*-hexane-$CHCl_3$ (2.0: 8.0) gave many spots on TLC, which on PTLC using solvent system *n*-hexane-$CHCl_3$ (3.0: 7.0) provided 5,6-dihydroxy 4,7-dimethoxy-2-methyl anthracene 9,10-dione (**15**) as a major compound while the other compounds were unable to isolate by the available chromatographic techniques.The fractions which were obtained from $CHCl_3$-MeOH (1.5: 8.5) were combined and rechromatographed over silica gel eluting with $CHCl_3$-MeOH provided three fractions. The fraction 2 which was obtained from $CHCl_3$-MeOH (1.7: 8.3) gave two spots on TLC, which on PTLC by using same solvent system afforded stigmasterol-3-*O*-β-D-glucopyranoside (**17**) and β-sitosterol-3-*O*-β-D-glucopyranoside (**18**), respectively.

1-Triacontanol (1): Colorless crystals, 41 mg, m.p. 86-87 °C. IR (KBr, ν_{max}, cm^{-1}): 3520, 3060, 810. EIMS: *m/z* (rel. int.) $[M]^+$ 438, 420, 405, 360. HR-EIMS *m/z* 438.4860 (calcd. for $C_{30}H_{62}O$, 438.4801). ^1H NMR ($CDCl_3$, 400 MHz)δ: 3.14 (2H, t, *J* = 7.1 Hz, H-1), 1.61 (2H, m, H-2), 1.62-1.29 (54 H, br. s, H-3-H-29), 0.91 (3H, t, *J* = 6.7 Hz, H-30). ^{13}C NMR ($CDCl_3$, 100 MHz)δ: 63.0 (C-1), 32.02 (C-2), 30.0 (C-3), 29.8 (C-4), 29.4-28.8, (C-5-C-28), 21.2 (C-29), 13.7 (C-30).

Hexacosanoic acid (2): White crystals, 38 mg, m.p. 88-89 °C. IR (KBr,ν_{max}, cm^{-1}): 3280-2620, 1716, 920. EIMS: *m/z* (rel. int.): $[M]^+$ 396 (58), 378 (100), 363 (41), 318, 303. HR-EIMS *m/z* 396.3901 (calcd. for $C_{26}H_{52}O_2$, 396.3967). ^1H NMR ($CDCl_3$, 400 MHz) δ: 10.57 (OH), 2.26 (2H, t, *J* = 7.4 Hz) 1.59 (2H, m) 1.29-1.18 (48 H, br, s, 24 × CH_2) 0.90 (3H, t, *J* = 6.4 Hz, CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 181.0 (C-1) 33.9 (C-2) 29.9 (C-3) 29.7-29.2 (C-4-C-25), 13.7 (C-26).

Tetracosanoic acid (3): Colorless needles, 22 mg. m.p. 87-88 °C. IR (KBr, ν_{max}, cm^{-1}): 3280-2610, 1710, 910. EIMS *m/z* (rel. int.): $[M]^+$ 368 (60), 350 (100), 335 (55), 290 (40). HR-EIMS *m/z* 368.3605 (calcd. for $C_{24}H_{48}O_2$, 368.3654). ^1H NMR ($CDCl_3$, 400 MHz) δ:10.47 (COO<u>H</u>), 2.21 (2H, t, *J* = 7.4 Hz, H-2), 1.57 (2H, m, H-3), 1.29-1.17 (40 H, br s, 20 × CH_2, H-4-H-23), 0.90 (3H, t, *J* = 6.4 Hz, H-24). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 180.3 (C-1) 34.2 (C-2) 29.6 (C-3) 29.2-29.0 (C-4-C-23), 13.7 (C-24).

Benzoic acid (4): White crystals, 28 mg, m.p. 122-123 °C. UV (CD_3OD) λ_{max}log ε: 228 (3.62), 272 (3.77), 300 (4.01) nm. HREIMS *m/z* (rel. int.): $[M]^+$ 122.0367 (calcd. for $C_7H_5O_2$, 122.0360). IR (KBr, ν_{max},cm^{-1}): 3260-2610 (COOH), 1705 (C=O). ^1H NMR ($CDCl_3$, 400 MHz) δ: 11.92 (1H, s, O-H), 8.12 (2H, t, *J* = 8.5 Hz, H-2 and H-6), 7.60 (1H, t, *J* = 8.5 Hz, H-4), 7.46 (2H, t, *J* = 8.5 Hz, H-3 and H-5). ^{13}C NMR (CD_3OD, 100 MHz) δ: 180.0 (C-7), 134.5 (C-4), 131.1 (C-1), 130.7 (C-2 and C-6), 130.7 (C-3 and C-5).

2-Hydroxybenzoic acid (5): White crystals, 18 mg, m.p. 159-160 °C. UV (MeOH) λ_{max} nm (log ε): 234 (3.67), 302 (3.98). IR ($CHCl_3$, ν_{max} , cm^{-1}): 3408, 3246, 2657, 1704, 1626, 812. EIMS *m/z* (rel. int.): $[M]^+$ 138 (93), 120 (100), 92 (94), 81 (27), 64 (65), 53 (61). HREIMS *m/z* 138.0291 (calcd. for $C_7H_6O_3$, 138.0316). ^1H NMR ($CDCl_3$, 400 MHz) δ: 7.84 (1H, δ, *J* = 8.5 Hz, H-6), 7.44 (1H, t, *J* = 8.5 Hz, H-4), 6.89 (1H, t, *J* = 8.5 Hz, H-5) and 6.85 (1H, d, *J* = 8.5 Hz, H-3). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 172.9 (C-7), 162.5 (C-2), 136.6 (C-4), 131.1 (C-6), 119.8 (C-5), 117.8 (C-3) 112.4 (H-1).

4-Ethoxy benzoic acid (6): White crystals, 25 mg, m.p. 195-196°C. IR ($CHCl_3$, ν_{max}, cm^{-1}): 3080, 2400, 1715, 1610 1540. EIMS *m/z* (rel. int.): $[M]^+$ 166 (10), 137 (50), 120 (100). HREIMS *m/z*: 166.0615 (calcd. for $C_9H_{10}O_3$, 166.0630). ^1H NMR ($CDCl_3$, 500 MHz) δ: 6.85 (2H, dd, *J* = 7.8, 1.6 Hz, H-2 and H-6), 7.95 (2H, dd, *J* = 7.8, 2.2 Hz, H-3 and H-5), 4.20 (2H, q, *J* = 4.9 Hz, OC<u>H</u>$_2$CH$_3$) and 1.10 (3H, t, *J* = 6.8 Hz, OCH_2CH_3). ^{13}C NMR ($CDCl_3$, 125 MHz)δ: 122.6 (C-1), 132.1 (C-2, C-6), 115.4 (C-3, C-5), 160.3 (C-4), 66.2 (OCH_2CH_3), 20.1 (OCH_2CH_3) and 167.1 (CO).

3,5-Dimethoxybenzoic acid (7): Colorless crystals, 20 mg m.p. 182.5 °C. UV (MeOH) λ_{max} nm (log ε): 220, 251, 285 nm. IR (KBr, ν_{max}, cm^{-1}): 3550 (OH), 1710 (C=O), 1625 (aromatic). EIMS *m/z* (rel. int. %): $[M]^+$ 182 (88), 151 (45), 120 (100), 92 (70), 64 (52), 53 (80). HREIMS *m/z*: 182.1739 (calcd. for

$C_9H_{10}O_4$, 182.1706). ^{1}H NMR (CD_3OD, 400 MHz) δ: 7.09 (2H, d, *J* = 2.0 Hz, H-2 and H-6), 6.72 (1H, d, *J* = 2.1 Hz, H-4), 3.82 (6H, s, 2 × OCH_3). ^{13}C NMR (CD_3OD, 100 MHz)δ: 167.5 (COOH), 161.5 (C-3 and C-5), 130.7 (C-1), 105.2 (C-2 and C-6), 101.3 (C-4)

β-Sitosterol (8): Crystallized from acetone, 25 mg m.p. 135 °C, $[\alpha]_D^{27}$ + 35.5° (c = 1.02, $CHCl_3$). IR (KBr, ν_{max}, cm^{-1}): 3446, 3050, 1650. EIMS *m/z* (rel. int. %): $[M]^+$ 414 (15), 399 (10), 396 (12), 381 (72), 329 (28), 275 (12), 273 (17), 255 (36). HREIMS *m/z*: 414.3845 (calcd. for $C_{29}H_{50}O$, 414.3861). ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 5.11 (1H, m, H-6), 3.36 (1H, m, H-3), 1.01 (3H, s, Me-19), 0.92 (3H, d, *J* = 6.2 Hz, Me-21), 0.84 (3H, t, *J* = 7.0 Hz, Me-29), 0.83 (3H, d, *J* = 6.5 Hz, Me-26), 0.81 (3H, d, *J* = 6.5 Hz, Me-27), 0.68 (3H, s, Me-18). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 140.9 (C-5), 121.9 (C-6), 71.9 (C-3), 56.8 (C-14), 56.2 (C-17), 50.8 (C-9), 50.4 (C-24), 42.6 (C-13), 42.4 (C-4), 40.3 (C-12), 37.3 (C-1), 36.6 (C-10), 36.3 (C-20), 34.0 (C-22), 32.3 (C-7), 32.0 (C-8), 31.8 (C-2), 29.3 (C-23), 28.2 (C-16), 26.2 (C-25), 24.3 (C-15), 23.1 (C-28), 21.1 (C-11), 19.8 (C-27), 19.4 (C-19), 19.1 (C-21), 18.8 (C-26), 11.9 (C-29), 11.4 (C-18).

β-Amyrin (9): Crystallized from ethanol, 30 mg, m.p. 197-198 °C, $[\alpha]_D^{25}$ + 100° (c = 1.15, $CHCl_3$). IR (KBr, ν_{max}, cm^{-1}): 3510, 3055, 1635, 820. EIMS *m/z* (rel. int. %) $[M]^+$: 426 (15), 411 (18), 408 (16), 393 (32), 257 (20), 218 (100), 207 (10), 203 (40) 189 (55). HREIMS *m/z* 426.3825 (calcd. for $C_{30}H_{50}O$, 426.3861). ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 5.11 (1H, m, H-12), 3.19 (1H, dd, *J* = 10.0, 4.5 Hz, H-3), 1.08 (3H, s, Me-27), 1.02 (3H, s, Me-23), 1.01 (3H, s, Me-24), 0.96 (3H, s, Me-25), 0.93 (3H, s, Me-29), 0.88 (3H, s, Me-26), 0.85 (3H, s, Me-30) and 0.80 (3H, s, Me-28). ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 144.3 (C-13), 124.1 (C-12), 78.8 (C-3), 54.4 (C-5), 47.7 (C-9), 47.3 (C-18), 46.9 (C-19), 42.3 (C-14), 41.6 (C-22), 40.9 (C-8), 39.4 (C-4), 39.0 (C-1), 37.0 (C-10), 34.0 (C-17), 33.3 (C-29), 33.2 (C-7), 32.9 (C-21), 31.7 (C-20), 28.2 (C-23), 28.0 (C-28),27.4 (C-2), 26.5 (C-16), 26.3 (C-15), 26.0 (C-27), 23.6 (C-11), 23.3 (C-30), 18.5 (C-6), 16.9 (C-26), 15.6 (C-25), 15.1 (C-24).

Stigmasterol (10): White crystals, 25 mg, m.p. 170-171 °C. $[\alpha]_D^{25}$ 51.5° (c = 1.06, $CHCl_3$). IR ($CHCl_3$, ν_{max}, cm^{-1}): 3432 (OH), 1648 (C=C). EIMS: *m/z* (rel. int. %): $[M]^+$ 412 (8), 396 (12), 394 (20), 379 (27), 369 (35), 351 (71), 327 (60), 301 (18), 300 (67), 273 (30), 270 (24). HREIMS *m/z*: 412.3920 (calcd. for $C_{29}H_{48}O$, 412.3926). ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 5.33 (1H, m, H-6), 5.15 (1H, dd, *J* = 15.2, 8.4 Hz, H-22), 5.02 (1H, dd, *J* = 15.2, 8.6 Hz, H-23), 3.28 (1H, m, H-3), 0.90 (3H, d, *J* = 6.5 Hz, Me-21), 0.83 (3H, d, *J* = 6.6 Hz, Me-26), 0.84 (3H, t, *J* = 7.0 Hz, Me-29), 0.81 (3H, d, *J* = 6.5 Hz, Me-27), 0.80 (3H, s, Me-19), 0.65 (3H, s, Me-18). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 140.9 (C-5), 138.4 (C-22), 129.4 (C-23), 121.7 (C-6), 71.9 (C-3), 57.0 (C-14), 56.0 (C-17), 51.3 (C-24), 50.3 (C-9), 42.5 (C-13), 42.2 (C-4), 40.5 (C-20), 39.7 (C-12), 37.5 (C-1), 36.6 (C-10), 32.2 (C-8), 32.0 (C-25), 31.9 (C-7), 31.4 (C-2), 28.9 (C-16), 25.4 (C-28), 24.4 (C-15), 21.6 (C-27), 21.3 (C-21), 21.0 (C-11), 19.4 (C-19), 19.0 (C-26), 12.4 (C-18), 12.0 (C-29).

5α,8α-Epidioxyergosta-6,9(11),22-trien-3β-ol (11): White amorphoussolid, 15 mg, $[\alpha]_D^{25}$-33.0 °C (c = 1.00, $CHCl_3$). IR (KBr, ν_{max}, cm^{-1}): 3440, 3024 and 1640. EIMS: *m/z* (rel. int.): $[M]^+$ 426 (28), 410 (35), 394 (100), 376 (15), 251 (38). HREIMS *m/z*: 426.641 (calcd. for $C_{28}H_{42}O_3$, 426.638). ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 6.61 (1H, d, *J* = 8.5 Hz, H-7), 6.30 (1H, d, *J* = 8.5 Hz, H-6), 5.43 (1H, dd, *J* = 6.0,1.8 Hz, H-11), 5.24 (1H, dd, *J* = 15.1, 8.0 Hz, H-22), 5.17 (1H, dd, *J* = 15.1, 8.5Hz, H-23), 3.98 (1H, m, H-3), 1.09 (3H, s, H-19), 1.01 (3H, d, *J* = 6.6 Hz, H-21), 0.92 (3H, d, *J* = 7.2 Hz, H-28), 0.84 (3H, d, *J* = 6.7 Hz, H-27), 0.83 (3H, d, *J* = 6.5 Hz, H-26) and 0.75 (3H, s, H-18). ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 142.8 (C-9), 135.8 (C-7), 135.5 (C-22), 132.5 (C-23), 135.2 (C-6), 119.8 (C-11), 83.0 (C-5), 78.5 (C-8), 66.4 (C-3), 56.0 (C-17), 48.3 (C-14), 43.7 (C-13), 42.8 (C-24), 41.3 (C-12), 39.9 (C-20), 38.1 (C-10), 36.2 (C-4), 33.1 (C-25), 32.8 (C-1), 30.8 (C-2), 28.7 (C-16), 25.5 (C-19), 22.8 (C-15), 20.9 (C-21), 20.0 (C-27), 19.7 (C-26), 17.5 (C-28) 12.8 (C-18).

Ursolic acid (12): Colorless needles, 25 mg, m.p. 283-285 °C, $[\alpha]_D^{28}$ + 62.5° (c = 1.00, $CHCl_3$). IR ($CHCl_3$, ν_{max}, cm^{-1}): 3510, 3050, 1697, 1635, 820. EIMS *m/z* (rel. int.): $[M]^+$ 456 (10), 411 (22), 248 (34), 203 (100), 189 (16). HREIMS *m/z*: 456.3599 (calcd. for $C_{30}H_{48}O_3$, 456.3603). ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 5.11 (1H, m, H-12), 3.19 (1H, dd *J* = 10.0 Hz, *J* = 4.5 Hz), 1.20 (3H, s, Me-27), 1.07 (3H, s, Me-23), 0.94 (3H, s, Me-25), 0.91 (3H, d, *J* = 6.6 Hz, Me-30), 0.86 (3H, s, Me-26), 0.81 (3H, s, Me-24), 0.80 (3H, d, *J* = 6.8 Hz, Me-29). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 176.2 (C-28), 138.7 (C-13), 125.8 (C-12), 79.1 (C-3), 55.2 (C-18), 52.4 (C-5), 48.9 (C-17), 47.4 (C-9), 42.0 (C-14), 39.6 (C-8), 38.5 (C-1), 37.0 (C-22), 36.1 (C-10), 33.2 (C-7), 30.5 (C-19), 32.3 (C-20), 29.4 (C-15), 27.5 (C-21), 24.6 (C-27), 24.5 (C-16), 27.4 (C-2), 24.0 (C-23) 23.9 (C-11), 23.8 (C-30), 22.4 (C-29), 18.3 (C-6), 17.6 (C-26), 15.7 (C-25) 15.4 (C-24).

Cyclostenol (13): Colorless crystals, 28 mg, m.p. 221-222 °C. $[\alpha]_D^{30}$ + 43.2° (c = 1.50, $CHCl_3$). IR (KBr, ν_{max}, cm^{-1}): 3435-3380 (OH), 1360-1380 (germinal dimethyl). EIMS *m/z* (rel. int.): $[M]^+$ 416 (35), 398 (48), 383 (100), 338 (30). HREIMS *m/z*: 416. 5210 (calcd. 416.5236 for $C_{28}H_{48}O_2$). ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 3.57 (1H, dd, *J* = 10.4, 4.9 Hz, H-3), 3.22 (1H, dt, *J* = 9.9, 7.6 Hz, H-6), 1.62 (2H), 1.06 (2H), 0.52 (2H, d, *J* = 4.7 Hz, H-19), 0.97 (3H, s, H-28), 0.92 (3H, d, *J* = 7.3 Hz, H-21), 0.87 (3H, s, H-18), 0.83 (6H, d, *J* = 6.8 Hz, H-26 and H-27). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 70.7 (C-6), 70.2 (C-3), 52.3 (C-17), 47.4 (C-14), 47.3 (C-8), 39.4 (C-24), 26.3 (C-11), 36.0 (C-22), 34.4 (C-12), 32.2 (C-15), 30.7 (C-1), 28.0 (C-25), 27.0 (C-16), 29.2 (C-10), 25.0 (C-19), 24.0 (C-23), 23.1 (C-26, 27), 18.8 (C-21), 18.7 (C-28), 17.8 (C-18).

Oleanolic acid (14): Colorless crystals, 22 mg, m.p. 305-306 °C. $[\alpha]_D^{25}$ + 78.9° (c = 0.07, $CHCl_3$). IR (KBr, ν_{max}, cm^{-1}): 3400-2640, 1700, 1660 and 820. EIMS *m/z* (rel. int.): 456 $[M]^+$ (4), 248 (98), 208 (12), 203 (60) and 133 (53). HREIMS *m/z*: 456.3610 (calcd. for $C_{30}H_{48}O_3$, 456.3603). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 5.24 (1H, t, *J* = 3.4 Hz, H-12), 3.60 (1H, dd, *J* = 4.1, 9.9 Hz, H-3), 1.12 (3H, s, Me-27), 1.03 (3H, s, Me-23), 0.98 (3H, s, Me-24), 0.96 (3H, s, Me-25), 0.91(3H, s, Me-29), 0.90 (3H, s, Me-26) and 0.89 (3H, s, Me-30). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 183.4 (C-28), 143.6 (C-13), 122.7 (C-12), 78.0 (C-3), 55.2 (C-5), 47.6 (C-9), 46.5 (C-17), 45.9 (C-19), 41.6 (C-14), 41.0 (C-18), 39.1 (C-8), 38.9 (C-4), 38.4 (C-1), 37.5 (C-10), 33.8 (C-21), 33.0 (C-29), 32.2 (C-7), 32.4 (C-22), 30.6 (C-20), 28.1 (C-23), 27.5 (C-15), 27.2 (C-2), 25.9

(C-27), 24.5 (C-30), 23.4 (C-11), 23.4 (C-16), 19.3 (C-6), 17.1 (C-26), 15.6 (C-24), 15.4 (C-25).

5, 6-Dihydroxy-4,7-dimethoxy-2-methylanthracene-9,10-dione (15): Orange crystals, m.p. 259 ° C. UV (MeOH): λ_{max} 306, 329, 405 nm. IR (KBr, ν_{max}, cm^{-1}) 3355, 1659, 1635, 1455. EIMS *m/z* (rel. int. %) $[M]^+$ 314 (66), 299 (29), 296 (35), 283 (19), 269 (30), 268 (40), 255 (15). HREIMS *m/z* (rel. int. %) 314.0785 (calcd. for $C_{17}H_{14}O_6$, 314.0790). ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 12.01 (1H, s, OH-5), 7.60 (1H, s, H-1), 7.23 (1H, s, H-8), 7.09 (1H, s, H-3), 6.50 (1H, s, OH-6), 3.95 (3H, s, OCH_3-7), 3.89 (3H, s, OCH_3-8), 2.49 (3H, s, CH_3-2). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 185.0 (C-10), 181.0 (C-9), 160.5 (C-4), 154.1 (C-5), 150.9 (C-7), 148.9 (C-2), 139.1 (C-6), 123.0 (C-3), 120.9 (C-1), 106.5 (C-8), 63.3 (OCH_3-4), 56.0 (OCH_3-7), 22.0 (CH_3-2).

Cycloart-24-ene-1,3-diol (16): White crystals, 16 mg, m.p. 140.5 °C, $[\alpha]_D^{25}$ + 78.9° (c = 0.07, $CHCl_3$). IR (KBr, ν_{max}, cm^{-1}) 3440-3380 (OH), 1360-1380 (geminaldimethyls). EIMS (rel. int. %) $[M]^+$ 442 (26), 424 (66), 406 (52), 391 (45), 346 (35). HREIMS (rel. int. %) $[M]^+$ 442 (calcd. 442.2413 for $C_{30}H_{50}O_2$), ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 5.10 (1H, t, *J* = 6.2 Hz, H-24), 3.22 (1H, dd, *J* = 6.9, 4.4 Hz, H-1), 3.31 (1H, dd, *J* = 9.2, 6.8 Hz, H-3), 1.54 (6H, s, Me-26, Me-27), 1.15 (3H, s, Me-18), 1.14 (3H, s, Me-28), 1.12 (3H, s, Me-30), 1.09 (3H, s, Me-29), 1.03 (3H, s, Me-21), 0.06 (2H, d, *J* = 4.4 Hz, H-18). ^{13}C NMR ($CDCl_3$, 100 MHz),130.0 (C-25), 124.1 (C-24), 76.2 (C-3), 54.3 (C-13), 53.1 (C-1), 51.0 (C-5), 50.9 (C-8), 46.0 (C-17), 41.4 (C-15), 39.5 (C-14), 37.4 (C-20), 36.9 (C-22), 36.2 (C-4),34.5 (C-11), 30.2 (C-10), 28.6 (C-19),27.3 (C-16), 25.9 (C-23), 24.9 (C-9), 22.6 (C-28), 21.8 (C-6), 21.8 (C-26), 20.7 (C-12), 20.6 (C-29),18.0 (C-30), 17.1 (C-27), 16.9 (C-21).

Stigmasterol 3-*O*-β-D-glucopyranoside (17): Amorphous solid, 30 mg, m.p. 289-290 ºC. $[\alpha]_D^{27}$-51.5º (c = 1.20, CH_3OH). IR (KBr, ν_{max}, cm^{-1}) 3454 (OH), 3024, 1646 (C=C). EIMS *m/z*: $[M\text{-Glc}]^+$ 412 (72), 397 (15), 394 (22), 379 (28), 369 (35), 351 (71), 327 (55), 301(15), 300 (67), 273 (21), 271 (26). HRFABMS (+ve) *m/z*: 575.4231 (calcd. for $C_{35}H_{59}O_6$, 575.4233). ^{1}H NMR (400 MHz, CD_3OD) δ: 5.23 (1H, br d, *J* = 5.4 Hz, H-6), 5.14 (1H, dd, *J* = 15.2, 8.4 Hz, H-22), 5.02 (1H, dd, *J* = 15.2, 8.6 Hz, H-23), 4.78 (1H, d, *J* = 7.4 Hz, H-1′), 3.84-4.44 (m, Glc-H), 3.83 (1H, m, H-3), 1.01 (3H, s, CH_3-19), 0.90 (3H, d, *J* = 6.2 Hz, CH_3-21), 0.83 (3H, d, *J* = 6.5 Hz, CH_3-26), 0.82 (3H, t, *J* = 7.0 Hz, CH_3-29), 0.80 (3H, d, *J* = 6.5 Hz, CH_3-27), 0.67 (3H, s, CH_3-18). ^{13}C NMR (125 MHz, CD_3OD) δ: 141.5 (C-5), 138.9 (C-22), 129.1 (C-23), 121.1 (C-6), 102.8 (C-1′), 79.8 (C-3), 76.9 (C-3′), 76.7 (C-5′), 74.2 (C-2′), 70.6 (C-4′), 62.2 (C-6′), 57.0 (C-14), 56.1 (C-17), 52.1 (C-24), 50.8 (C-9), 43.9 (C-4), 43.1 (C-13), 40.5 (C-20), 39.9 (C-12), 37.8 (C-1), 36.9 (C-10), 32.9 (C-25), 32.8 (C-2), 31.9 (C-7), 31.7 (C-8), 28.9 (C-16), 25.6 (C-28), 24.5 (C-15), 21.9 (C-21), 21.7 (C-27), 21.5 (C-11), 19.5 (C-19), 19.1 (C-26), 12.6 (C-18), 12.1 (C-29).

β-Sitosterol 3-*O*-β-D-glucopyranoside (18): Amorphous solid, 18 mg, m.p. 279-280 ºC, $[\alpha]_D^{28}$-14.5º (c = 1.32, MeOH). IR (KBr, ν_{max}, cm^{-1}) 3452, 3044,1646. EIMS *m/z*: $[M\text{-Glc}]^+$ 414 (19), 399 (15), 396 (24), 381 (70) 329 (25), 275 (11), 273 (24), 255 (35). HRFABMS (+ve) *m/z*: 577.4386 $[M + H]^+$ (calcd. for $C_{35}H_{61}O_6$, 577.4389). ^{1}H NMR (300 MHz, CD_3OD) δ: 5.33 (1H, d, *J* = 7.2 Hz, H-1′), 5.12 (1H, br d, *J* = 5.4 Hz, H-6), 3.85 (1H, m, H-3), 3.82-4.42 (m, Glc-H), 1.01 (3H, s, CH_3-19), 0.92 (3H, d, *J* = 6.2 Hz, CH_3-21), 0.84 (3H, t, *J* = 7.0 Hz, CH_3-29), 0.83 (3H, d, *J* = 6.5 Hz, CH_3-26), 0.81 (3H, d, *J* = 6.5 Hz, CH_3-27), 0.68 (3H, s, CH_3-18). 13C-NMR (100 MHz, CD_3OD) δ: 142.0 (C-5), 122.1 (C-6), 103.2 (C-1′), 80.9 (C-3), 76.8 (C-3′), 76.6 (C-5′), 74.2 (C-2′), 70.2 (C-4′), 62.0 (C-6′), 56.9 (C-14), 56.5 (C-17), 50.5 (C-24), 50.4 (C-9), 43.9 (C-4), 43.0 (C-13), 40.8 (C-12), 39.5 (C-22), 38.7 (C-1), 37.2 (C-20), 37.1 (C-10), 33.0 (C-7), 32.9 (C-8), 29.9 (C-2), 29.7 (C-16), 29.5 (C-23), 26.0 (C-25), 25.8 (C-15), 23.7 (C-28), 21.5 (C-11), 20.1 (C-27), 19.7 (C-21), 19.5 (C-19), 18.2 (C-26), 12.2 (C-18), 11.9 (C-29).

RESULTS AND DISCUSSION

The methanolic extract of *Tribulus longipetalus* was divided into *n*-hexane, chloroform, ethyl acetate, *n*-butanol and water soluble fractions. A series of column chromatographic techniques applied to chloroform and ethyl acetate fractions resulted in the isolation and structural elucidation of eighteen known compounds and were identified as 1-triacontanol[4] (**1**), hexacosanoic acid[5] (**2**), tetracosanoicacid[6] (**3**), benzoic acid[7] (**4**), 2-hydroxybenzoic acid[8] (**5**), 4-ethoxy benzoic acid[9] (**6**), 3,5-dimethoxybenzoic acid[10] (**7**), β-sitosterol[4] (**8**), β-amyrin[11] (**9**), stigmasterol[12] (**10**), 5α,8α-epidioxyergosta-6, 9(11), 22- trien-3β-ol[13] (**11**), ursolic acid[4,10] (**12**), cyclostenol[14] (**13**), olea-nolic acid[15] (**15**), 5,6-dihydroxy-4, 7-dimethoxy-2-methy-lanthracene-9,10-dione[16] (**15**), 1,3-dihydroxycyclolanosterol[17] (**16**), stigmasterol 3-O-β-D-glucopyranoside[15] (**17**), β-sitosterol 3-O-β-D-glucopyranoside[18] (**18**), respectively. All of these compounds have been purified for the first time from *Tribulus longipetalus Viv*.

REFERENCES

1. A. Gafoor, Flora of West Pakistan, Printas Printing Press, Karachi, Pakistan, p. 21 (1974).
2. S.R. Baquar, Medicinal And Poisonous Plants of Pakistan, Printas Printing Press, Karachi, Pakistan, p. 451 (1989).
3. A. Temraz, D.O. El Gindi, H.A. Kadry, N. De Tommasi and A. Braca, *Phytochemistry*, **67**, 1011 (2006).
4. E. Ahmed, A. Malik, N. Riaz and A. Sharif, *J. Chem. Soc. Pak*., **26**, 389 (2004).
5. S. Watanabe, *Z. Naturforsch. C*, **30**, 825 (1975).
6. L. Marosi, *Annalen*, 584 (1973).
7. E. Ahmed, A. Malik, M.M. Ahmad, M.A. Munawar, S.A. Nagar, J. Anwar, M.A. Qazi, A. Sharif, N. Afza and M. Ashraf, *J. Chem. Soc. Pak*., **30**, 936 (2008).
8. Aldrich Library of ^{13}C-and ^{1}H-FT-NMR Spectra, 2, 1065B, 1065C, 1251B, 1252A, 1414C, 1511A, 1512A (1992).
9. K.N. Scott, *J. Am. Chem. Soc*., **94**, 8564 (1972).
10. K. Jabeen, A. Javaid, E. Ahmad and M.M. Athar, *Nat. Prod. Res*., **25**, 264 (2011).
11. S. Hussain, E. Ahmed, A. Malik and A. Jabbar, *J. Chem. Soc. Pak*., **27**, 296 (2005).
12. H.L. Holland, P.R.P. Diakow and G.J. Taylor, *Can. J. Chem*., **56**, 3121 (1978).
13. A.A.C. Gunatilaka, Y. Gopichand, F.J. Schmitz and C. Djerassi, *J. Org. Chem*., **46**, 3860 (1981).
14. H.W. Kircher and H.L. Bird Jr., *Phytochemistry*, **21**, 1705 (1982).
15. E. Ahmed, A. Sharif, S. Hussain, A. Malik and M. Ul Hassan, *J. Chem. Soc. Pak*., **33**, 676 (2011).
16. A.G. Gonzalez, J.B. Barrera, B.B. Davila, E. Valencia and X.A. Dominguez, *Phytochemistry*, **31**, 255 (1992).
17. T. Shen, H.Q. Yuan, W.Z. Wan, X.L. Wang, X.N. Wang, M. Ji and H.X. Lou, *J. Nat. Prod*., **71**, 81 (2008).
18. E. Ahmed, A. Sharif, H. Nawaz and M. Ul Hassan, *J. Chem. Soc. Pak*., **33**, 682 (2011).