Phytochemical and Antimicrobial Studies of *Grewia tenax*

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Summary: For the first time eleven compounds have been isolated from the ethyl acetate soluble fraction of whole plant of *Grewia tenax* namely, *β*-sitosterol (**1**), *β*-sitosteryl acetate (**2**), *β*-amyrin (**3**), *β*-amyrin acetate (**4**), 5*α*,8*α*-epidioxyergosta-6,22-diene-3*β*-ol (**5**), 5*α*,8*α*-epidioxyergosta-6,9(11),22-trien-3*β*-ol (**6**), *α*-taraxerol (**7**), betulin (**8**), stigmasterol (**9**), oleanolic acid (**10**) and stigmasterol 3-*O*-*β*-D-glucoside (**11**) respectively. All the compounds were evaluated for their antimicrobial activity.

Introduction

Grewia tenax Forsk. (Syn. *G. populifolia* Vahl) belongs to family Tiliaceae. It is a shrub distributed in the warmer parts of the world. The genus *Grewia* consists of about 150 species, distributed in the tropical and sub-tropical zones of Africa, Asia and Australia. *Grewia tenax* is sub erect to erect shrub that occurs in Pakistan at Kohat, Dera Ismail Khan, Sargodha, and Thatta. It is also known as Gangu [1-3] and used against hepatitis virus (HCV) [4] by local practitioners in folk medicines [5-8]. No phytochemical work has been so far been reported on this species. This prompted us to carry out phytochemical studies on this species. The methanolic extract of *G. tenax* showed strong phytotoxicity. On further fractionation the major phytotoxicity was detected in the ethyl acetate fraction. Column chromatography of the ethyl acetate fraction have resulted in the isolation and structure elucidation of eleven compound for the first time as β-sitosterol (**1**), [9-10] *β*-sitosteryl acetate (**2**) [11], β-amyrin (**3**), [12, 13], *β*-amyrin acetate (**4**) [14], 5α,8α-epidioxyergosta-6,22-diene-3*β*-ol (**5**) [15], 5*α*,8*α*-epidioxyergosta-6,9(11),22-trien-3*β*-ol (**6**) [15], α-taraxerol [16] (**7**), betulin [17] (**8**), stigmasterol (**9**), [18, 19] oleanolic acid (**10**) [20] and stigmasterol 3-*O*-β-D-glucoside (**11**) [18, 19], respectively. The isolated compounds were evaluated for their antimicrobial activity against *Bacillus subtilis, Shigella flexneri, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi, Trichophyton longifusus, Candida albicans, Microsporum canis, Fusarium solani,* and *Candida glabrata*.

Results and Discussion

Column chromatography of the ethyl acetate fraction of *G. tenax* have resulted in the isolation and structure elucidation of β-sitosterol (**1**), [9, 10] *β*-sitosteryl acetate (**2**) [11], β-amyrin (**3**), [12, 13], *β*-amyrin acetate (**4**) [14], 5α,8α-epidioxyergosta-6,22-diene-3*β*-ol (**5**) [15], 5*α*,8*α*-epidioxyergosta-6,9(11),22-trien-3*β*-ol (**6**) [15], α-taraxerol [16] (**7**), betulin [17] (**8**), stigmasterol (**9**), [18, 19] oleanolic acid (**10**) [20] and stigmasterol 3-*O*-β-D-glucoside (**11**) [18, 19], respectively. All of these have been reported for the first time from *G. tenax*. The compounds **1-11** were respectively screened for antimicrobial assay against *Bacillus subtilis, Shigella flexneri, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi, Trichophyton longifusus, Candida albicans, Microsporum canis, Fusarium solani,* and *Candida glabrata,* using agar well diffusion and agar tube dilution protocol [21]. All the isolated compounds showed weak to moderate activity (Table-1).

Experimental

General

Column chromatography (CC): silica gel 70-230 mesh. Flash chromatography (FC): silica gel

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230-400 mesh. TLC: pre-coated silica gel 60 F_{254} plates. Optical rotations: Jasco-DIP-360 digital polarimeter. UV and IR spectra: Hitachi-UV-3200 and Jasco-302-A spectrophotometers, respectively. ^{1}H- and ^{13}C-NMR, spectra: Bruker spectrometers operating at 500, 400, 125 and 100 MHz; chemical shifts δ in ppm relative to $SiMe_4$ as internal standard and coupling constants *J* in Hz. HREIMS, HRFABMS: JEOL JMS-HX-110 and JMS-DA-500 mass spectrometers, *m/z* (rel. int). The purity of the isolated compounds was check on pre-coated high performance thin layer chromatography (HPTLC) plates of E-Merck. Miconazole and imipenem were purchased from sigma chemical company (St. Louis, MO, USA). Nutrient agar was purchased from Oxoid Ltd. (Basingstoke, Hampshire, England) while DMSO, used for antimicrobial activity was purchased from BDH Laboratory supplies (Poole, England).

Plant Material

The whole plant of *Grewia tenax* Forsk (25 kg) was collected from Thatta (Pakistan) and identified from Department of Botany, University of Karachi where a voucher specimen has been deposited.

Isolation

The shade dried and powdered whole plant (5 kg) was extracted thrice with MeOH (3×15 L) at room temperature. The combined methanolic extract was evaporated under reduced pressure to obtain a thick gummy mass (300 g). It was suspended in water and successively extracted with *n*-hexane (125g), ethylacetate (55g), *n*-butanol (40g) and water (60g). The ethyl acetate soluble fraction (55g) was subjected to column chromatography over silica gel eluting with *n*-hexane-chloroform, chloroform-methanol and methanol in increasing order of polarity to obtain 14 fractions. The fractions 3 obtained from *n*-hexane-chloroform 7.0: 3.0 provided two major and one miner spot on TLC having nearby same Rf value, were combined and again chromatographed over $AgNO_3$ impregnated PTLC using solvent system *n*-hexane-chloroform (6.5:3.5) gave β–sitosterol (**1**), stigmasterol (**9**) and oleanolic acid (**10**).

Similarly the fraction **4**, obtained from *n*-hexane-chloroform 6.0: 4.0 provided four major spots with different Rf value was again rechromatographed over silica gel PTLC using the same solvent system (*n*-hexane-chloroform: 6.0: 4.0) gave β-amyrin (**3**), β-amyrin acetate (**4**) α-taraxerol (**7**). The fraction **5** obtained from *n*-hexane-chloroform 5.0: 5.0 showed two major spots on TLC column was again chromatographed over silica gel using solvent system *n*-hexane-EtOAc (6.0: 4.0) purified β-sitosteryl acetate (**2**) and betulin (**8**). Similarly the fraction **6** obtained from *n*-hexane-chloroform 4.0: 6.0 showed two spots on TLC was again chromatographed over PTLC using solvent system *n*-hexane: ethyl acetate (5.0: 5.0) purified 5α, 8α-Epidioxergosta 6-22-diene 3β-ol (**5**) and 5α, 8α epidioxyergosta-6, 9(11), 22-diene-3β-ol (**9**) having Rf 0.5 and 0.45 respectively. The fraction 10 was obtained at polarity of 100 % chloroform showed several miner and one major spot on TLC was rechromatographed over silicia gel PTLC using solving solvent system chloroform-methanol (9.5: 0.5) gave stigmasterol 3-O-β- D-glucoside (11), in a 28 mg amount.

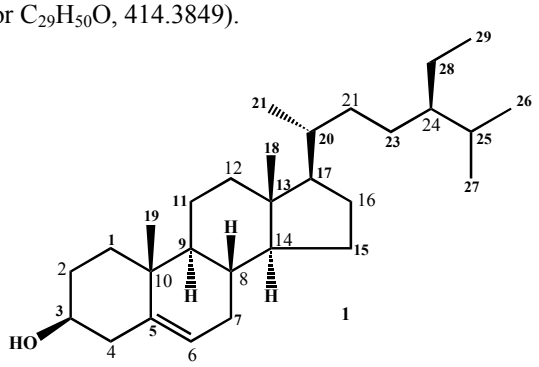
Table-1: Antimicrobial activity of Compounds **1-11** from *Gravia tanix.*

Microorganism	**Zone of inhibition diameter (mm)**											
Bacteria	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**Standard drugs imipenem**
Gram-positive												
Bacillus subtilis	**10**	**22**	**5**	**15**	**20**	**15**	**12**	**18**	**14**	**12**	**10**	**33**
Shigella flexneri	**07**	**05**	**05**	**08**	**05**	**08**	**05**	**05**	**05**	**07**	**12**	**27**
Staphylococcus aureus	**09**	**11**	**07**	**13**	**10**	**08**	**10**	**08**	**08**	**05**	**10**	**33**
Gram-negative												
Escherichia coli	**08**	**15**	**07**	**12**	**8**	**05**	**18**	**10**	**10**	**10**	**08**	**30**
Pseudomonas aeruginosa	**05**	**10**	**04**	**05**	**10**	**05**	**15**	**06**	**05**	**12**	**05**	**24**
Salmonella typhi	**05**	**05**	**0**	**05**	**0**	**0**	**0**	**0**	**05**	**07**	**05**	**25**
Fungi												**Miconazole**
Trichophyton longifusus	**35**	**22**	**20**	**20**	**25**	**20**	**20**	**15**	**10**	**25**	**35**	**70**
Candida albicans	**10**	**20**	**32**	**28**	**10**	**35**	**30**	**18**	**22**	**25**	**40**	**110.8**
Aspergillus flavus	**0**	**05**	**0**	**0**	**0**	**05**	**0**	**0**	**04**	**05**	**0**	**20**
Microsporum canis	**15**	**22**	**35**	**10**	**10**	**10**	**05**	**30**	**05**	**20**	**20**	**98.4**
Fusarium solani	**25**	**05**	**05**	**20**	**20**	**30**	**15**	**10**	**22**	**25**	**20**	**73.25**
Candida glabrata	**35**	**25**	**30**	**45**	**40**	**40**	**10**	**10**	**0**	**10**	**10**	**110.8**

Key: Values are inhibition zones (mm) and an average of triplicate; Concentration of sample = 3 μg/ ml of DMSO. Standard drug for antibacterial activity = imipenem (10 μg/ disc). Size of well = 6 mm (diameter). Concentration of Sample for antifungal activity = 200 μg/ ml of DMSO. Incubation temperature = 27 (28 ± 1°C). Incubation period = 7 days.

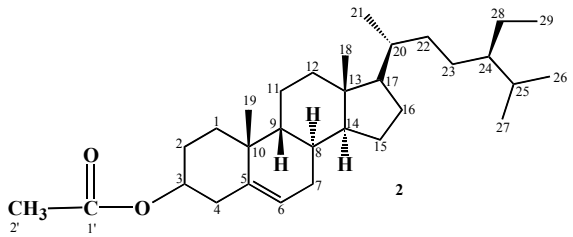
β-Sitosterol (**1**)

White crystals, 18 mg, m.p. 136-136.5 °C; $[\alpha]_D^{25}$: + 35.5° (*c* = 1.0, $CHCl_3$), IR (KBr) ν_{max} cm^{-1}: 3440 , 3055, 1659; ^{1}H-NMR ($CDCl_3$, 400 MHz): δ: 5.21 (1H, m, H-6), 3.39 (1H, m, H-3), 1.20 (3H, s, Me-19), 0.93 (3H, d, *J* = 6.0 Hz, Me-21), 0.85 (3H, t, *J* = 7.2 Hz, Me-29), 0.84 (3H, d, *J* = 6.5 Hz, Me-26), 0.82 (3H, d, *J* = 6.0 Hz, Me-27), 0.69 (3H, s, Me-18). ^{13}C-NMR ($CDCl_3$, 100 MHz), *δ*: 140.5 (C-5), 121.5 (C-6), 71.2 (C-3), 56.1 (C-14), 56.0 (C-17), 50.8 (C-9), 50.6 (C-24), 42.9 (C-13), 42.5 (C-4), 40.5 (C-12), 37.5 (C-1), 36.0 (C-10), 36.8 (C-20), 34.1 (C-22), 32.4 (C-7), 32.5 (C-8), 31.9 (C-2), 29.1 (C-23), 28.4 (C-16), 26.5 (C-25), 24.8 (C-15), 23.9 (C-28), 21.0 (C-11), 19.5 (C-27), 19.1 (C-19), 19.4 (C-21), 18.4 (C-26), 11.5 (C-29) and 11.3 (C-18). N.B**:** *m/z* 414 (20), 399 (18), 396 (29), 381 (80), 329 (45), 275 (35), 273 (15), 255 (50). HREIMS: *m/z* 414.3855 (calcd for $C_{29}H_{50}O$, 414.3849).



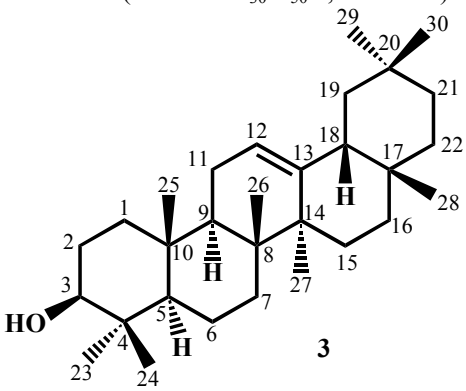
β-Sitosteryl acetate (**2**)

White crystals, 22 mg, m.p. 119-120 °C; $[\alpha]_D^{25}$ –38.5° (*c* = 0.06, $CHCl_3$). IR ν_{max} ($CHCl_3$): 3025, 1725, 1635 and 809 cm^{-1}. ^{1}H-NMR ($CDCl_3$, 500 MHz), *δ*: 3.52 (1H, m, H-3), 5.20 (1H, m, H-6), 0.67 (3H, s, Me-18), 1.03 (3H, s, Me-19), 0.90 (3H, d, *J* = 6.0 Hz, Me-21), 0.85 (3H, d, *J* = 6.4 Hz, Me-26), 0.82 (3H, d, *J* = 6.5 Hz, Me-27), 0.86 (3H, t, *J* = 7.2 Hz, Me-29) and 2.07 (3H, s, CH_3-COO), ^{13}C-NMR ($CDCl_3$, 125 MHz), *δ*: 37.5 (C-l), 31.2 (C-2), 77.1 (C-3), 42.5 (C-4), 139.9 (C-5), 122.2 (C-6), 32.1 (C-7), 32.0 (C-8), 50.9 (C-9), 36.9 (C-10), 21.9 (C-11), 40.9 (C-12), 42.8 (C-13), 56.3 (C-14), 24.5 (C-15), 28.2 (C-16), 56.6 (C-17), 11.3 (C-18), 19.5 (C-19), 36.1 (C-20), 19.6 (C-21), 34.7 (C-22), 29.5 (C-23), 45.4 (C-24), 26.6 (C-25), 18.2 (C-26), 19.2 (C-27), 23.4 (C-28), 11.5 (C-29), 20.4 ($\underline{C}H_3CO$) and 171.2 ($\underline{C}O$). EIMS *m/z* (rel. int.): [M^+] 456 (25), 441 (35), 396 (100), 381 (20), 329 (50), 315 (75), 303 (80), 275 (50), 273 (50), 255 (70) and 213 (40). HREIMS *m/z*: 456.3955 (calcd for $C_{31}H_{52}O_2$, 456.3967).



β-Amyrin (**3**)

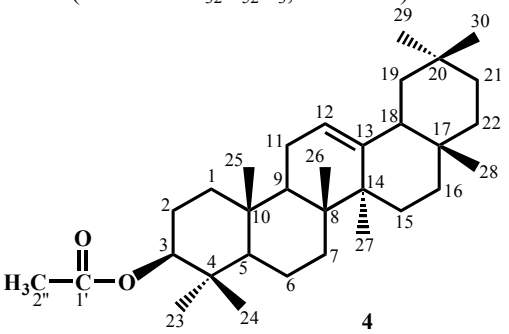
Crystals, 18mg, m.p 197-198°C; $[\alpha]_D^{25}$: +100° (*c* = 0.08, $CHCl_3$). IR ($CHCl_3$) ν_{max} (cm^{-1}): 3500-3520, 3040, 1630 and 810; ^{1}H-NMR ($CDC1_3$, 400 MHz) δ: 5.14 (m, H-12), 3.10 (dd, *J*= 10.5, 4.8 H_Z, H-3), 1.03, 1.00, 1.07, 0.94, 0.92, 0.86, 0.84 and 0.81 (3H each, s, Me). ^{13}C-NMR ($CDCl_3$, 100 MHz): *δ* 144.0 (C-13), 123.9 (C-12), 78.5 (C-3), 54.0 (C-5), 47.9 (C-9), 47.5 (C-18), 47.1 (C-19), 42.5 (C-14), 41.8 (C-22), 40.1 (C-8), 39.5 (C-4), 39.4 (C-1), 37.9 (C-10), 34.8 (C-17), 33.7 (C-29), 33.6 (C-7), 33.1 (C-21), 31.5 (C-20), 28.6 (C-23), 28.8 (C-28), 27.0 (C-2), 26.9 (C-16), 26.5 (C-15), 26.2 (C-27), 23.5 (C-11), 23.9 (C-30), 18.8 (C-6), 16.8 (C-26), 15.8 (C-25) and 15.6 (C-24). EIMS *m/z* (rel. int.): 426 $[M]^+$ (25), 411 (32), 408 (36), 393 (60), 257 (40), 218 (100), 207 (20), 203 (70) and 189 (80). EIHRMS showed $[M]^+$ at *m/z* 426.3833 (calcd for $C_{30}H_{50}O$, 426.3861).



β-Amyrin acetate (**4**)

Crystalls, 28 mg, m.p**.:** 244-245 °C. $[\alpha]_D^{25}$: + 81.0° (*c* = 1.0, $CHCl_3$). IR (KBr) ν_{max} cm^{-1}: 3045, 1720, 1680, 1470, 1390, 1150 and 808 cm^{-1}. ^{1}H-NMR ($CDCl_3$, 500 MHz): *δ* 5.20 (1H, m, H-12), 4.15 (1H, dd, *J* = 10.2, 4.0 Hz, H-3), 2.15 (3H, s, OAc), 1.01, 1.09, 0.99, 0.98 (3H, each s, Me), 0.92 (6H, s, H-29 and H-30), 0.88 and 0.85 (3H, each s, Me). ^{13}C-NMR ($CDCl_3$, 125 MHz), *δ*: 170.5 (CO), 139.0 (C-13), 124.0 (C-12), 79.9 (C-3), 59.8 (C-18), 55.2 (C-5), 47.0 (C-9), 42.8 (C-14), 41.6 (C-22), 40.0 (C-8), 39.2 (C-19), 39.7 (C-20), 39.1 (C-4), 39.0 (C-1), 37.0 (C-10), 34.0 (C-17), 33.2 (C-7), 31.6 (C-21), 28.1 (C-15), 28.5 (C-28), 28.6 (C-23), 27.9 (C-2), 26.0 (C-

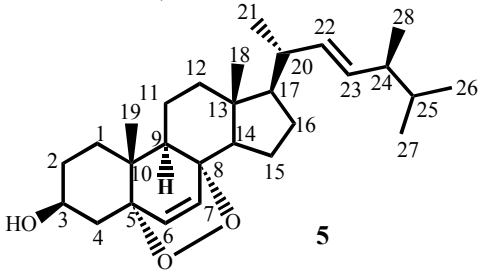
16), 23.5 (C-29), 23.4 (C-17), 21.5 (C-30), 20.0 (CH_3CO), 18.5 (C-6), 17.8 (C-11), 16.7 (C-26), 15.9 (C-24) and 15.0 (C-25). EIMS *m/z* (rel. int.): 468 $[M]^+$ (30), 426 (55), 411 (28), 408 (20), 257 (40), 218 (100), 207 (20), 203 (70) and 189 (85). HREIMS *m/z*: 468.3931 (calcd. for $C_{32}H_{52}O_3$, 468.3916).



4

5α,8α-Epidioxyergosta-6,22-diene-3β-ol (**5**)

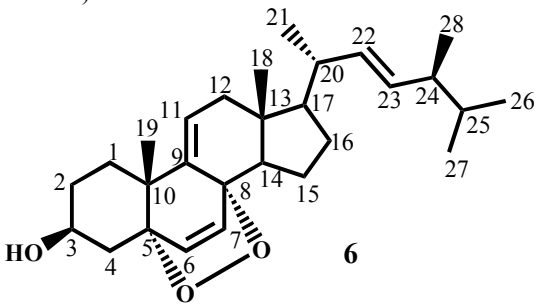
Colorless crystals, 30 mg, m.p. 176-178 °C. $[\alpha]_D^{25}$: –31.0° (*c* = 1.5, $CHCl_3$). IR (KBr) λ_{max} cm^{-1}, 3425, 3050 and 1660. ^{1}H-NMR ($CDCl_3$, 500 MHz), *δ*: 6.55 (1H, d, *J* = 8.0 Hz, H-7), 6.22 (1H, d, *J*= 8.0 Hz, H-6), 5.28 (1H, dd, *J* = 15.0, 8.2 Hz, H-22), 5.19 (1H, dd, *J* = 15.0, 8.6 Hz, H-23), 3.70 (1H, m, H-3), 1.08 (3H, d, *J* = 7.5 Hz, H_3-21), 0.94 (3H, d, *J* = 6.0 Hz, H_3-28), 0.87 (3H, s, H_3-19), 0.85 (3H, d, *J* = 6.2 Hz, H_3-27), 0.60 (3H, s, H_3-18) and 0.80 (3H, d, *J* = 6.3 Hz, H_3-26). ^{13}C-NMR ($CDCl_3$, 125 MHz), *δ*: 135.9 (C-6), 135.6 (C-22), 132.5 (C-23), 130.9 (C-7), 82.2 (C-5), 79.9 (C-8), 66.6 (C-3), 56.8 (C-17), 51.2 (C-14), 51.9 (C-9), 44.4 (C-13), 42.3 (C-24), 39.1 (C-20), 39.8 (C-12), 37.6 (C-4), 36.5 (C-10), 34.7 (C-1), 33.2 (C-25), 30.0 (C-2), 28.9 (C-16), 23.3 (C-15), 20.4 (C-21), 20.2 (C-11), 20.0 (C-27), 19.0 (C-26), 18.3 (C-19), 17.4 (C-28) and 12.3 (C-18). EIMS *m/z* (rel. int.): $[M]^+$ 428 (30), 410 (50), 396 (100), 363 (48), 253 (41). HREIMS *m/z*: 428.3340 (calcd for $C_{28}H_{44}0_3$, 428.3290).



5

5α,8α-Epidioxyergosta-6,9(11),22-trien-3β-ol (**6**)

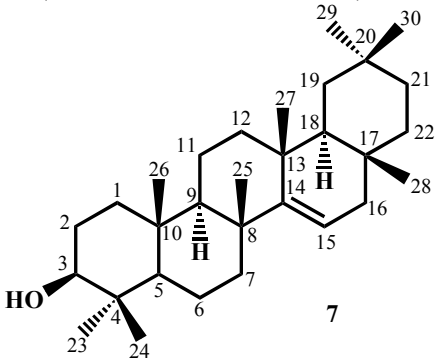
Gummy solid, 24 mg, IR (KBr) υ_{max} cm^{-1}: 3445, 3000 and 1636. ^{1}H-NMR ($CDCl_3$, 400 MHz), *δ*: 6.65 (1H, d, *J* = 8.5 Hz, H-7), 6.32 (lH, d, *J* = 8.0 Hz, H-6), 5.43 (1H, dd, *J* = 6.2, 1.5 Hz, H-11), 5.26 (1H, dd, *J* = 15.0, 8.2 Hz, H-22), 5.19 (1H, dd, *J* = 15.0, 8.0 Hz, H-23), 3.92 (1H, m, H-3), 1.07 (3H, s, H_3-19), 1.01 (3H, d, *J*= 6.6 Hz, H_3-21), 0.92 (3H, d, *J*= 7.2 Hz, H_3-28), 0.82 (3H, d, *J*= 6.1 Hz, H-27), 0.83 (3H, d, *J*= 6.5 Hz, H_3-26) and 0.72 (3H, s, H-18). ^{13}C-NMR ($CDCl_3$, 100 MHz), δ: 142.4 (C-9), 135.5 (C-7), 135.2 (C-22), 132.1 (C-23), 135.8 (C-6), 119.6 (C-11), 83.5 (C-5), 78.9 (C-8), 66.3 (C-3), 56.6 (C17), 48.8 (C-14), 43.4 (C-13), 42.6 (C-24), 41.9 (C-12), 39.4 (C-20), 38.3 (C-10), 36.8 (C-4), 33.6 (C-25), 32.6 (C-l), 30.7 (C-2), 25.6 (C-16), 25.9 (C-19), 22.8 (C-15), 20.5 (C-21), 20.5 (C-27), 19.2 (C-26), 17.4 (C-28) and 12.4 (C-18). EIMS *m/z* (rel. int.): $[M]^+$ 426 (35), 410 (55), 394 (100), 376 (28), 251 (56). HREIMS *m/z:* 426.6350 (calcd. for $C_{28}H_{42}O_3$, 426.6381).



6

α-taraxerol (**7**)

14 mg, m.p. 271-271.5 °C; IR (KBr) ν_{max} (cm^{-1}): 3640, 3060, 1640 and 820; ^{1}H-NMR ($CDC1_3$, 400 MHz): δ: 5.54 (1H, t, *J* = 6.2 Hz, H-15), 3.36 (1H, t, *J* = 3.2 Hz, H-3), 1.15 (6H, s, Me-26 and me- 27), 0.94 (6H, s, Me-23 and Me-30), 0.91 (6H, s, Me-25 and Me-29), 0.85 (3H, s, Me-28) and 0.80 (3H, s, Me-24). HREIMS (rel. int.): $[M]^+$ *m/z* 426.3825 (calcd for $C_{30}H_{50}O$, 426.3861).

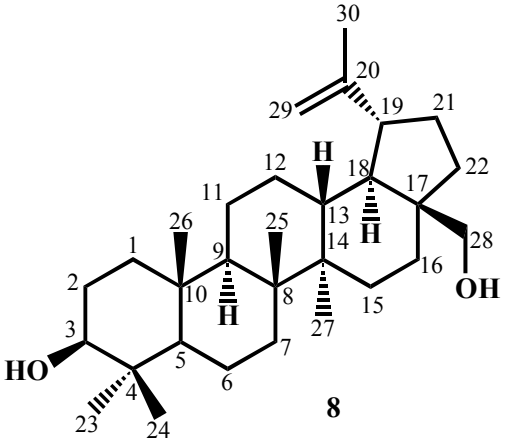


7

Betulin (**8**)

White crystals, 10 mg, m.p. 251-252 °C; IR (KBr) ν_{max} cm^{-1}: 3450, 3055, 1630 and 850; ^{1}H-NMR ($CDCl_3$, 500 MHz): δ: 4.60 (2H, m, H-29), 3.70 (1H, dd, *J* = 10.2, *J* = 4.0 Hz, H-3), 3.81, 3.42 (1H each, d,

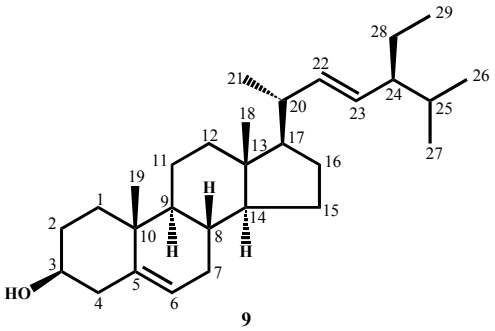
J = 11.5 Hz, H-28), 1.60 (3H, brs, Me-30), 1.00 (3H, s, Me-26), 0.96 (3H, s, Me-27), 0.90 3H, s, Me-23), 0.86 (3H, s, Me-25), 0.85 (3H, s, Me-24). HREIMS (rel. int.):: *m/z* 442.3820 (calcd for $C_{30}H_{50}O_2$, 414.3849).



8

Stigmasterol (**9**)

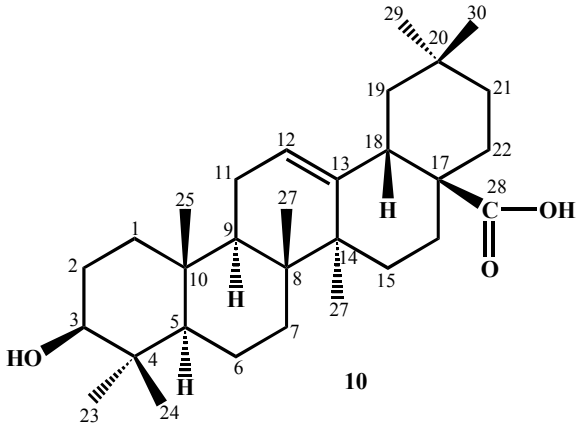
White crystals, 12 mg, m.p. 170 -171 °C, $[\alpha]_D^{25}$ – 51.9° (*c* = 0.15, $CHCl_3$). IR ($CHCl_3$) υ_{max} (cm $^{-1}$): 3433 (OH), 3027, and 1649 (C=C); ^{1}H NMR ($CDCl_3$, 300 MHz) 5.31 (1H, m, H-6), 5.58 (1H, dd, *J* = 15.0, 8.0 Hz, H-22), 5.20(1H, dd, *J* = 15.0, 8.0 Hz, H-23), 3.25 (1 H, m, H-3), 0.92 (3H, d, *J* = 6.0 Hz, Me-21), 0.82 (3H, d, *J* = 6.4 Hz, Me-27), 0.84 (3H, t, *J* = 7.2 Hz, Me-29), 0.80 (3H, d, *J* = 6.2 Hz, Me-26), 0.78 (3H, s, Me-19), 0.68 (3H, s, M-18). ^{13}C-NMR ($CDCl_3$, 100 MHz), *δ*: 140.5 (C-5), 138.0 (C-22), 129.0 (C-23), 121.2 (C-6), 71.4 (C-3), 57.2 (C-14), 56.4 (C-17), 51.0 (C-24), 50.5 (C-9), 42.9 (C-13), 42.1 (C-4), 40.0 (C-20), 39.5 (C-12), 37.2 (C-1), 36.3 (C-10), 32.4 (C-8), 32.4 (C-25), 31.4 (C-7), 31.4 (C-2), 28.5 (C-16), 25.0 (C-28), 24.2 (C-15), 21.0 (C-27), 21.4 (C-21), 21.4 (C-11), 19.5 (C-19), 19.5 (C-26), 12.3 (C-18), 12.2 (C-29). EIMS *m/z* (rel. int. %): $[M]^+$ 412 (13), 396 (20), 394 (30), 379 (41), 369 (44), 351 (81), 327 (80), 301 (30), 300 (57), 273 (40), 270 (37). EIHRMS showed $[M]^+$ at *m/z* 412.3900 (calcd. for $C_{29}H_{48}O$, 412.3926).



9

Oleanolic acid (**10**)

(14 mg, colorless needles, $[\alpha]_D^{25}$: + 78.1° (c = 1.0, $CHCl_3$). m.p. 305-306 °C; IR (KBr) ν_{max} (cm^{-1}); 3410, 1702, 1665, and 825; ^{1}H-NMR ($CDCl_3$, 400 MHz); δ: 5.24 (1H, t, *J* = 3.0 Hz, H-12), 3.62 (1H, dd, *J* = 9.5 Hz, *J* = 4.0 Hz, H-3l), 1.10 (3H, s, Me-27), 1.00 (3H, s, Me-23), 0.99 (3H, s, Me-30), 0.97 (3H, s, Me-25), 0.94 (3H, s, Me-26), 0.92 (3H, s, Me-29), and 0.90 (3H, s, Me-24). ^{13}C-NMR ($CDCl_3$, 100 MHz): *δ* 183.0 (C-28), 143.0 (C-13), 122.2 (C-12), 79.4 (C-3), 55.4 (C-5), 47.1 (C-9), 46.9 (C-17), 45.5 (C-19), 41.2 (C-14), 41.4 (C-18), 39.3 (C-8), 38.6 (C-4), 38.6 (C-1), 37.4 (C-10), 33.6 (C-21), 33.2 (C-29), 32.2 (C-7), 32.2 (C-22), 30.5 (C-20), 28.3 (C-23), 27.9 (C-15), 27.5 (C-2), 25.3 (C-27), 23.5 (C-30), 23.3 (C-11), 23.6 (C-16), 18.0 (C-6), 17.0 (C-26), 15.2 (C-24), and 15.2 (C-25). EIMS *m/z* (rel. int.): 456 $[M]^+$ (22), 248 (100), 208 (42), 203 (70) and 133 (59). EIHRMS; *m/z* 456.3603 (calcd for $C_{30}H_{48}O_3$, 456.3595).



10

Stigmasterol 3-O-β-D-glucoside (**11**)

15 mg, Colorless crystals, m.p 289-290 °C, $[\alpha]_D^{25}$: – 14.7° (*c* = 1.3, MeOH), IR (KBr) υ_{max} cm^{-1} 3430 (OH), 3020, 1645 (C=C) ,^{1}H NMR (CD_3OD, 400MHz) *δ*: 5.22 (1H, br d, *J* = 5.0 Hz, H-6), 5.14 (1 H ,dd *J* = 15.2, 8.4 Hz H-22), 5.02 (1H, dd, *J* = 15.2, 8.6 Hz, H-23), 4.72 (1H, dd, *J* = 7.0 Hz, H-1), 3.85 (1H, m, H-3), 3.82-4.40 (m, Glc.-H), 1.00 (3H, s, Me-19), 0.92 (3H, d, *J* = 6.4 Hz, Me-21), 0.84 (3H, d, 6.5 Hz, Me-26), 0.82 (3H, t, *J* = 7.2 Hz, Me-29), 0.80 (3H, d, *J* = 6.8 Hz, Me-27), 0.68 (3H, s, Me-18). ^{13}C-NMR ($CDCl_3$, 100 MHz), *δ*: 142.3 (C-5), 122.6 (C-6), 103.4 (C-1′), 80.7 (C-3), 77.6 (C-3′), 77.5 (C-5′), 75.2 (C-2′), 71.6 (C-4′), 61.8 (C-6′), 56.7 (C-14), 56.9 (C-17), 50.3 (C-24), 49.6 (C-9), 43.5 (C-4), 43.7 (C-13), 40.9 (C-12), 39.0 (C-22), 38.5 (C-1), 37.4 (C-20), 37.9 (C-10), 33.0 (C-7), 32.5 (C-8), 29.7 (C-2),

29.2 (C-16), 29.4 (C-23), 25.9 (C-25), 25.0 (C-15), 23.5 (C-28), 21.7 (C-11), 19.4 (C-27), 19.3 (C-19), 19.0 (C-21), 18.4 (C-26), 12.2 (C-18) and 11.6 (C-29). EIMS: *m/z* $[M]^+$ 414 (25), 399 (20), 396 (18), 381 (81), 329 (38), 275 (45), 273 (47), 255 (39). EIHRMS showed M^+ peak at *m/z* 575.4201 (calcd for C_{35} $H_{59}O_6$, 575.4233).

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