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Phytochemical Studies on *Amberboa ramosa*

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ABSTRACT
Twelve compounds have been isolated for the first time from *Amberboa ramosa* namely, octacosanoic acid (**1**), cinnamicacid (**2**), 7,8-dihydroxycoumarin (**3**), 5-hydroxy-7,8-dimethoxyflavone(**4**), 5-hydroxy-3,6,7,4′-tetramethoxyflavone (**5**), 5,7,3',4'-tetrahydroxyflavone (**6**), β-amyrin (**7**), β-amyrin acetate (**8**), marrusidin A (**9**) marrusidin B (**10**), polyodonine (**11**), stigmasterol 3-*O*-β-D-glucopyranoside (**12**)respectively. Their structures have been elucidated by EI-MS, HR-EI-MS, HR-FAB-MS, ^{1}H-NMR and ^{13}C-NMR spectroscopic data. Compounds **2-6** showed moderate antioxidant activity.

Keywords**:** NMR; *Amberboa ramosa*; Compositeae; Antioxidant activity; Compositeae.

1. INTRODUCTION
The genus *Amberboa* is a small genus of family Compositeae, comprising six species. These are annuals or biennials extending from Mediterranean to Central Asia[1-2]. This plant is used as tonic, aperient, febrifuge, deobstruent, cytotoxic and antibacterial activities. A decoction of leaves is given to relieve swelling and to dissolve kidney stones, treating vomiting, paralysis and diseases of spleen. A paste of this plant is used on open wounds and other skin diseases. Its roots and flowers are reported to possess medicinal properties against rheumatism and kidney troubles[3-5]. Shifting of literature revealed that triterpenoids, flavonoids, steroids and sesquiterpene lactones have previously been reported from this species[6-8]. The chemotaxonomic and ethnopharmacological significance of the genus *Amberboa* promted us to reinvestigate the constituents of *A. ramosa*. As a result, we now report the isolation and structural elucidation of octacosanoic acid (**1**), cinnamic acid (**2**), 7,8-dihydroxycoumarin (**3**), 5-hydroxy-7,8-dimethoxyflavone(**4**), 5-hydroxy-3,6,7,4′-tetramethoxyflavone (**5**), 5,7,3',4'-tetrahydroxyflavone (**6**), β-amyrin (**7**), β-amyrin acetate (**8**), marrusidin A (**9**) marrusidin B (**10**), polyodonine (**11**), stigmasterol 3-*O*-β-D-glucopyranoside (**12**), respectively. The compounds **2 – 6** showed moderate antioxidant activity.

2. RESULTS AND DISCUSSION
The methanolic extract of the whole plant of *Amberboa ramosa* was subsequently divided into *n*-hexane, chloroform, ethylacetate, *n*-butanol and water-soluble fractions. The ethyl acetate soluble fraction was subjected to a series of column and flash chromatographic techniques as described in the experimental to obtain twelve compounds reported for the first time from this species. These could be identified octacosanoic acid (**1**), cinnamic acid (**2**), 7,8-dihydroxycoumarin (**3**), 5-hydroxy-7,8-dimethoxyflavone(**4**), 5-hydroxy-3,6,7,4′-tetramethoxyflavone (**5**), 5,7,3',4'-tetrahydroxyflavone (**6**), β-amyrin (**7**), β-amyrin acetate (**8**), marrusidin A (**9**) marrusidin B (**10**), polyodonine (**11**), stigmasterol 3-*O*-β-D-glucopyranoside (**12**) respectively, on the basis of their respective spectral data.

3. EXPERIMENTAL

3.1 *General*
Optical rotations were measured on a JASCO DIP-360 polarimeter. IR spectra were recorded on a 460 Shimadzu spectrometer. EI-MS and HR-FAB-MS were recorded on JMS-HX-110 and JMS-DA 5000 mass spectrometers. The ^{1}H-NMR, ^{13}C-NMR, HMQC, and HMBC spectra were recorded on Bruker spectrometers operating at 400 MHz for ^{1}H- and 100 MHz for ^{13}C-NMR, respectively. The chemical shift values are reported in ppm (δ) units and the coupling constants (*J*) are in Hz. Aluminum sheets precoated with silica gel 60 F_{254} (20 × 20 cm, 0.2 mm thick; E-Merck) were used for TLC and silica gel (230-400 mesh) was used for column chromatography. Visualization of the TLC plates was carried out under UV at 254 and 366 nm and by spraying with ceric sulfate reagent (with heating). Melting points were determined on a Gallenkemp apparatus and are uncorrected. For antioxidant assay all the chemicals were purchased from Sigma Chemical Company (St. Louis, MO, USA).

3.2 *Plant Material*
The whole plant of *Amberboa ramosa* Jafri (Compositae) was collected in June 2002, from Karachi (Pakistan) and identified by Dr. SurraiyaKhatoon, Plant Taxonomist, Department of Botany, University of Karachi, where a voucher specimen (no. KU 312 b) has been deposited.

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3.3 *Extraction and Isolation*

The shade dried plant material (22 kg) was extracted with methanol (3x40 L) for ten days at room temperature. The solvent was evaporated under reduced pressure and the residue (217 g) was partitioned between *n*-hexane and water. The water soluble fraction was further extracted with chloroform, ethyl acetate and *n*-butanol. The column chromatography of the ethyl acetate soluble fraction (90 g) over silica gel and elution with *n*-hexane-ethyl acetate in increasing order of polarity afforded six major fractions (A-F). The fraction A which was obtained from n-hexane: $CHCl_3$ (8:2) was further purified by column chromatography over silica gel eluting successively with hexane and chloroform (8.6:1.4) to afford **1** and (7.5:2.5) to furnished compound **2**. The fraction B obtained from n-hexane-$CHCl_3$ (7.0:3.0) was a mixture of three components, which were separated by column chromatography using solvent system *n*-hexane-$CHCl_3$ (7.7:2.3) to afford compound **4**, *n*-hexane-$CHCl_3$ (7.4:2.6) to afford compounds **7** and *n*-hexane-$CHCl_3$ (7.2:2.8) compound **8**, respectively. The fraction C obtained from *n*-hexane-$CHCl_3$ (5.0:5.0) was further purified by column chromatography eluting with *n*-hexane-$CHCl_3$ (5.6:4.4) to afford compound **3** and mixture of two compounds which was further purified by column chromatography using solvent n-hexane-$CHCl_3$ (4.9:5.1) as eluent to obtain compounds **5** and **11**. The fraction D obtained from n-hexane-$CHCl_3$ (2.0:8.0) was rechromatographed over silica gel and eluted with n-hexane-$CHCl_3$ (2.3:7.7) afford compounds **9** and **10** from the top and tail fractions, respectively. The fraction E obtained from $CHCl_3$ was rechromatographed and eluted with $CHCl_3$-MeOH (9.9:0.1) to afford compound **6**. The fraction F obtained from $CHCl_3$-MeOH (9.5:0.5) was subjected to column chromatography eluting with $CHCl_3$-MeOH (9.8:0.2) to obtain compound **12**.

3.3.1 *Octacosanoic acid*

Amorphous solid (21 mg); 135-136 °C; IR (KBr) ν_{max} cm^{-1}: 2780 and 1705; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 2.32 (2H, t, *J*= 7.5 Hz, H-2), 1.61 (2H, q, H-3), 1.23-1.28 (48H, br s, H-4-H27) and 0.84 (3H, t, *J*= 6.4 Hz, Me-28); EI-MS *m/z* (rel. int.): 424 (M^+), 368 (80), 325 (9), 269 (16), 185 (14), 111 (31), 101 (13), 87 (23), 73 (100), 60 (55); HR-EI-MS *m/z*: 424.4276 (calcd for $C_{28}H_{56}O_2$, 424.4280).The physical and spectral data showed complete agreement with those reported in the literature[9].

3.3.2 *Cinnamic acid*

White solid (8 mg), m.p. 132-133 ^oC; UV ($CHCl_3$) λ_{max} (log ε) nm: 326 (3.9), 285 (3.06); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 7.52 (1H, d, *J* = 15.9 Hz, H-1′), 7.36 (m), 7.29 (m), 7.21 (m), 6.25 (1H, d, *J* =15.9 Hz, H-2′); HR-EI-MS, *m/z*:148.6151 (calcd for $C_9H_8O_2$, 148.6158), El-MS *mlz*(rel. int.): 148 $[M]^+$ (21), 117 (25), 107 (100), 105 (16). The physical and spectral data showed complete agreement with those reported in the literature[10].

3.3.3 *7,8-Dihydroxycoumarin*

Amorphous solid (14 mg), m.p. 113-114°C; UV ($CHCl_3$) λ_{max} (log ε) nm: 312 (3.77), 243 (3.82), 218 (4.08); IR (KBr) ν_{max} cm^{-1}: 3108, 1713, 1607, 1595, 1525, 1503; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 7.61 (1H, d, *J* = 9.5 Hz, H-4), 6.85 (1H, d, *J* = 8.4 Hz, H-5), 6.75 (1H, d, *J* = 8.4 Hz, H-6), 6.10 (1H, d, *J* = 9.5 Hz, H-3); HR-EI-MS, *m/z:* 178.0261 (calcd for $C_9H_6O_4$, 178.0267), El-MS *mlz*(rel. int.): 178 (100), 150 (84), 122 (14), 94 (28), 66 (43), 51 (14). The physical and spectral data were much closed to the reported values[11].

3.3.4 *5-Hydroxy-7,8-dimethoxyflavone*

Yellowish crystalline solid (18 mg); M.P.:97-98 °C; UV (MeOH)λ_{max} nm (log ε): 247 (3.28), 215 (3.50), 315 (4.52) IR(KBr) $\nu_{max}$$cm^{-1}$:3519, 2925, 1660, 1610, 157o;^{1}H-NMR(CD_3OD, 400 MHz) δ: 6.68 (s, H-6), 7.85 (1H, d, *J* = 7.6 Hz, H-2′, H-6′), 7.53 (3H, m, H-3′, H-4′, H-5′), 6.58 (1H, s, H-3), 3.92, 3.98 (6H, s, MeO-7, 8) and 12.69 (chelated hydroxyl group at C-5); EI-MS*m/z* (rel. int.): 298 (37), 255 (28), 181 (15), 153 (53), 125 (17), 102 (19), 93 (21), 77 (25), 69 (100); HR-EI-MS: $[M]^+$ peak at*m/z*: 298.0829 (calcd for $C_{17}H_{14}O_5$; 298.0831). The physical and spectral data were in closed agreement to the reported values[12].

3.3.5 *5-Hydroxy-3,6,7,4′-tetramethoxyflavone*

Amorphous powder (12 mg), m.p. 178-180 ºC; UV ($CHCl_3$) λ_{max} (log ε) nm: 331 (3.92), 275 (3.89), 252 (4.06); IR (KBr) ν_{max} cm^{-1}: 3502, 2954, 1672, 1621, 1564, 1509; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ : 8.05 (2H, d, *J* = 8.5 Hz, H-2', H-6′), 6.99 (2H, d, *J* = 8.5, H-3', H-5′), 6.48 (1H, s, H-8), 3.93, 3.90, 3.87, 3.84 (12H, s, MeO-6, 7, 3, 4'), and 12.60 due to chelated hydroxyl group at C-5; HR-EI-MS showed $[M]^+$ at *m/z* 358.1123 (calcd for $C_{19}H_{18}O_7$, 358.1129), E-IMS *m/z* (rel. int.): 358 (100), 344 (57), 316 (11), 300 (18), 226 (20), 165 (16), 135 (28), 83 (25). The physical and spectral data identified it as *5-hydroxy-3,6,7,4′-tetramethoxyflavone*[13-14].

3.3.6 *5,7,3',4'-Tetrahydroxyflavone*

Yellow crystals (10 mg), m.p. 328°C; UV ($CHCl_3$) λ_{max} (log ε) nm: 256 (3.64), 265 (3.62), 360 (4.12) and 306 (4.41); IR (KBr) ν_{max} cm^{-1}: 3300 (OH), 1655 (C=O) and 1593 (C=C); ^{1}H-NMR (C_5D_5N, 400 MHz) δ: 6.72 (1H, d, *J* = 1.8 Hz, H-6), 6.91 (1H, d, *J* = 1.8 Hz, H-8), 6.79 (1H, s, H-3), 7.54 (1H, dd, *J* = 8.2, 2.2 Hz, H-6'), 7.27 (1H, d, *J* = 8.2 Hz, H-5'), 7.51 (1H, d, *J* = 2.2 Hz, H-2'), 13.78 (1H, br s, 5-OH); ^{13}C-NMR (C_5D_5N, 100 MHz) δ : 181.7 (C-4), 166.9 (C-7), 157.9 (C-5), 101.8 (C-9), 162.6 (C-2) , 114.7 (C-3), 150.4 (C-3′), 151.9 (C-4′), 147.8 (C-6′), 116.8 (C-1′), 119.4 (C-

5′), 122.7 (C-2′), 164.0 (C-10); HR-EI-MS, *m/z*:286.2321 (calcd for $C_{15}H_{10}O_6$, 286.2365),EI-MS *m/z* (rel. int.): 286 (100), 270 (34), 153 (16) and 69 (18). The physical and spectral data showed complete agreement with those reported in literature[15].

3.3.7 *β-Amyrin*

Crystallized from ethanol (33 mg); MP: 197-198 ^oC; $[\alpha]_D^{25}$: +100(*c* = 0.21, $CHCl_3$); IR (KBr) ν_{max} cm^{-1}: 3510, 3055, 1635 and 820; ^{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), 1.02, 1.01, 1.08, 0.96, 0.93, 0.88, 0.85 and 0.80 (3H, each s, Me); EI-MS *m/z* (rel. int.): 426 $[M]^+$ (15), 411 (18), 408 (16), 393 (32), 257 (20), 218 (100), 207 (10), 203 (40) and 189 (55); HR-EI-MS *m/z*: 426.3825 (calcd. for $C_{30}H_{50}O$, 426.3861). The physical and spectral data were in close agreement to the reported values[16-17].

3.3.8 *β-Amyrin acetate*

Crystallized from MeOH (34 mg); MP: 244-245 ^oC; $[\alpha]_D^{25}$: + 81.4(*c* = 0.20, $CHCl_3$); IR (KBr) $\nu_{max}$$cm^{-1}$: 3055, 1710, 1660, 1460, 1382, 1180 and 810; ^{1}H-NMR ($CDCl_3$, 500 MHz) δ:5.10 (1H, m, H-12), 4.05 (1H, dd, *J*= 10.0, 4.5 Hz, H-3), 2.13 (3H, s, OAc), 1.00, 1.07, 0.98, 0.96 (3H, each s, Me), 0.90 (6H, s, H-29 and H-30), 0.86 and 0.81 (3H, each s, Me); EI-MS *m/z* (rel. int.): 468 $[M]^+$ (27), 426 (35), 411 (11), 408 (12), 257 (26), 218 (100), 207 (8), 203 (55) and 189 (75); HR-EI-MS *m/z*: 468.3931 (calcd. for $C_{32}H_{52}O_3$, 468.3916). The physical and spectral data coincided with the literature[18].

3.3.9 *Marrusidin A*

Amorphous powder (38 mg); M.p. 228-230°C; $[\alpha]^{25}_{D}$ = -42.2(c =0.045, $CHCl_3$); IR (KBr)ν_{max} cm^{-1}: 2975, 1782, 1765, 1475, 1180 and 1050; ^{1}HNMR ($CDCl_3$, 400 MHz)*δ*: 1.51–1.53 (1H, m, H-1a), 1.29-1.30 (1H, m, H-1b), 1.78-1.80 (1H, m, H-2a), 1.71-1.73 (1H, m, H-2b), 2.10-2.13 (1H, m, H-3a), 1.42-1.44 (1H, m, H-3b), 2.05 (1H, d, *J*=4.5 Hz, H-5), 4.68 (1H, t, *J*=5.4 Hz, H-6), 2.15-2.17 (1H, m, H-7a), 1.75-1.77 (1H, m, H-7b), 2.14-2.15 (1H, m, H-8), 2.07-2.08 (1H, m, H-11a), 1.86-1.87 (1H, m, H-11b), 2.37-2.39 (1H, m, H-12a), 2.17-2.19 (1H, m, H-12b), 2.41 (1H, dd, *J*=13.4, 5.5, H-14a), 2.21-2.24 (1H, m, H-14b), 5.32 (1H, dd, *J*=9.2, 5.7, H-15), 1.10 (3H, d, *J*=6.3 Hz, H-17), 1.27 (3H, s, H-18), 1.04 (3H, s, H-20), 3.45 (3H, s, OMe at C-15); EI-MS: *m/z* 378 (34), 335 (10), 320 (7), 211 (100); HR-EI-MS: *m/z*378.1340 ($C_{21}H_{30}O_6$; calc. 378.1346). Its physical and spectral data showed complete agreement with those reported in literature[19].

3.3.10 *Marrusidin B*

Amorphous powder (38 mg); M.p.230-232°; $[\alpha]_D^{25}$ = +21.0 (c 0.045, $CHCl_3$); IR (KBr)ν_{max} cm^{-1}: 2974, 1780, 1768, 1473, 1178 and 1055; ^{1}HNMR ($CDCl_3$, 400 MHz) *δ*: 1.48–1.50 (1H, m, H-1a), 1.30-1.32 (1H, m, H-1b), 1.76-1.77 (1H, m, H-2a), 1.66-1.69 (1H, m, H-2b), 2.05-2.06 (1H, m, H-3a), 1.40-1.43 (1H, m, H-3b), 2.14 (1H, d, *J*=4.5 Hz, H-5), 4.67 (1H, t, *J*=5.2 Hz, H-6), 2.21-2.23 (1H, m, H-7a), 1.70-1.74 (1H, m, H-7b), 2.09-2.2.11 (1H, m, H-8), 2.24-2.25 (1H, m, H-11a), 1.87-1.88 (1H, m, H-11b), 2.33-2.55 (1H, m, H-12a), 2.15-2.17 (1H, m, H-12b), 2.55 (1H, dd, *J*=13.6, 5.6, H-14a), 2.18-2.19 (1H, m, H-14b), 5.36 (1H, dd, *J*=9.0, 5.8, H-15), 0.84 (3H, d, *J*=6.0 Hz, H-17), 1.25 (3H, s, H-18), 1.02 (3H, s, H-20), 3.48 (3H, s, OMe at C-15); EI-MS: *m/z* 378 (36), 335 (9), 320 (6), 211 (100); HR-EI-MS: *m/z*378.1340 ($C_{21}H_{30}O_6$; calc. 378.1346). The physical and spectral data showed complete resemblance with the reported values[19].

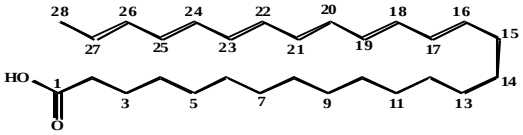
3.3.11 *Polyodonine*

White crystalline solid (38 mg); M.p 200-201^oC; $[a]_D^{25}$ = +78^o (c 0.1, $CHCl_3$); IR (KBr)ν_{max} cm^{-1}:1760 cm^{-1} and 1740, 3092 and 1602 cm^{-1}; ^{1}HNMR ($CDCl_3$, 400 MHz): *δ*: 1.71(m, 1H), 1.31(m, 1H), 1.59(m, 1H), 1.83(m, 1H), 2.17(m, 1H), 1.52 (m, 1H), 2.28 (d, 4.4 Hz, 1H), 4.75 (dd, 4.4, 6.0 Hz), 1.73 (m, 1H), 2.23 (m, 1H), 2.15 (m, 1H), 2.37 (d, 18.4 Hz, 1H), 2.80 (d, 18.4 Hz, 1H), 4.99 (d, 2.5 Hz, 1H), 6.62 (dd, 2.5, 0.5 Hz, 1H), 4.33 (d, 10.7 Hz, 1H), 4.47 (dd10.7, 0.5 Hz, 1H), 0.72 (d, 6.4 Hz, 3H), 1.32 (s, 3H), 1.07 (s, 3H); ^{13}C-NMR ($CDCl_3$, 100 MHz) *δ*: 28.3 (C-1), 17.7 (C-2), 28.1 (C-3), 44.0(C-4), 44.3 (C-5), 75.5 (C-6), 31.1 (C-7), 31.3 (C-3), 84.6 (C-9), 38.5 (C-10), 40.0 (C-11), 212.5 (C-12), 92.7 (C-13), 102.7 (C-14), 151.4 (C-15), 77.9 (C-16). 15.6 (C-17), 22.5 (C-18), 183.4 (C-19), 23.7 (C-20); EIMS: *m/z* (rel. intensity): 346.2 (13), 329 (6), 234 (47), 207 (3) and 173 (39). The physical and spectral data showed complete agreement with literature[20].

3.3.12 *Stigmasterol 3-O-β-D-glucopyranoside*

Colorless crystals (38 mg); MP: 289-290 ^oC; $[\alpha]_D^{25}$:–51.5 (*c* = 0.22, CH_3OH); IR (KBr) ν_{max} cm^{-1}: 3454 (OH), 3024, 1646 (C=C); ^{1}H-NMR (CD_3OD, 400 MHz) δ: 5.23 (lH, br d, *J* = 5.4 Hz, H-6), 5.14 (lH, dd, *J* = 15.2, 8.4 Hz, H-22), 5.02 (lH, dd, *J*= 15.2, 8.6 Hz, H-23), 4.78 (lH, d, *J*= 7.4Hz, H-l′), 3.83 (lH, m, H-3), 3.84-4.44 (m, Glc-H′), 1.01 (3H, s, Me-19), 0.90.(3H, d, *J* = 6.2 Hz, Me-21), 0.83 (3H, d, *J* = 6.5 Hz, Me-26), 0.82 (3H, t, *J* = 7.0 Hz, Me-29), 0.80 (3H, d, *J* = 6.5 Hz, Me-27), 0.67 (3H, s, Me-18); ^{13}C-NMR (CD_3OD, 125 MHz) 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-l), 36.9 (C-10), 32.9 (C-25), 32.8 (C2), 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); EI-MS *m/z* (rel. int.): 412 $[M\text{-}Glu]^+$ (72), 397 (15), 394 (22), 379 (28), 369 (35), 351 (71), 300 (67), 327 (55), 301 (15), 273 (21), 271 (26); HR-FAB-M\S *m/z*: 575.4231 $[M+H]^+$ (calcd for $C_{35}H_{59}0_6$, 575.4233). It was identified through physical and spectral data as stigmasterol 3-O-β-D-glucopyranoside[21-22].



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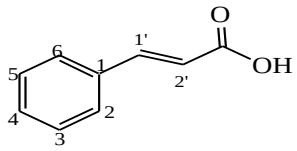

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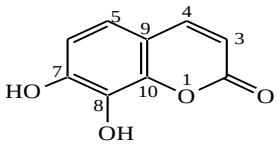

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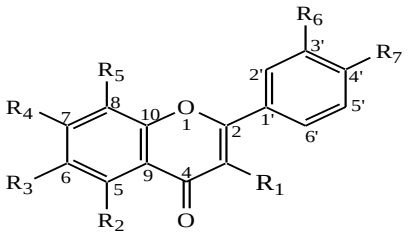
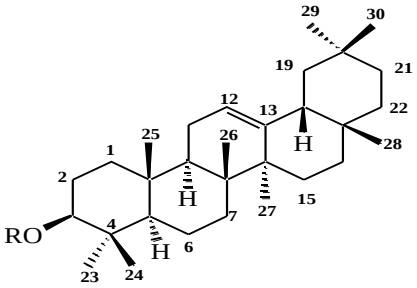
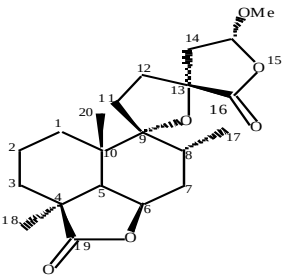


4. R_1,R_3,R_6,R_7=H, R_2=OH, R_4,R_5=OCH_3
5. R_5,R_6=H, R_2= OH, R_1,R_3,R_4,R_7=OCH_3
6. R_1,R_3,R_5=H, R_2,R_4,R_6,R_7=OH.



7. R = H,
8. R = $COCH_3$



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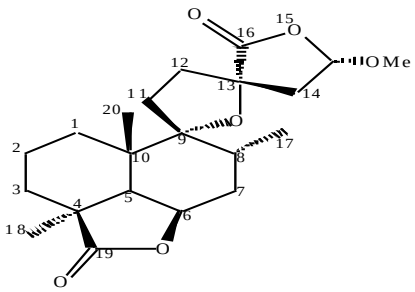
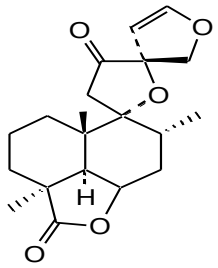
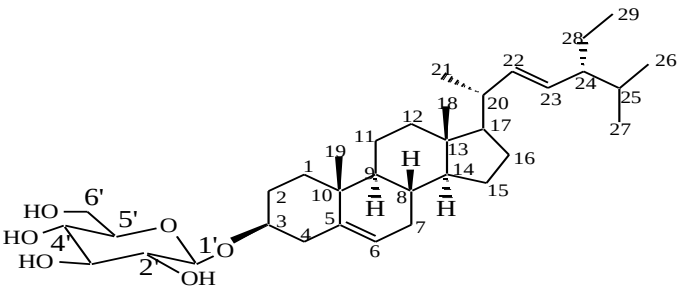


10



11



12

Fig-1: Structure of compounds 1-12 isolated from Amberboa ramosa

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