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Tyrosinase inhibitory cycloartane type triterpenoids from the methanol extract of the whole plant of *Amberboa ramosa* Jafri and their structure–activity relationship

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Abstract—New tyrosinase inhibitory cycloartane triterpenoids have been discovered from the methanol extract of the whole plant of *Amberboa ramosa* (Roxb.) Jafri, which is a member from the Compositae family. Utilizing the conventional spectroscopic techniques, including 1D and 2D NMR analysis, and also by comparing the experimental with literature data, the isolated compounds proved to be cycloartane type triterpenoids. These cycloartanes are: (22*R*)-cycloart-20, 25-dien-2α3β22α triol (**1**), (22*R*)-cycloart-23-ene-3β, 22α, 25-triol (**2**), cycloartenol (**3**), cycloart-23-ene-3β, 25-diol (**4**), cycloart-20-ene-3β, 25-diol (**5**), cycloart-25-ene-3β, (22*R*) 22-diol (**6**), 3β, 21, 22, 23-tetrahydroxy-cycloart-24 (31), 25 (26)-diene (**7**), and (23*R*)-5α-cycloart-24-ene-3β, 21, 23-triol (**8**). Out of these eight compounds, compound **3** did not show any activity against the enzyme tyrosinase. Among them compound **7** was found to be the most potent (1.32 μM) when compared with the standard tyrosinase inhibitors kojic acid (16.67 μM) and L-mimosine (3.68 μM). Finally in this paper, we have discussed the structure–activity relationships of these molecules.

1. Introduction

The genus *Amberboa* belongs to the family Compositae and comprises six species. One of these is *A. ramosa* (Roxb.) Jafri (syn: *A. divaricata* Kuntaze; *Volutarelle divaricala* DC Benth and Hook; *V. ramosa* (Roxb.) Sant.), which is an annual herbaceous plant found in India and Pakistan. The plant has tonic, aperient, febrifuge, deobstruent, cytotoxic, and antibacterial effects.[1] Triterpenoids, flavanoids, steroids, and sesqiterpene lactones have been reported previously from the *Amberboa* species.[1–3] In the present investigation, a methanolic extract of the *A. ramosa* showed positive activity in the brine shrimp lethality test.[4]

As the ^{1}H NMR of the crude chloroform fraction exhibited the presence of cycloartane type compounds. With regard to further fractionation process we tested this chloroform-soluble fraction of crude methanol extract. In our continuous efforts toward isolation, we obtained eight compounds from this chloroform fraction. These compounds on extensive spectroscopic studies proved to be cycloartane type triterpenoids and were also compared to their previously published literature data. In further studies, we studied the potentials of these compounds against the enzyme tyrosinase.

Tyrosinase (EC 1.14.18.1) is a multifunctional copper-containing enzyme, which is widely distributed in plants and animals. It catalyzes the oxidation of monophenols, *o*-diphenols, and *o*-quinones. Tyrosinase is known to be a key enzyme for melanin biosynthesis in plants and animals. Tyrosinase inhibitors therefore can be clinically useful for the treatment of some dermatological disorders associated with melanin hyperpigmentation. They also find uses in cosmetics for whitening and depigmentation after sunburn. In addition, tyrosinase is known to

Abbreviations: EC, enzyme commission; KA, kojic acid; LM, L-mimosine; NMR, nuclear magnetic resonance; SAR, structure–activity relationship.

Keywords: Tyrosinase inhibitor; Hyperpigmentation; Melanocytes; Structure–activity relationship; *Amberboa ramosa*; Cycloartane triterpenoid.

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be involved in the molting process of insect and adhesion of marine organisms.[5]

The present paper describes the tyrosinase inhibitory activities and the structure–activity relationships (SAR) of the eight cycloartane triterpenoids isolated from the chloroform fraction of the whole plant of *A. ramosa*, including their general isolation procedures and characterization utilizing conventional spectroscopic techniques and the previously reported literature data.

2. Spectral data of the cycloartane type triterpenes

2.1. Compound 1—(22R)-cycloart-20,25-dien-2α3β22α triol

White crystalline, $[\alpha]_D^{30}$ +36.4° (c = 0.022, $CHCl_3$). Mp: 186–188 °C. IR: (KBr) ν_{max}: 3600–3450, 3045, 1650, 890 cm^{-1}. EI-MS: *m/z* (%): M^+ 456 (24), 438 $[M-H_2O]^+$ (35), 423 $[M-H_2O-Me]^+$ (25), 420 $[M-2H_2O]$ (10), 387 $[M-C_5H_9]$ (8), 369 $[M-C_5H_9-H_2O]$ (14), 313 [M−18-side chain] (90), 282 $[C_{21}H_{32}O-H_2O]$, 175 $[C_{21}H_{32}O-C_8H_{13}O]$ (50). ^1H NMR: δ 0.35 (1H, d, J = 4.2 Hz, H-19b), 0.56 (1H, d, J = 4.2 Hz, H-19a), 0.80 (1H, dddd, $J_1 = J_2 = J_3$ = 12.5, J_4 = 2.5 Hz, H-6b), 0.81 (3H, s, Me-28), 0.93 (3H, s, Me-18), 0.97 (3H, s, Me-30), 1.01 (3H, s, Me-29), 1.70, 1.72 (each 3H, d, J = 1.2 Hz, Me-26, Me-27), 3.29 (1H, m, H-3a), 3.63 (1H, dd, J = 11, J_2 = 5 Hz, H-21b), 3.79 (1H, dd, J = 11, J_2 = 3 Hz, H-21a), 4.60 (1H, ddd, $J_1 = J_2$ = 9, J_3 = 2.5 Hz, H-23), 5.25 (1H, dqq, J_1 = 9, $J_2 = J_3$ = 1.2 Hz, H-24). ^{13}C NMR: ($CDCl_3$–CD_3OD, 1:1) δ: 31.7 (C-1), 29.3 (C-2), 78.7 (C-3) 40.2 (C-4), 47.0 (C-5), 20.7 (C-6), 47.6 (C-7), 19.8 (C-8), 26.1 (C-9), 26.2 (C-10), 32.0 (C-11), 45.1 (C-12), 45.1 (C-13), 48.7 (C-14) 35.2 (C-15), 27.3 (C-16), 46.1 (C-17), 171.1 (C-18), 29.7 (C-19), 39.7 (C-20), 63.7 (C-21), 39.4 (C-22), 65.5 (C-23), 128.3 (C-24), 132.8 (C-25), 17.4 (C-26), 24.9 (C-27), 29.9 (C-28), 13.5 (C-29), 19.0 (C-30).

2.2. Compound 2—(22R)-cycloart-23-ene-3β, 22α, 25-triol

White crystalline. $[\alpha]_D^{30}$ +43.4° (c = 0.023, $CDCl_3$). Mp: 194–196 °C. IR: (KBr) ν_{max}: 3590, 3440, 3040, 1650–1645, 950 cm^{-1}. EI-MS: *m/z* (%): M^+ 458 (12), 443 $[M-Me]^+$ (10), 440 $[M-H_2O]^+$, 425 $[M-Me-H_2O]^+$ (18), 422 $[M-2H_2O]^+$ (21), 318 [M−ring A] (13), 300 $[C_{21}H_{34}O_2-H_2O]^+$ (14), 297 [M−18-side chain]$^+$ (85), 175 $[C_{21}H_{34}O_2-C_8H_{15}O_2]^+$. ^1H NMR: ($CDCl_3$, 500 MHz) δ: 4.70 and 4.60 (br s, H_2-21), 3.20 (dd, J = 9.9, 4.5 Hz), 2.14 (m, H_2-22), 1.33 and 1.31 (s, H_3-26 and H_3-27), 0.98 (s, H_3-30), 0.96 (s, H_3-18), 0.88 (s, H_3-28), 0.86 (s, H_3-29), 0.50 (d, J = 4.5 Hz, H-19a) 0.30 (d, J = 4.5 Hz, H-19b). ^{13}C NMR: ($CDCl_3$, 100 MHz) δ: 31.8 (C-1), 30.26 (C-2), 76.8 (C-3), 40.6 (C-4), 47.1 (C-5), 21.0 (C-6), 28.1 (C-7), 47.9 (C-8) 20.3 (C-9), 26.1 (C-10), 26.0 (C-11), 35.6 (C-12) 45.5 (C-13), 48.4 (C-14), 33.0 (C-15), 26.5 (C-16), 51.9 (C-17), 18.2 (C-18), 29.9 (C-19), 156.4 (C-20), 106.6 (C-21), 33.0 (C-22), 34.2 (C-23), 39.1 (C-24), 70.9 (C-25), 29.3 (C-26), 29.4 (C-27), 19.3 (C-28), 25.3 (C-29), 14.8 (C-30).

2.3. Compound 3—cycloartenol

This compound cycloartenol has been reported several times. First, it was published by Rees et al.[6] They have discussed about the cyclization of this compound from 2,3-oxidosqualene.[6] And very recently He et al.[7] have revised the structure of cycloartenol by several spectroscopic techniques including 2D NMR. He et al. also mentioned that cycloartenol was the first triterpene isolated from the *Pinellia* genus.[7]

2.4. Compound 4—cycloart-23-ene-3β,25-diol

Colorless crystal. Mp: 198–199 °C. $[\alpha]_D^{20}$ +33.95°C ($CHCl_3$, c = 1.1). IR: ν_{max} ($CHCl_3$): 3590, 3430, 3040 cm^{-1}. MS: *m/z* (rel. int. %): $[M]^+$ 442 (12), $[M-Me]^+$, 427 (10), $[M-H_2O]^+$ 424 (17), $[M-H_2O-Me]^+$ 409 (2.1), $[M-2H_2O-Me]^+$ 391 (19), $[M-H_2O-C_5H_9]^+$ 355 (10) $[M-C_8H_{15}O]^+$ 315 (35), $[M-C_8H_{15}O-2H]^+$ 313 (18), $[M-C_9H_{16}O]^+$ 302 (42), $[M-C_8H_{15}O-H_2O]^+$ 297 (11), $[M-C_9H_{16}O-H_2O]^+$ 284 (18), $[M-C_8H_{15}O-C_9H_{16}O]^+$ 175 (80). ^1H NMR: ($CDCl_3$, 400 MHz) δ: 0.54–0.60 (dd, J = 3.7 Hz, H_2-19), 0.79 (s, H_3-29), 0.84 (d, J = 6.9 Hz, H_3-21), 0.87 (s, H_3-14), 0.95 (s, H_3-30 and H_3-18), 1.3 (s, H_3-26 and H_3-27), 3.27 (dd, Jax, ax = 10.9 Hz, Jax, eq = 4.5 Hz, 3H), 5.60 (m, H-23 and H-24). ^{13}C NMR: ($CDCl_3$, 100 MHz) δ: 31.8 (C-1), 30.2 (C-2), 78.7 (C-3), 40.3 (C-4), 46.0 (C-5), 20.9 (C-6), 27.9 (C-7), 47.8 (C-8), 19.8 (C-9), 25.9 (C-10), 25.8 (C-11), 35.4 (C-12), 45.2 (C-13), 48.7 (C-14), 32.6 (C-15), 26.3 (C-16), 51.9 (C-17), 17.9 (C-18), 29.7 (C-19), 36.2 (C-20), 18.6 (C-21), 38.9 (C-22), 125.5 (C-23), 139.2 (C-24), 70.6 (C-25), 29.7 (C-26), 29.8 (C-27), 19.1 (C-28), 25.3 (C-29), 13.8 (C-30).

2.5. Compound 5—cycloart-20-ene-3β,25-diol

Colorless crystalline. Mp: 169–170 °C. $[\alpha]_D^{20}$ −18° (CH_3Cl_3, c = 0.03). IR: ν_{max} ($CHCl_3$) 3580, 3440, 3045, 1380, 1640, 890 cm^{-1}. HRMS: *m/z* (rel. int. %) $[M]^+$ 442.3866 ($C_{30}H_{50}O_2$) (30), $[M-H_2O]^+$ 424.3728 ($C_{30}H_{48}O$) (45), $[M-H_2O-Me]^+$ 409.3481 ($C_{29}H_{45}O$) (36), $[M-2H_2O]$ 406.3666 ($C_{30}H_{46}$) (12), $[M-2H_2O-Me]^+$ 391.3311 ($C_{29}H_43$) (10), $[M-H_2O-C_3H_7]^+$ 381.3219 ($C_{27}H_{41}O$) (6), $[M-H_2O-C_5H_9]^+$ 355.3062 ($C_{25}H_{39}O$) (16), $[M-C_8H_{15}O]^+$ 315.2701 ($C_{22}H_{35}O$) [17] $[M-C_9H_{16}O]^+$ 302.2650 ($C_{21}H_{34}O$) (40), $[M-C_8H_{15}O-H_2O]^+$ 297.2573 ($C_{22}H_{33}$) (11), $[M-C_{19}H_{16}O-H_2O]^+$ 284.2524 ($C_{21}H_{32}$) (13), $[M-C_9H_{16}O-C_8H_{15}O]^+$ 175.1483 ($C_{13}H_{19}$) (55). ^1H NMR: ($CDCl_3$, 500 MHz) δ: 4.70 and 4.60 (br s, H_2-21), 3.20 (dd, J = 9.9, 4.5 Hz), 2.14 (m, H_2-22), 1.33 and 1.31 (s, H_3-26 and H_3-27), 0.98 (s, H_3-30), 0.96 (s, H_3-18), 0.88 (s, H_3-28), 0.86 (s, H_3-29), 0.50 (d, J = 4.5 Hz, H-19a) 0.30 (d, J = 4.5 Hz, H-19b). ^{13}C NMR: ($CDCl_3$, 100 MHz) δ: 31.8 (C-1), 30.26 (C-2), 76.8 (C-3), 40.6 (C-4), 47.1 (C-5), 21.0 (C-6), 28.1 (C-7), 47.9 (C-8) 20.3 (C-9), 26.1 (C-10), 26.0 (C-11), 35.6 (C-12) 45.5 (C-13), 48.4 (C-14), 33.0 (C-15), 26.5 (C-16), 51.9 (C-17), 18.2 (C-18), 29.9 (C-19), 156.4 (C-20), 106.6 (C-21), 33.0 (C-22), 34.2 (C-23), 39.1 (C-24), 70.9 (C-25), 29.3 (C-26), 29.4 (C-27), 19.3 (C-28), 25.3 (C-29), 14.8 (C-30).

2.6. Compound 6—cycloart-25-ene-3β,(22*R*)22-diol

Colorless needles. Mp: 180–181 °C. $[\alpha]_D^{20}$ +20.6° ($CHCl_3$, $c = 0.87$). IR: ν_{max} ($CHCl_3$). 3450–3600, 3054, 1650, 1380, 890 cm^{-1}. HRMS: *m*/*z* (rel. int. %) $[M]^+$ 442.3824 ($C_{30}H_{50}O_2$) (33), $[M-H_2O]^+$, 424.3706 ($C_{30}H_{48}O$) (46), $[M-H_2O-Me]^+$ 409.3484 ($C_{29}H_{45}O$) (38), $[M-2H_2O-Me]^+$ 391.3325 ($C_{29}H_{43}$) (12), $[M-H_{20}C_4H_8]^+$ 386.3199 ($C_{26}H_{42}O_2$) (1), $[M-H_2O-C_3H_7]^+$ 381.3155 ($C_{27}H_{41}O$) (8), $[M-H_2O-C_5H_9]^+$ 355.3004 ($C_{25}H_{39}O$) (14), $[M-C_8H_{15}O]^+$ 315.2682 ($C_{22}H_{35}O$) (13), $[M-C_8H_{17}O]^+$ 313.2654 ($C_{22}H_{33}O$) (10), $[M-C_9H_{16}O]^+$ 302.2621 ($C_{21}H_{34}O$) (42), $[M-C_8H_{15}O-H_2O]^+$ 297.2562 ($C_{22}H_{33}$) (11), $[M-C_9H_{16}O-H_2O]^+$ 284.2528 ($C_{21}H_{32}$) (15), $[M-C_9H_{16}O-C_8H_{15}O]^+$ 175.1465 ($C_{13}H_{19}$) (58). ^1H NMR: ($CDCl_3$, 500 MHz) δ: 4.80 and 4.49 (br s, H_2-26), 4.06 (ddd, $J = 6.6$, 6.2, 1.0 Hz, H-22), 3.20 (dd, $J = 9.8$, 4.4 Hz, H-3), 1.60 (s, H_3-27), 1.43 (dq, $J = 6.4$, 1.0 Hz, H-20), 0.96 (s, H_3-30), 0.95 (s, H_3-18) 0.80 (s, H_3-29), 0.54 (d, $J = 4.2$ Hz, H-19a), 0.31 (d, $J = 4.2$ Hz, H-19b). ^{13}C NMR: ($CDCl_3$, 100 MHz) δ: 31.70 (C-1), 30.40 (C-2), 78.9 (C-3), 40.58 (C-4), 47.1 (C-5), 21.4 (C-6), 28.2 (C-7), 47.9 (C-8), 20.4 (C-9), 29.2 (C-10), 26.0 (C-11), 35.6 (C-12), 45.3 (C-13), 48.8 (C-14), 32.1 (C-11), 26.6 (C-16), 52.3 (C-17), 18.0 (C-18), 29.9 (C-19), 36.0 (C-20), 18.3 (C-21), 76.7 (C-22), 28.1 (C-23), 32.9 (C-24), 149.7 (C-25), 111.3 (C-26), 17.3 (C-27), 19.6 (C-28), 25.4 (C-29), 14.2 (C-30).

2.7. Compound 7—3β,21,22,23-tetrahydroxy-cycloart-24(31),25(26)-diene

Colorless crystals. Mp: 143–145 °C. $[\alpha]_D^{20}$ +18.2° (MeOH; $c = 1.20$). IR: ν_{max} KBr cm^{-1}: 3500–3450 (OH), 3045 (cyclopropane ring), 1660 (C=C). HREIMS: *m*/*z* (rel. int. %) $[M]^+$ 486.3705 $C_{31}H_{50}O_4$ (30), $[M-H_2O]^+$ 468 (45), $[M-2H_2O]^+$ 450 (5), $[M-C_5H_6]^+$ 420 (40), $[M-C_{22}H_{36}O]^+$ 316 (42). ^1H NMR: ($CDCl_3$) δ: 0.30 (d, 1H, $J = 4.0$ Hz), 0.50 (d, 1H, $J = 4.0$ Hz), 0.80 (s, 3H), 1.20 (s, 3H), 1.30 (s, 3H), 1.85 (s, 3H), 3.60 (m, 1H), 3.80 (m, 2H), 4.20 (m, 1H), 4.50 (br s, 1H), 4.57 (br s, 1H), 4.80 (s, 1H), 4.88 (br s, 1H). ^{13}C NMR: δ 32.1 (C-1), 31.0 (C-2), 77.9 (C-3), 41.1 (C-4), 47.9 (C-5), 21.2 (C-6), 27.6 (C-7), 47.5 (C-8), 20.1 (C-9), 26.1 (C-10), 26.2 (C-11), 35.5 (C-12), 45.5 (C-13), 48.3 (C-14), 32.1 (C-15), 26.4 (C-16), 43.3 (C-17), 17.9 (C-18), 29.7 (C-19), 46.5 (C-20), 61.6 (C-21), 75.7 (C-22), 76.0 (C-23), 150.6 (C-24), 147.0 (C-26), 110.0 (C-26), 18.4 (C-27), 19.4 (C-28), 25.4 (C-29), 14.6 (C-30), 109.8 (C-31).

2.8. Compound 8—(23*R*)-5α-cycloart-24-ene-3β,21,23-triol

Colorless crystals. Mp: 204–205 °C. $[\alpha]_D^{20}$ +38° ($CHCl_3$; $c = 0.6$). IR: ν_{max} KBr cm^{-1}: 3600–3400 (OH), 3045 (cyclopropane ring), 1660 (C=C). HREIMS: *m*/*z* (rel. int. %) $[M]^+$ 458.3762 $C_{30}H_{50}O_3$ (30), $[M-H_2O]^+$ 440 (45), $[M-C_4H_7]^+$ 403 (5), [M−side chain]$^+$ 315 (40), $[M-C_{13}H_{19}]^+$ 175 (42). ^1H NMR: δ 0.35 (1H, d, $J = 4.2$ Hz, H-19b), 0.56 (1H, d, $J = 4.2$ Hz, H-19a), 0.80 (1H, dddd, $J_1 = J_2 = J_3 = 12.5$, $J_4 = 2.5$ Hz, H-6b), 0.81 (3H, s, Me-28), 0.93 (3H, s, Me-18), 0.97 (3H, s, Me-30), 1.01 (3H, s, Me-29), 1.70, 1.72 (each 3H, d, $J = 1.2$ Hz, Me-26, Me-27), 3.29 (1H, m, H-3a), 3.63 (1H, dd, $J = 11$, J_2, = 5 Hz, H-21b), 3.79 (1H, dd, $J = 11$, $J_2 = 3$ Hz, H-21a), 4.60 (1H, ddd, $J_1 = J_2 = 9$, $J_3 = 2.5$ Hz, H-23), 5.25 (1H, dqq, $J_1 = 9$, $J_2 = J_3 = 1.2$ Hz, H-24). ^{13}C NMR: ($CDCl_3$–CD_3OD, 1:1) δ: 31.7 (C-1), 29.3 (C-2), 78.7 (C-3) 40.2 (C-4), 47.0 (C-5), 20.7 (C-6), 47.6 (C-7), 19.8 (C-8), 26.1 (C-9), 26.2 (C-10), 32.0 (C-11), 45.1 (C-12), 45.1 (C-13), 48.7 (C-14) 35.2 (C-15), 27.3 (C-16), 46.1 (C-17), 171.1 (C-18), 29.7 (C-19), 39.7 (C-20), 63.7 (C-21), 39.4 (C-22), 65.5 (C-23), 128.3 (C-24), 132.8 (C-25), 17.4 (C-26), 24.9 (C-27), 29.9 (C-28), 13.5 (C-29), 19.0 (C-30).

3. Results and discussion

Tyrosinase (also known as polyphenol oxidase, PPO, monophenols, and *o*-diphenol oxygen oxidoreductase) (EC 1.14.18.1) referred with the trivial name tyrosinase is a metalloenzyme with a dinuclear copper active site and related to the active site of the oxygen transporter protein hemocyanin that shows in some forms also diphenol oxidase activity. This enzyme is engaged in many biological processes such as defense, mimetism, shielding from UV light, consolidating of cell walls in fungi or exoskeleton in Arthropods, and in general the production of melanins.[8]

Tyrosinase is at the moment a well-characterized enzyme. It oxidizes phenol in two steps: phenol is oxidized to catechol (*o*-benzenediol), which is consequently oxidized (by tyrosinase) to *o*-quinone. Tyrosinase shows no activity for the oxidation of *p*- and *m*-benzenediols. Laccase, which catalyzes the oxidation of *o*-, *m*-, and *p*-benzenediols to the corresponding *o*-, *m*-, and *p*-quinones, is used for the detection of these benzenediols. Thus, co-immobilization of tyrosinase and laccase allows the detection of several phenolic compounds.[9]

The compounds **1** and **2** exhibited moderate inhibition against the enzyme tyrosinase, when the inhibitions have been compared with the standard tyrosinase inhibitors (here we used kojic acid, KA, and L-mimosine, LM, reference tyrosinase inhibitors) and the values of IC_{50} were 5.25 and 8.90 μM, respectively (see Table 1). The compound **2** did not show potent inhibition like **1**, which

Table 1. Tyrosinase inhibitory activities of the compounds

Compound	$IC_{50} \pm SEM$ (μM)
1	7.92 ± 0.387
2	15.94 ± 1.93
3	NA[a]
4	8.32 ± 0.097
5	12.09 ± 1.03
6	22.21 ± 1.94
7	1.32 ± 0.373
8	4.93 ± 0.197
KA[b]	16.67 ± 0.519
LM[b]	3.68 ± 0.02234

[a] Not active.
[b] Reference inhibitors.

may be due to the absence of the –OH group at the C-2 position. It means maybe this –OH group contributes to the potency of the cycloartane type triterpenoids against the enzyme tyrosinase.

The compound **3** did not show any kind of inhibition against the enzyme tyrosinase. Interestingly, compounds **4** and **5** exhibited moderate inhibition against tyrosinase, though these two compounds are very much similar to **3**.

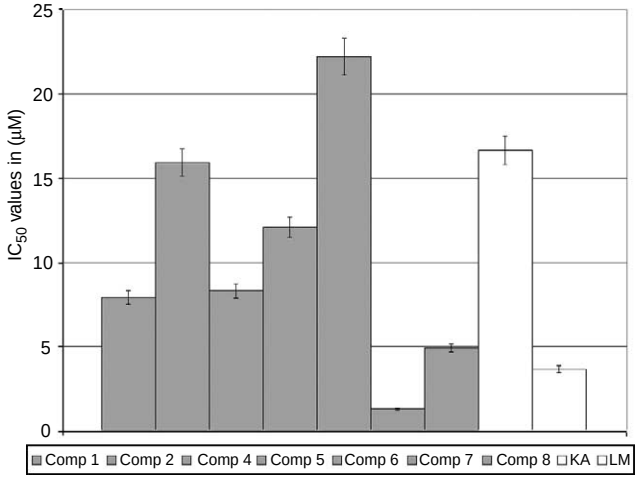

Figure 1. Graphical presentation of the IC_{50} (μM) values of different compounds isolated from the *A. ramosa* Jafri, against the enzyme tyrosinase.

The only dissimilarity is that they have –OH functionality at the C-25 position. Moreover, potency of the compound **4** shown to be more than that of **5** may be due to the presence of double bond at C-23 and C-24 positions.

Similarly, compound **6** exhibited mild inhibitions against tyrosinase. This also may be due to the presence of the –OH functional group at C-22 positions like compounds **1** and **2**, although it does contain any –OH at the C-25 position.

The compound **7** exhibited an extremely potent (IC_{50} = 1.32 μM) inhibition against the enzyme tyrosinase, when compared with the standard tyrosinase inhibitor KA (IC_{50} = 16.67 μM). This compound even exhibited better inhibition than that of LM (IC_{50} = 3.68 μM) (see Table 1). Structurally very similar compound **8** also exhibited potent inhibition and the IC_{50} value was found to be 4.93 μM. This is may be due to absence of the –OH functionality at the C-22 position.

Figure 1 presents the potentials of the compounds against the enzyme tyrosinase and Figure 2 shows the molecular structures of all the compounds isolated from the *A. ramosa* Jafri.

From these compounds it has been found that for the inhibition against the enzyme tyrosinase, the presence of –OH group(s) plays a vital role. Of course, the position of the –OH group is also playing an important role,

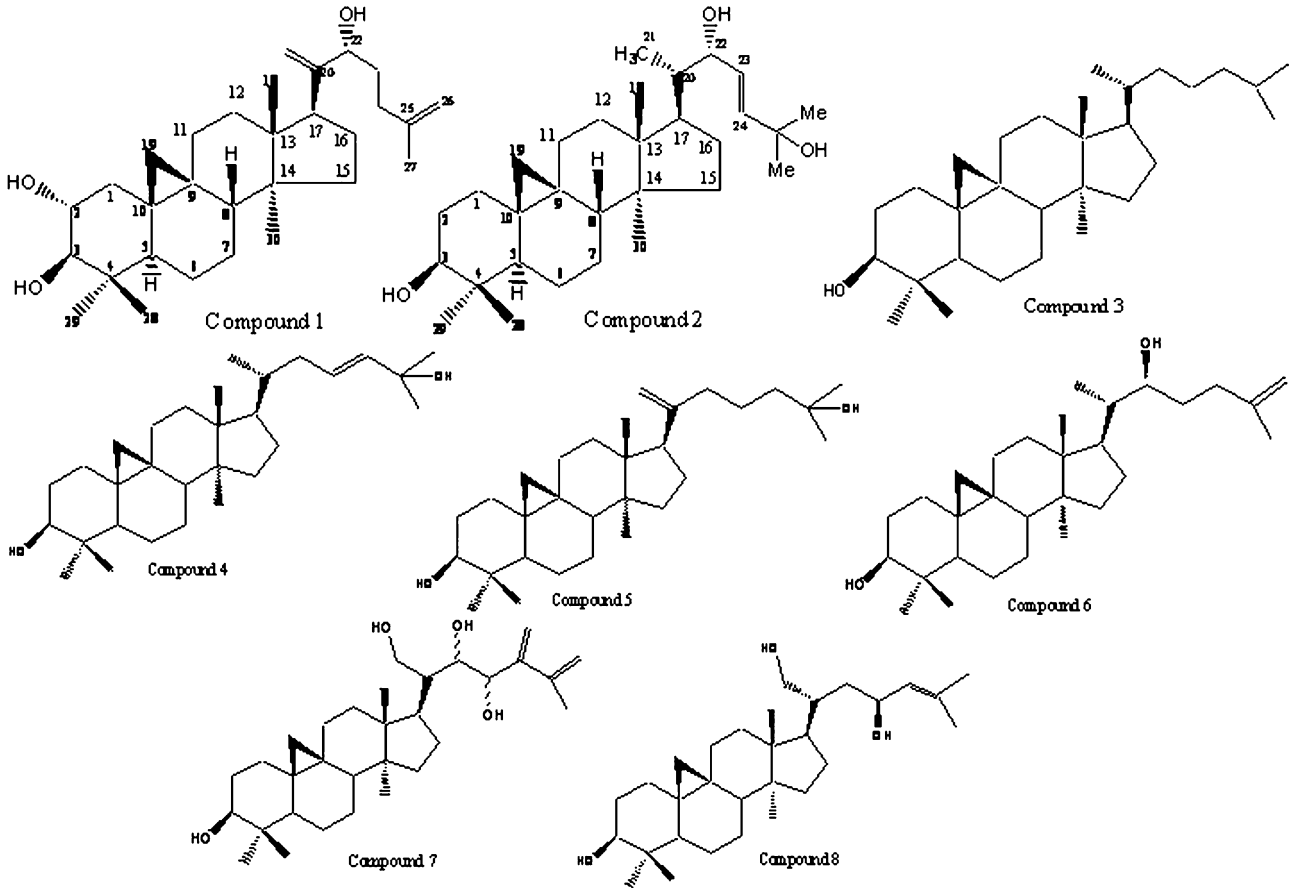

Figure 2. Molecular structures of the compounds isolated form the *A. ramosa* Jafri.

like C-22. Sometimes double bond is also having some contributions, like compound **4**.

Very recently we have reported two long chain esters, including some other compounds, from the same chloroform-soluble fraction of *A. ramosa* and their structures assigned to be methyl 2β (2*S*)-hydroxyl-7(*E*)-tritriacontenoate and methyl 2β (2*S*)-*O*-β-D-galactopyranosyl-7(*E*)-tetratriacontenoate.[10] These two compounds exhibited strong to moderate inhibitory activity against tyrosinase.[10]

Melanin is a heteropolymer of indole compounds that is produced inside melanosomes by the action of the tyrosinase enzyme on the tyrosinase precursor material in melanocytes. It has recently been shown that other factors such as metal ions and the tyrosinase related protein 1 (TRP-1) and tyrosinase related protein 2 (TRP-2) enzyme also contribute to the production of melanin. However, tyrosinase plays a critical regulatory role in melanin biosynthesis. Therefore, many tyrosinase inhibitors that suppress melanogenesis have been actively studied with the aim of developing preparations for the treatment of hyperpigmentation.[11] The production of abnormal melanin pigmentation (e.g., melasma, freckles, ephelide, senile lentigines, etc.) is a serious esthetic problem in human beings. In fungi, the role of melanin is correlated with the differentiation of reproductive organs and spore formation, virulence of pathogenic fungi, and tissue protection after injury. In addition, tyrosinase is responsible for the undesired enzymatic browning of fruits and vegetables that takes place during senescence or damage at the time of postharvest handling, which makes the identification of novel tyrosinase inhibitors extremely important.[12]

From these studies it can be concluded that the compound **7** can be the potential candidate for the treatment of melanin biosynthesis related skin diseases, likely hyper- and hypo-pigmentation of human as well as animals.

4. Experimental

4.1. Plant material

Amberboa ramosa (Compositae), whole plant was collected in June 2002, from Karachi (Pakistan) and identified by Dr. Surraiya Khatoon, Plant Taxonomist, Department of Botany, University of Karachi, where a voucher specimen has been deposited.

4.2. General spectroscopic and related procedures

Optical rotations were measured on a JASCO DIP-360 polarimeter. IR spectra were recorded on a 460 Shimadzu spectrometer. EIMS and HRMS were recorded on JMS-HX-110 with a data system and on JMS-DA 500 mass spectrometers. The ^{1}H and ^{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 pre-coated with silica gel 60 F_{254} (20 × 20 cm, 0.2 mm thick; E-Merck) were used for TLC and flash silica (230–400 mesh) was used for column chromatography.

4.3. Extraction and isolation approaches

The shade dried whole plant (20 kg) material has been extracted thrice with methanol. The residue from the methanolic extract was then partitioned between *n*-hexane and water. The water-soluble fraction was further extracted with chloroform, ethylacetate, and *n*-butanol. The chloroform-soluble fraction (55 g) was subjected to column chromatography over flash silica eluting with *n*-hexane–ethylacetate, ethylacetate, ethylacetate–methanol, and methanol in increasing order of polarity. The fractions which were obtained from *n*-hexane–ethylacetate (8.5:1.5) were combined and further subjected to column chromatography using *n*-hexane–ethylacetate (8.8:1.2) as eluent to afford the pure compounds **3** (15 mg) and **4** (26 mg). The fraction, which eluted with *n*-hexane–ethylacetate (4:1), showed three majors and two minor spots on TLC. It was further subjected to column chromatography using *n*-hexane–ethylacetate (8.3:1.7) as eluent to afford pure compounds **1** (15 mg), **2** (22 mg), and **5** (16 mg), respectively.

4.4. Tyrosinase inhibition assays

Tyrosinase inhibition assays were performed in a 96-well micro-plate format using SpectraMax® 340 (Molecular Devices, CA, USA) micro-plate reader according to the developed method described earlier by Hearing.[13] Briefly, all the compounds were dissolved in DMSO to a concentration of 2.5%. Thirty units of mushroom tyrosinase (28 nM) was first pre-incubated with the compounds, in 50 nM Na-phosphate buffer (pH 6.8) for 10 min at 25 °C. Then, the L-DOPA (0.5 mM) was added to the reaction mixture and the enzyme reaction was monitored by measuring the change in absorbance at 475 nm (at 37 °C) of the formation of the DOPAchrome for 10 min. The percent inhibition of the enzyme and IC_{50} values of the active compounds were calculated using a program developed with Java and Macro Excel® 2000 (Microsoft Corp., USA) for this purpose. The following equation has been followed:

$$\text{Percent inhibition}(\%) = [B - S/B] \times 100$$

Here the B and S are the absorbances for the blank and samples, respectively. All the studies have been carried out at least in triplicates and the results here represents means ± SEM (standard error of the mean). Here in these experiments kojic acid (KA) and L-mimosine (LM) are used as standard tyrosinase inhibitors. All the reagents, enzyme, substrate, and reference compounds were purchased from Sigma Chem. Co., MO, USA.

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