Phytochemical Studies on *Galinsoga parviflora*

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Summary: Seven compounds have been isolated for the first time from *Galinsoga parviflora* namely, β-sitosterol (**1**), octacosanoic acid (**2**), ursolic acid (**3**), 4-hydroxybenzoic acid (**4**), 3,4-dihydroxybenzoic acid (**5**), gallic acid (**6**) and β-sitosterol 3-*O*-β-d-glucopyranoside (**7**), respectively. Their structures have been elucidated by EIMS, HREIMS, FAB, HRFABMS, and ^{1}H NMR spectroscopic data.

Introduction

The genus *Galinsoga* belongs to the family Asteraceae which consists of only 3 species, occurring mainly in New Zealand, United Kingdom, Brazil, South Asia and USA. In Pakistan, it is very common in Baluchistan, Dir, Hunza, Swat, Gilgat, Muree and Kashmir [1]. *Galinsoga parviflora* is one of the important species of the genus *Galinsoga*. The flowers of this plant are pink, with pink or red-tipped ray florets and yellow disk florets [2]. The fruit is sparsely with hairy achences (seeds). Leaves explored pleasant-smelling when crushed. The plant can grow in semi-shade or moist soil. The roots are useful in treating in nettle by rubbing. The juice of the whole plant is applied to treat wounds. It helps to coagulate the blood of fresh cuts and wounds [2]. Literature survey revealed that only an aliphatic ketone [3] has been reported from this species. This prompted us to carry out the phytochemical studies on this species. Herein we report the isolation and structure elucidation of β-sitosterol (**1**), octacosanoic acid (**2**), ursolic acid (**3**), 4-hydroxybenzoic acid (**4**), 3,4-dihydroxybenzoic acid (**5**) gallic acid (**6**) and β-sitosterol 3-*O*-β-D-glucopyranoside (**7**).

Results and Discussion

The methanolic extract of the whole plant showed strong toxicity in brine shrimp lethality test. It was subsequently divided into *n*-hexane, ethyl acetate, *n*-butanol and water-soluble fractions. Out of these the ethyl acetate soluble fraction showed strong toxicity in brine shrimp lethality test. This fraction was, therefore, subjected to a series of column and flash chromatographic techniques as described in the experimental section to obtain eight compounds reported for the first time from this species. These could be identified as of β-sitosterol (**1**), octacosanoic acid (**2**), ursolic acid (**3**), 4-hydroxybenzoic acid (**4**), 3,4-dihydroxybenzoic acid (**5**), gallic acid (**6**) and β-sitosterol 3-*O*-β-D-glucopyranoside (**7**) on the basis of their spectral data. All these compounds individually showed lower level of toxicity compared to the crude ethyl acetate extract thereby indicating that the strong toxicity of the latter is due to combined effect of various constituents present therein.

Experimental

General

Column chromatography was carried out using silica gel of 70-230 and flash chromatography on silica gel 230-400 mesh. Aluminium sheets precoated with silica gel 60 F_{254} (20×20 cm, 0.2 mm thick; E-Merck) were used for TLC to check the purity of the compounds and were visualized under UV light (254 and 366 nm) followed by ceric sulfate as spraying reagent. Optical rotations were measured on a Jasco DIP-360 digital polarimeter. The UV spectra were recorded on a Hitachi UV-3200 spectrometer (λ_{max} in nm). IR spectra were recorded

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on Shimadzu IR-460 spectrophotometer (ν in cm^{-1}). EIMS, HREIMS, FABMS and HRFABMS spectra were recorded on Jeol JMS-HX 110 spectrometer with data system. The ^1H NMR spectra were recorded on Bruker AMX-400 MHz instruments using TMS as an internal reference. The chemical shift values are reported in ppm (δ) units and the scalar coupling constants (J) are in Hz.

Plant Material

The whole plant of *C. parviflora* Cav. was collected from District Ziarat (Balochistan) in April, 2006 and identified by Dr. Rasool Bakhsh Tareen, Plant Taxonomist, Department of Botany, Balochistan University, Quetta, where a voucher specimen (GP-0062-06) has been deposited.

Isolation

The dried whole plant (10 kg) was extracted thrice with MeOH (40L) at room temperature. The combined methanolic extract was evaporated under reduced pressure to obtain a thick gummy mass (400 g). It was suspended in water and successively extracted with *n*-hexane, ethyl acetate and *n*-butanol. The ethyl acetate soluble fraction (50 g) was subjected to column chromatography over silica gel eluting with *n*-hexane, ethyl acetate, ethyl acetate, ethyl acetate-methanol and methanol in increasing order of polarity. The fractions obtained from *n*-hexane-ethyl acetate (8.5:1.5) showed two spots on TLC which were combined and chromatographed over silica gel using *n*-hexane-ethyl acetate (8.4:1.6) to obtain β-sitosterol (**1**) and octacosanoic acid (**2**). The fractions obtained from *n*-hexane-ethyl acetate (8:2) gave single spot on TLC, which on final purification by the same solvent system provided ursolic acid (**3**). The fractions obtained from *n*-hexane-ethyl acetate (7:3) were combined and chromatographed over silica gel using *n*-hexane-ethyl acetate (6.8:3.2) to obtain 4-hydroxybenzoic acid (**4**). The fractions obtained from *n*-hexane-ethyl acetate (6:4) showed two spots on TLC which were combined and chromatographed over silica gel using *n*-hexane-ethyl acetate (4.5:5.5) to yield 3,4-dihydroxybenzoic acid (**5**) and gallic acid (**6**). The fractions obtained from *n*-hexane-ethyl acetate (2:8) were combined and re-chromatographed over silica gel using *n*-hexane-ethyl acetate (1.8:8.2) as eluent to obtain β-sitosterol 3-O-β-D-glucopyranoside (**7**).

β-Sitosterol (1)

Crystallized from acetone (24 mg), m.p. 136 - 137°C; $[\alpha]_D$ - 36° (*c* 0.0021, $CHCl_3$); IR (KBr) ν_{max} cm^{-1}: 3441, 3055, 1652; ^1H-NMR ($CDCl_3$, 400 MHz) δ: 5.22 (1H, m, H-6), 3.31 (lH, 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); HREIMS, *m/z:* 414.3861 (calcd. for $C_{29}H_{50}O$, 414.3849). The physical and spectral data showed complete agreement with those reported in the literature [4-6].

Octacosanoic acid (2)

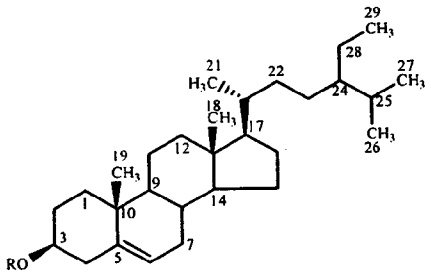
Crystallized from acetone (18 mg), m.p. 90 - 91°C; IR (KBr) ν_{max} cm^{-1}: 3300-2610, 1705, 825; ^1H-NMR ($CDCl_3$, 400 MHz) δ: 2.25 (2H, t, J = 6.7 Hz, H-2), 1.51 (2H, m, H-3), 1.20 (56H, br s, CH_2-4–CH_2-26), 0.88 (2H, m, H-27), O.65 (3H, t, J = 6.6 Hz, CH_3-28); HREIMS, *m/z:* 424.4285 (calcd. for $C_{28}H_{56}O_2$, 424.4280). The physical and spectral data corresponded to the reported values [7].

Ursolic acid (3)

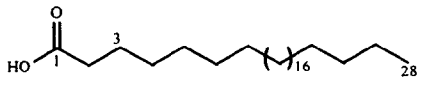
Amorphous powder (25 mg), m.p. 291 - 292°C, $[\alpha]_D$ + 66.2° (*c* 0.01, $CHCl_3$); IR (KBr) ν_{max} cm^{-1}: 3510, 3050, 1697, 1635, 820; ^1H-NMR (CD_3OD, 400 MHz) δ: 5.11 (1H, m, H-12), 3.19 (1H, dd, $J_{ax,ax}$ = 10.0 Hz, $J_{ax,eq}$ = 4.5 Hz, H-3α), 1.20 (3H, s, Me-27), 1.07 (3H, s, Me-23), 0.94 (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); HREIMS, *m/z:* 456.3603 (calcd. for $C_{30}H_{48}O_3$, 456.3595). The physical and spectral data showed complete resemblance with those reported in the literature [8,9].

4-Hydroxybenzoic acid (4)

Crystallized in acetone (22 mg), m.p. 213 - 214°C; UV (MeOH) λ_{max} (log ε) nm: 311 (3.8), 290 (4.06), 218 (3.9); IR (KBr) ν_{max} cm^{-1}: 3510, 3330-2720, 1705; ^1H-NMR (CD_3OD, 400 MHz) δ: 8.19 (1H, d, J = 7.9 Hz, H-2,6), 7.58 (1H, d, J = 7.9 Hz, H-3,5); HREIMS showed $[M]^+$ at *m/z* 138.0316 (calcd. for $C_7H_6O_3$, 138.0309) The physical and spectral data corresponded to the reported values [10].



1 R = H
7 R = Glucose



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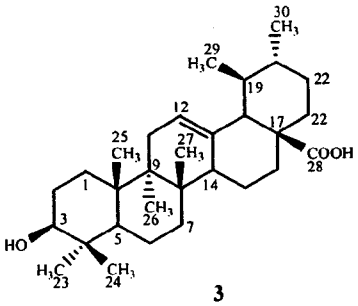
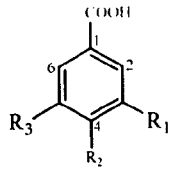


3



4 R_1 = H, R_2 = OH, R_3 = H
5 R_1 = OH, R_2 = OH, R_3 = H
6 R_1 = OH, R_2 = OH, R_3 = OH

Fig. 1: Structure of the compounds isolated from *G. parviflora.*

3,4-Dihydroxybenzoic acid (5)

Crystallized in methanol (16 mg), m.p. 195 - 196°C; UV (MeOH) λ_{max} (log ε) nm: 316 (3.2), 297 (3.5); IR (KBr) ν_{max} cm^{-1}: 3417, 3233-2629, 1704; ^{1}H-NMR (CD_3OD, 400 MHz) δ: 7.50 (1H, d, J = 2.2 Hz, H-2), 7.39 (1H, dd, J = 7.5, 2.2 Hz, H-6), 6.85 (1H, d, J = 7.5 Hz, H-5); HREIMS showed $[M]^+$ at *m/z* 154.0275 (calcd. for $C_7H_6O_4$, 154.0266). The physical and spectral data showed complete agreement to those reported in literature [11].

Gallic acid (6)

Crystallized in methanol (16 mg), m.p. 235 - 238°C; UV (MeOH) λ_{max} (log ε) nm: 325 (4.1), 275 (3.7); IR (KBr) ν_{max} cm^{-1}: 3323-2699, 1705; ^{1}H-NMR (CD_3OD, 400 MHz) δ: 7.03 (2H, s, H-2,6); HREIMS showed $[M]^+$ at *m/z* 170.0221 (calcd. for $C_7H_6O_5$, 170.0215). The physical and spectral data corresponded to the literature values [12].

β-Sitosterol 3-O-β-D-glucopyranoside (7)

It was obtained as a colorless amorphous powder (29 mg), m.p. 232-235°C, $[\alpha]_D$-45° (*c* 0.023, MeOH); IR (KBr) ν_{max} cm^{-1}: 3452, 3044, 1648. ^{1}H-NMR ($CDCl_3$+CD_3OD, 400 MHz) δ: 5.13 (1H, m, H-6), 4.57 (1H, d, J = 7.3 Hz, H-1'), 3.85 (1H, m, H-3), 3.24-3.80 (m, Glc-H), 1.01 (3H, s, Me-19), 0.92 (3H, d, J = 6.1 Hz, Me-21), 0.87 (3H, t, J = 7.0 Hz, Me-29), 0.87 (3H, d, J = 6.4 Hz, Me-26), 0.81 (3H, d, J = 6.4 Hz, Me-27) and 0.68 (3H, s, Me-18); HRFABMS, *m/z*: 577.4444 $[M+H]^+$ (calcd. for $C_{35}H_{61}O_6$, 577.4438). The physical and spectral data were in agreement with those reported in literature [4-6].

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