Research Article

New Potential Allelochemicals from Galinsoga purviflora CAV

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Abstract

Two new potential allelochemicals m.f. C34H42O21, m.p. 223-225 0C and [M+] 786(A) and m.f. C33H40O21, m.p. 216-219 0C and [M+] 772(B) have been isolated from the acetone soluble fraction of the 95% ethanolic extract of the stems of Galinsoga purviflora Cav. along with two known compounds Quercetin (C) and Kaempferol (D). The structure of two new compounds were characterized as 3,5,3',5' tetra hydroxy 7,4' dimethoxy flavone-3-O-α-L-rhamnopyranosyl-(1→3)-O-α-L-arabinopyranosyl-3'-O-β-D-galactopyranoside (A) and 3,5,7,8,4' pentahydroxy 3'methoxyflavone-3-O-α-L-rhamnopyranosyl-7-O-β-D-glucopyranosyl-(1→4)-O-β-D-xylopyranoside (B) by various color reactions, spectral analysis and chemical degradations.

Keywords: *Galinsoga purviflora* Cav., Compositae, stems, allelochemicals, antimicrobial activity

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Introduction

Galinsoga purviflora Cav.[1-5] belongs to family Compositae which is commonly known as "Patter Suva" in Hindi. It is found throughout in India from West Himalaya up to 8,000ft., Assam, Malaya, Sumatra and Java. The species of this plant are used in folk medicine for the treatment of inflammation, and also used for fodder. Extracts from this herb are applied in dermatological diseases, eczemas and lichens. It helps to coagulate the blood from fresh cut and wounds. Earier workers[6-9] have reported various flavonoids from this plant. In the present paper we report the isolation and structural elucidation of two new allelochemicals 3, 5, 3', 5' tetra hydroxy 7, 4' dimethoxy flavone-3-O-*α*-L-rhamnopyranosyl-(1→3)-O-*α*-L-arabinopyranosyl-3'-O-*β*-D-galactopyranoside and 3, 5, 7, 8, 4' pentahydroxy 3' methoxy flavone-3-O-*α*-L-rhamnopyranosyl-7-O-*β*-D-glucopyranosyl-(1→4)-O-*β*-D-xylopyranoside along with two known compounds Quercetin and Kaempferol. from ethenolic extract of the stems of this plants.

Experimental

All of the melting point were determined on a thermo electrical melting points apparatus and are uncorrected. The IR spectra were recorded in KBr disc on FT-IR spectrophotometer, Shimadzu 8400S. The NMR spectral data were obtained on Bruker DRX (300 MHz and 75 MHz for ^{13}C NMR) in DMSO-d_6 with tetramethyl silane as internal standard. The FABMS was recorded on Jeol - SX (102) mass spectrometer.

Plant Material

The stems of the plants were collected locally around Sagar region and were taxonomically authenticated by

taxonomist, Department of Botany, Dr. H. S. Gour Central University, Sagar (M.P.) India. A voucher specimen has been deposited in the Natural Products Laboratory, Department of Chemistry of this university.

Extraction and Isolation

Air dried and powdered stems (4.0 kg) of the plant were extracted with 95% ethanol in a Soxhlet apparatus for 74 h. The ethanolic extract of stems of the plant was further successively partitioned with chloroform, ethyl acetate, acetone and methanol. The acetone soluble fraction was further concentrated under reduced pressure to give brown viscous mass (2.35 g), which was subjected to TLC examination using nBAW (4:1:5) as solvent and I_2 vapours as visualizing agent. It gave four spots, indicating it to be mixture of four compounds designated on A, B, C, and D. These compounds were separated by TLC and purified by column chromatography over silica gel using $CHCl_3$: MeOH as eluents and studied separately.

Study of Compound A

It was crystallized from methanol to give light brownish compounds 1.45 g. It has m.p. 223-225^{0}C, m.f. $C_{34}H_{42}O_{21}$, [M^+] 786 (FABMS); found (%): C 51.84, H 5.28, O 42.69 calcd. For m.f. $C_{34}H_{42}O_{21}$: C 51.90, H 5.34, O 42.74. UVλ_{max} (nm) :(MeOH) , 265, 366 (+$AlCl_3$) 303, (+NaOMe)322. IR (KBr) υ_{max} 3474, 2918, 1720, 1680,1660, 1600, 986. ^{1}H NMR (300MHz, DMSO-d_6) δ (ppm); 6. 35 (1H,d, *J* 2.2 Hz, H-6), 6.41 (1H, d, *J* 1.9 Hz, H-8), 9.207 (1H, s, 3-OH),12.38 (1H, s, 5-OH), 3.83(3H, s, 7-OCH_3), 7.08(1H,d , *J* 8.5 Hz, H-2', H-6'), 8.48 (1H, , s, 3', 5'-OH),3.93 (3H, s, 4'- OCH_3), 5.64(1H, d, *J* 5.8 Hz, H-1"),3.80-5.64 (4H, m, H-2", H-3", H-4", H-5"),5.12 (1H, d, *J* 1.25 Hz, H-1'"), 3.81-5.12 (4H, m, H-2'", H-3'", H-4'", H-5'"),1.06 (3H, d, *J* 6.1 Hz, 6'"-OCH_3),5.18 (1H,d, *J* 7.8 Hz, H-1""), 3.32-3.76 (4H, m, H-2"", H-3"", H-4"", H-5""), 3.84 (1H,dd, *J* 10.8,6.8 Hz, H-6a""), 4.28 (1H, dd, *J* 11.4, 4.1 Hz, H-6b"").^{13}C NMR (75 MHz, DMSO-d_6), δ(ppm), : 157.2 (C-2), 134.2(C-3), 182.6 (C-4), 162.1(C-5), 98.4 (C-6),165.2 (C-7) ,92.9 (C-8), 156.3 (C-9), 105.3 (C-10),112.9 (C-2') ,147.3(C-3'), 151.3(C-4'),111.8 (C-5'),119.4 (C-6'), 58.4 (7-OCH_3), 55.8 (4'-OCH_3), 101.6 (C-1"), 77.2 (C-2"), 72.9 (C-3"),68.3 (C-4"),66.3 (C-5"), 102.8 (C-1'"), 72.9(C-2'"), 72.1(C-3'"), 74.3(C-4'"), 69.7(C-5'"), 102.8(C-1""), 73.4(C-2""), 73.2(C-3""), 68.2(C-4""), 76.2(C-5""), 61.2(C-6"").

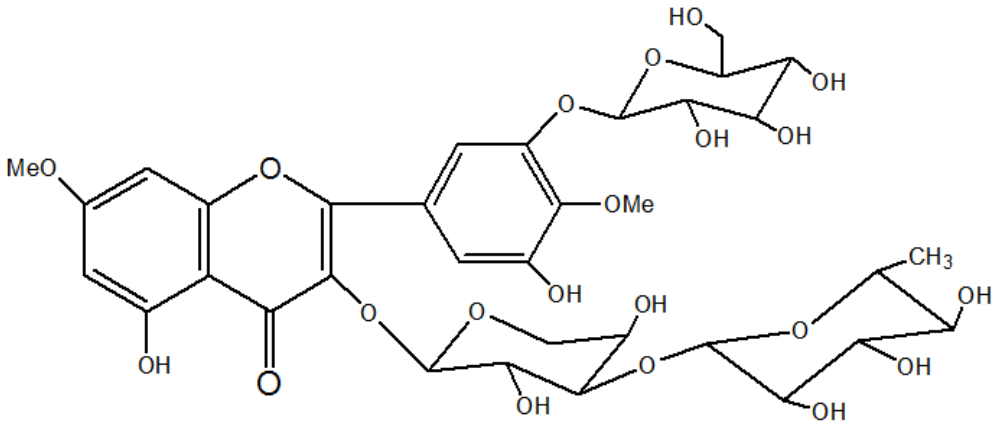

Figure 1 Chemical structure of Compound A

Acid Hydrolysis of Compound A

250 mg of the compound was dissolved in ethanol (25ml) and refluxed with 40ml of H_2SO_4 on water bath for 5-6 h. The reaction mixture was concentrated and allowed to cool and residue was extracted with diethyl ether (Et_2O). The ether layer was washed with water and evaporated to dryness. The residue was subjected to column chromatography over silica gel column using $CHCl_3$: MeOH (4:8) to give compound A-1 identified as 3, 5, 3', 5', tetra hydroxy7, 4', dimethoxy flavone. The aqueous hydrolysate was neutralized with $BaCO_3$ and $BaSO_4$ was filtered off. The filtrate was concentrated and subjected to paper chromatography examination using nBAW (4:1:5) solvent and aniline hydrogen

phthalate as spraying reagent, showed the presence of L- rhamnose (R_f 0.36), L- arabinose (R_f 0.22), D-galactose (R_f 0.15).

Study of Compound A-1

It has molecular formula $C_{17}H_{14}O_8$, m.p. 242-244 ^{0}C, [M^+] 520 (EIMS); found (%): C 39.27, H 2.65, O 16.31 calcd (%) for m.f. $C_{17}H_{14}O_8$, C 39.23, H 2.69, O 16.28; UVλ_{max} (nm):(MeOH) , 269, 356 (+$AlCl_3$) 310, (+NaOMe)342. IR (KBr) υ_{max} 3476, 2916, 1717, 1675,1665, 1605, 975.^{1}HNMR (300MHz, DMSO-d_6) δ (ppm); 6. 37 (1H,d, *J* 2.1 Hz, H-6), 6.43 (1H, d, *J* 1.6 Hz, H-8), 9.210 (1H, s, 3-OH),12.32 (1H, s, 5-OH), 3.79(3H, s, 7-OCH_3), 7.10(1H,d , *J* 8.9 Hz, H-2', H-6'), 8.46 (1H, , s, 3', 5'-OH),3.91 (3H, s, 4'- OCH_3). ^{13}CNMR (75MHz, DMSO-d_6), δ(ppm), : 157.4 (C-2), 134.6(C-3), 182.7 (C-4), 162.7(C-5), 98.3 (C-6),164.2 (C-7) ,91.9 (C-8), 155.9 (C-9), 105.6 (C-10),111.9 (C-2') ,147.5(C-3'), 152.3(C-4'),110.9 (C-5'),119.7 (C-6'), 59.8 (7-OCH_3), 55.6 (4'-OCH_3).

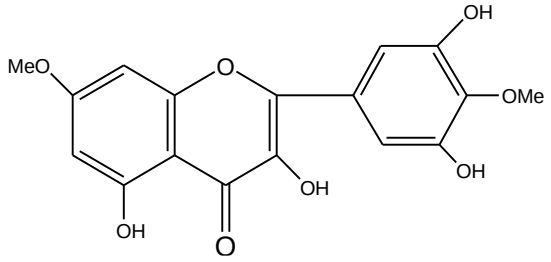

Figure 2 Chemical structure of Compound A-1

Permethylation of Compound A

Compound A (25 mg) was refluxed with MeI (15 ml) and Ag_2O (10ml) in DMF (20mg) for two days and then filtered. The filtrate was hydrolyzed with 10% ethanolic H_2SO_4 for 7-8 h, to give methylated aglycone identified as 3, 3',-dihydroxy-5 7 4' 5' tetramethoxy flavone and methylated sugars, which were identified as 2, 3, 4- tri- O-methyl- L- rhamnose (R_G1.02), 2, 4- di- O-methyl- L- arabinose (R_G0.65), 2, 3,4, 6-tetra-O-methyl-D-galactose (R_G 0.86).

Enzymatic Hydrolysis of Compound A

Compound 3.5gm was dissolved in MeOH (20ml) and hydrolysed with equal volume of takadiastase enzyme. The reaction mixture was allowed to stay at room temperature for 2 days and filtered. The hydrolysate was concentrated and subjected to paper chromatography examination using nBAW (4:1:5) as solvent, which showed the presence of L-rhamnose (R_f 0.36), L-arabinose (R_f 0.22)(Co-PC). The proaglycone was dissolved in MeOH (20ml) and further hydrolysed with equal volume of almond emulsion yielded D-galactose (R_f 0.15) and aglycone, identified as 3, 5, 3', 5'- tetrahydroxy-7, 4'-dimethoxy flavone.

Study of Compound B

It was crystallized from methanol to give light brownish compounds 1.10 g. It has m.p. 217-219^{0}C, m.f. $C_{33}H_{40}O_{21}$, [M^+] 772 (FABMS); found (%): C 51.46, H 5.21, O 42.98 calcd.for m.f. $C_{34}H_{42}O_{21}$:C 51.29, H 5.1, O 43.52. UVλ_{max} (nm):(MeOH) 205,, 274, 304(+$AlCl_3$) 204, 275, 323, 347. IR (KBr) υ_{max} 3402, 1644, 1619, 1568,1517, 1443, 1382, 1329, 1271, 1207, 1185, 1034, 872, 795. ^{1}H NMR (300MHz, DMSO-d_6) δ (ppm); 6.89 (1H,d, *J* 2.1Hz, H-6), 9.48 (1H, s, 3-OH),12.39 (1H, s, 5-OH), 3.82(1H, s, 7-OH), 10.26(1H, s, 8-OH), 7.73(1H,d , *J* 8.5 Hz, H-2',),6.95 (1H,d , *J* 8.4Hz, H-5',), 7.75 (1H,d , *J* 8.4Hz, H-6',), 9.43 (1H, , s , 4'-OH),3.85 (3H, s, 3'- OCH_3), 5.26(1H, d, *J* 7.5 Hz, H-1"),4.23 (1H, m, H-2"),3.19-3.76 (3H, m, H-3", H-4", H-5"), 0.94(3H, s, *J* 5.8 Hz, CH_3-6"),5.02 (1H, d, *J* 6.9Hz, H-1'"), 3.57-3.84 (3H, m, H-2'", H-3'", H-4'"),3.87 (1H,d , *J* 8.5 Hz, H-5'"), 4.37 (1H,d, *J* 4.7Hz, H-1""), 3.72-4.37 (5H, m, H-2"", H-3"", H-4"", H-5"", H-6"").^{13}C NMR (75 MHz, DMSO-d_6), δ(ppm), : 147.2 (C-2), 133.2(C-3), 181.0 (C-4), 144.7(C-5), 110.2 (C-6),158.3 (C-7) ,128.6 (C-8), 149.8 (C-9), 102.8 (C-10),110.9 (C-2'),147.8(C-3'), 148.5(C-4'),115.3 (C-5'),122.7 (C-6'), 55.8 (3'-OCH_3), 104.6 (C-1"), 73.1 (C-2"), 72.3 (C-3"),73.4 (C-4"),72.2 (C-5"), 18.2 (C-6"), 103.5 (C-1'"), 84.2(C-2'"), 73.4(C-3'"), 70.3(C-4'"), 65.1(C-5'"), 102.3(C-1""), 72.9(C-2""), 78.1(C-3""), 68.9(C-4""), 77.5(C-5""), 62.8(C-6"").

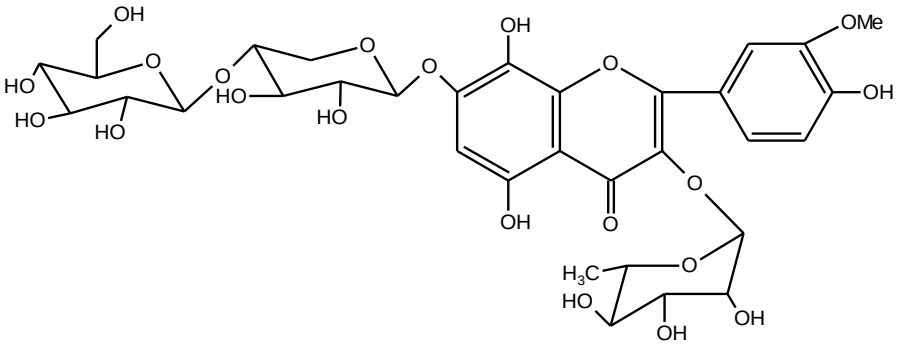

Figure 3 Chemical structure of Compound B

Acid Hydrolysis of Compound B

300 mg of the compound was dissolved in ethanol (30ml) and refluxed with 25ml of H_2SO_4 on water bath for 5-6 h. The reactive mixture was concentrated and allowed to cool and residue was extracted with diethyl ether (Et_2O). The ether layer was washed with water and evaporated to dryness. The residue was subjected to column chromatography over silica gel column using $CHCl_3$: MeOH (3:6) to give compound A-1 identified as 3, 5, 7, 8, 4' pentahydroxy 3' methoxy flavone. The aqueous hydrolysate was neutralized with $BaCO_3$ and $BaSO_4$ was filtered off. The filtrate was concentrated and subjected to paper chromatography examination using nBAW (4:1:5) solvent and aniline hydrogen phthalate as spraying reagent, showed the presence of L- rhamnose (R_f 0.35), D - glucose (R_f 0.17), D-xylose (R_f 0.29).

Study of Compound B-1

It has molecular formula $C_{16}H_{14}O_8$, m.p. 248-251 ^{0}C, [M^+] 334 (EIMS); found C 57.42, H 4.18 O 38.34, calcd (%) for m.f. $C_{17}H_{14}O_8$, C 57.48, H 4.19, O 38.32; UVλ_{max} (nm):(MeOH) 210, 281, 307(+$AlCl_3$) 203, 272, 327, 342. IR (KBr) υ_{max} 3404, 1648, 1615, 1570,1512, 1433, 1378, 1320, 1266, 1203, 1187, 1036, 845, 798. ^{1}H NMR (300MHz, DMSO-d_6) δ (ppm); 6.89 (1H,d, *J* 2.1Hz, H-6), 9.48 (1H, s, 3-OH),12.39 (1H, s, 5-OH), 3.82(1H, s, 7-OH), 10.26(1H, s, 8-OH), 7.73(1H,d , *J* 8.5 Hz, H-2',),6.95 (1H,d , *J* 8.4Hz, H-5',), 7.75 (1H,d , *J* 8.4Hz , H-6',), 9.43 (1H, , s , 4'-OH),3.85 (3H, s, 3'- OCH_3).. ^{13}C NMR (75 MHz, DMSO-d_6), δ(ppm), : 147.2 (C-2), 133.2(C-3), 181.0 (C-4), 144.7(C-5), 110.2 (C-6),158.3 (C-7) ,128.6 (C-8), 149.8 (C-9), 102.8 (C-10),110.9 (C-2'),147.8(C-3'), 148.5(C-4'),115.3 (C-5'),122.7 (C-6'), 55.8 (3'-OCH_3).

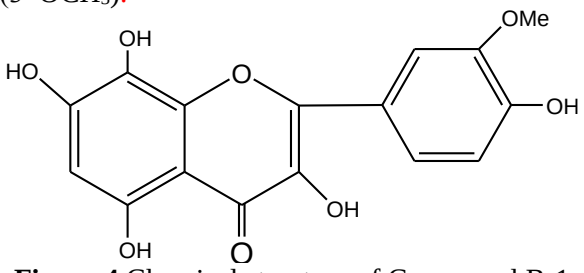

Figure 4 Chemical structure of Compound B-1

Permethylation of Compound B

Compound A (30 mg) was refluxed with MeI (10 ml) and Ag_2O (10ml) in DMF (20mg) for two days and then filtered. The filtrate was hydrolyzed with 10% ethanolic H_2SO_4 for 7-8 h, to give methylated aglycone identified a 3, 7,-dihydroxy-5 8 3' 4' tetramethoxy flavone and methylated sugars, which were identified as 2, 3, 4- tri- O-methyl- L-rhamnose (R_G1.03), 2, 3,4, 6-tetra-O-methyl-D-glucose (R_G 0.83). 2, 3- di- O-methyl-D- xylose (R_G0.66).

Enzymatic Hydrolysis of Compound B

Compound 25 mg was dissolved in MeOH (20ml) and hydrolysed with equal volume of takadiastase enzyme. The reaction mixture was allowed to stay at room temperature for 2 days and filtered. The hydrolysate was concentrated and subjected to paper chromatography examination using nBAW (4:1:5) as solvent, which showed the presence of

L-rhamnose (R_f 0.35),(CoPC). The proaglycone was dissolved in MeOH (20ml) and further hydrolysed with equal volume of almond emulsin yielded D-glucose(R_f 0.17), D-xylose (R_f 0.29) and aglcone, identified as 3, 5, 7, 8, 4' pentahydroxy 3' methoxy flavone.

Study of Known Compound C

It was crystallized from acetone to give 340 mg. It has m.f. $C_{15}H_{10}O_7$ m.p. 327-329^{0}C found (%): C 59.69, H 3.32, O 37.06 calcd (%) for m.f. $C_{15}H_{10}O_7$ C 59.60, H 3.31, O 37.086 UVλ_{max} (nm):(MeOH) 206, 257, 265, 346 nm. . IR (KBr) υ_{max} 3426, 2921, 1656, 1619, 1504, 1363, 1257 cm^{-1}.^{1}HNMR (300MHz, DMSO-d_6) δ (ppm); 6.15(1H, s , H-6), 6.42 (1H,d , H-8), 10.56(1H, s, 3-OH), δ 12.26 (2H, s, 5-OH and 7-OH), (7.59 1H, s, H-2'), 6.98(1H, d, *J* 4.62 Hz,H-5'), 7.35(1H, s, H-6'), δ 12.56 (2H, s, 3'-OH and 4'-OH). ^{13}CNMR (75MHz, DMSO-d_6), δ (ppm); 158.2 (C-2), 135.4(C-3), 172.5 (C-4), 159.3(C-5), 98.4 (C-6),164.3 (C-7) ,92.3 (C-8),158.3 (C-9), 104.2 (C-10),121.5 (C-1'),106.9 (C-2') ,146.3(C-3'), 147.3(C-4'),116.8 (C-5'),121.4 (C-6'). Then it was identified as quercetin by comparison of its spectral data with reported literature[10].

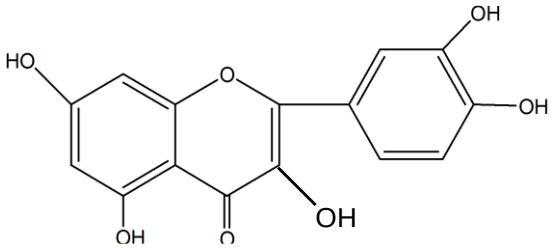

Figure 5 Chemical structure of Compound C

Study of Known Compound D

It was crystallized from acetone to give 285mg. It has m.f. $C_{15}H_{10}O_6$, m.p.352-354^{0}C[M^+] 286 (EMIS) found (%): C 63.84, H 3.12, O 33.06 calcd (%) for m.f. $C_{15}H_{10}O_7$ C 62.93, H 3.49, O 33.56. UVλ_{max} (nm):(MeOH) 263, 342 (+NaOMe) 262, 387 (+$AlCl_3$) 274, 301, 349, 386. IR (KBr) υ_{max} 3434, 1659, 1621, 1086, 836 . ^{1}HNMR (300MHz, DMSO-d_6) δ (ppm); 6.23 (1H, d, *J* 2.0 Hz, H-6),6.43 (1H,d , *J* 1.2 Hz, H-8), 11.4(1H, s, 3-OH), 12.6(1H, s, 5-OH), 10.64(1H, s, 8-OH), 7.94(2H, d, *J* 9.0 Hz, H-2'and H-6'), 6.86(1H, d, *J* 8.2 Hz,H-3' and H-5'), 9.65(1H, s, 3'-OH). ^{13}CNMR (75MHz, DMSO-d_6), δ(ppm), : 154.2 (C-2), 134.3(C-3), 172.6 (C-4), 162.1(C-5), 100 .5 (C-6), 164.5(C-7) ,98.3 (C-8), 157.3(C-9), 106.2(C-10),122.5 (C-1'),128.5 (C-2' and C-6') ,116.3(C-3' and C-5'), 161.8(C-4'). Then it was identified as kaempferol by comparison of its spectral data with reported literature[11].

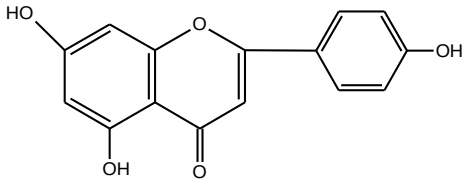

Figure 6 Chemical structure of Compound D

Antimicrobial activity of compound A and B

Ethanol soluble fraction of compound A and B of the stems of *galinsoga purviflora* Cav. were used for the evaluation of antimicrobial activity. Antimicrobial study of compound A and B has been carried out by disc diffusion method. Compound A and B were dissolved in ethanol and further diluted to obtain desired concentrations (10, 20, 40, 60 and 80 µg/ml). Gentamycin (10 µg/disc) was used as positive control.

The bacterial strains were activated by inoculating a loopful of the strains in the nutrient broth (10 ml), and incubated for 6 h to maintain McFarland standard turbidity (10^6 cells/ml) at 37°C to obtained spore (10^4/100 µl) suspensions. Later, 0.1 ml of inoculum of each bacterial strains was spread uniformly into the M.H.A. petri plate.

Each test sample (40 µl) was introduced on the disc (6 mm) of Hi-Media. The disc was impregnated on the seeded agar plates. The plates were allowed to stand for 1 h for pre-diffusion of the extract (Esimone*et al*.,1998). The plates were prepared in triplicates and incubated at 37°C for 24 h for bacterial . The antimicrobial activity was taken on the basis of diameter of zone of inhibition (mm). All the experiments were done in triplicates. The results are recorded in table no. 1.

Table 1 Antibacterial activity of compound A
****=** Values are mean of three replicates; **NI:** No inhibition; **Gen:** Gentamycin.

Bacteria	**Concentration (µg/ml) of compound A**					**Gen**
	10	20	40	60	80	
E. coli	03.2±0.32	07.0±0.22	09.5±0.16	14.0±0.49	17.2±0.40	24.5±0.44
P. aeruginosa	NI	NI	NI	09.0±0.37	11.2±0.36	25.7±0.11
K. pneumonia	NI	NI	05.0±0.36	10.0±0.23	13.2±0.12	22.7±0.21
S. aureus	NI	06.6±0.19	10.0±0.25	14.5±0.18	18.2±0.2	23.9±0.45
S. subtilis	03.0±0.09	06.0±0.49	08.5±0.21	10.5±0.44	12.0±0.30	22.4±0.19

Table 2 Antibacterial activity of compound B
****=** Values are mean of three replicates; **NI:** No inhibition; **Gen:** Gentamycin

Bacteria	**Concentration (µg/ml) of compound B**					**Gen**
	10	20	40	60	80	
E. coli	06.5±0.47	10.0±0.05	14.4±0.08	17.0±0.21	18.5±0.22	25.2±0.14
P. aeruginosa	03.2±0.37	05.5±0.04	08.2±0.36	13.3±0.35	16.7±0.44	24.6±0.29
K. pneumonia	04.1±0.09	05.8±0.30	10.6±0.40	13.5±0.44	16.7±0.48	27.7±0.01
S. aureus	03.1±0.16	07.2±0.40	10.2±0.33	12.4±0.31	14.8±0.43	25.3±0.20
B. subtilis	NI	NI	5.6±0.15	8.9±0.16	13.2±0.23	23.1±0.06

Results and Discussion

Two new allelochemicals **A** and **B** have been isolated from acetone soluble fraction of ethenolic extract of the stems of this plant. Compound **A** has molecular formula $C_{34}H_{42}O_{21}$, mp.223-225^{0}C, [M^+] 786 (FABMS). It gave Molisch and Shinoda tests[12] showing its flavonoidal glycosidic nature. Its IR spectra showed strong absorbtion bands at 3474, 2918, 1720, 1680, 1660, 1600 and 986 cm^{-1}. In UV spectrum two bands at 265 and 366 nm showed its flavonoidal skeleton. The bathochromic shift of 58 nm with NaOMe and 38nm $AlCl_3$ relative to methanol showed the presence of OH groups at C-3 and C-5 position in the aglycone A-1.

In ^{1}H NMR spectrum of compound a singlet at δ 9.20 confirmed the present of OH groups at C-3 position. Two singlets at δ12.38 and δ 8.49 confirmed the present of OH groups at C-5 and C-5 ' position. Two singlets at δ3.83 and δ 3.93 confirmed the present of OMe group at C-7 and C-4' position. In ^{1}HNMR spectrum of the agylcone A-1 a two doublates at δ 6. 35 and δ6.41 were assigned to H-6 and H-8 of ring A. A doublet at δ7.08 were assigned to H-2 ' H-6 ' in ring B. The anomeric proton at δ 5.64(1H, d, *J* 5.8 Hz), δ 5.12 (1H, d, *J* 1.25 Hz) and δ 5.18 (1H,d, *J* 7.8 Hz) were assigned for H-1", H-1"', H-1"" of L- arabinose, L- rhamnose, and D- galactose respectively.

In the mass spectrum of compound A, characteristic ion peaks at *m/z* 786 [M^+], 640 [M^+- L- rhamnose], 508 [M^+- L- arabinose], and 346 [M^+- D- galactose aglycone] were found by subsequent losses from the molecular ion of each molecule of L- rhamnose, L- arabinose, and D- galactose showing L- rhamnose was terminal sugar, L- arabinose was linked to aglycone at C-3 position and D- galactose was attached at C-3' position of aglycone.

Acid hydrolysis of compound A with 10% ethanolic H_2SO_4 gave aglycone A-1 m.p. 242-244 ^{0}C, m.f. $C_{17}H_{14}O_8$ [M^+] 346 (EIMS) and sugar moiety (ies). These were separated and studied separately. The aglycone **A-1** was identified as 3, 5, 3', 5' tetra hydrox-7, 4' dimethoxy flavone (see in Experimental section).

The aqueous hydrolysate after the removal of aglycone was neutralized with $BaCO_3$ and the BaSO4 was filtered off. The filtrate was concentrated subjected to paper chromatography examination and sugars were identified as L- rhamnose(R_f 0.38), L- arabinose (R_f 0.23), and D- galactose (R_f 0.15) (Co-PC)[13]. Periodate oxidation of compound A, confirmed that all the sugars were present in the pyranose form[14].

The position of sugar moieties in compound A were determined by permethylation[15] followed by acid hydrolysis yielded methylated aglycone identified as 3, 3' dihydroxy 5,7,4',5' tetramethoxy flavone showed that glycosidation was involved at 3, 3' position of the aglycone and methylated sugars were identified as 2, 3, 4- tri- O-methyl- L- rhamnose (R_G1.02), 2, 4- di- O-methyl- L- arabinose (R_G0.65), 2, 3,4, 6-tetra-O-methyl-D-galactose (R_G 0.86) indicating that C-1'''-OH of L- rhamnose was linked to C-3''-OH of L-arabinose and C-1''-OH of the L-arabinose was attached with C-3 position of the aglycone, C-1''''-OH of D-galactose was linked to C-3'position of the aglycone. therefore it was concluded that interlinkage (1→3) was found between L-rhamnose and L-arabinose which was further confirmed by ^{13}C-NMR spectra (see in experimental section).

Enzymatic hydrolysis of compound A with takadiastase enzyme liberated L- rhamnose (R_f 0.38) first followed by L- arabinose (R_f 0.23) and proaglycone identified as 3, 5, 3', 5' tetra hydroxy 7, 4'-di methoxy flavone - 3'-0-*β*-D galactopyranoside showed the presence of α linkage between L- rhamnose and L-arabinose as well as between L-arabinose and proaglycone. Proaglycone on further hydrolysis with almond emulsin enzyme liberated D-galactose (R_f 0.15) and aglycone suggesting the presence of β linkage between D- galactose and aglycone.

On the basis of above evidences the structure of compound A was characterized as 3,5 3',5' tetra hydroxyl-7,4'dimethoxyflavone 3-O-*α*-L-rhamnopyranosyl-(1→3)-O-*α*-L-arabinopyranosyl-3'-O-*β*-D-galactopyranoside.

Compound **B** has molecular formula $C_{33}H_{40}O_{21}$, mp. 216-219 ^{0}C, [M^+] 772 (FABMS). It gave Molisch and Shinoda test[12] showing its flavonoidal glycosidic nature. Its IR spectra showed strong absorbtion bands at 3402, 1644, 1619, 1568,1517, 1443, 1382, 1329, 1271, 1207, 1185, 1034, 872, 795. In UV spectrum band at 274 and 347nm showed its flavonoidal skeleton.

In ^{1}H NMR spectrum of compound B four singlets at δ9.48, δ12.39, δ 3.82 and δ10.26 confirmed the present of OH groups at C-3, C-5, C-7, and C-8 position. A singlet at δ9.48 confirmed the present of OH group at C-4' position One singlet at δ3.85 confirmed the present of OMe group at C-3' position. In ^{1}HNMR spectrum of the agylcone B-1 a singlet at δ 6.89 was assigned to H-6 of ring A. A doublet at δ 5.95 was assigned to H-5' of ring B. Two doublets at δ7.3 and δ7.75 were assigned to H-2 ' H-6 ' in ring B. The anomeric proton at δ 5.26 (1H, d, *J* 7.5 Hz), δ 5.02 (1H, d, *J* 6.9 Hz) and δ 4.37 (1H,d, *J* 4.7 Hz) were assigned for H-1'', H-1''', H-1'''' of L- rhamnose, D- xylose and D- glucose respectively.

In the mass spectrum of compound 1, characteristic ion peaks at *m/z* 772 [M^+], 626 [M^+- L- rhamnose], 464 [M^+- D glucose], and 332 [M^+- D- xylose aglycone] were found by subsequent losses from the molecular ion of each molecule of L- rhamnose, D- glucose , and D- xylose showing D- glucose was terminal sugar D- xylose was linked to aglycone at C-7 position and L- rhamnose was attached at C-3 position of aglycone.

Acid hydrolysis of compound B with 10% ethanolic H_2SO_4 gave aglycone B-1 m.p. 248-251^{0}C m.f. $C_{16}H_{14}O_8$, [M^+] 334 (EIMS) and sugar moiety (ies). These were separated and studied separately. The aglycone **B-1** was identified as 3, 5, 7, 8, 4' pentahydroxy 3' methoxy flavone. (see in Experimental section).

The aqueous hydrolysate after the removal of aglycone was neutralized with $BaCO_3$ and the $BaSO_4$ was filtered off. The filtrate was concentrated subjected to paper chromatography examination and sugars were identified as L-rhamnose (R_f 0.35), D-glucose(R_f 0.17), D-xylose (R_f 0.29)(Co-PC)[13]. Periodate oxidation of compound B, confirmed that all the sugars were present in the pyranose form[14].

The position of sugar moieties in compound B were determined by permethylation[15] followed by acid hydrolysis yielded methylated aglycone identified as 3, 7,-dihydroxy-5 8 3' 4' tetramethoxy flavone showed that

glycosidation was involved at 3, 7 position of the aglycone and methylated sugars were identified as 2, 3, 4- tri- O-methyl- L- rhamnose (R_G1.03), 2, 3,4, 6-tetra-O-methyl-D-glucose (R_G 0.83). 2, 3- di- O-methyl-D- xylose (R_G0.66) indicating that C-1"-OH of L- rhamnose was linked to C-3position of the aglycone L-arabinose and C-1""-OH of the L-arabinose was attached with C-3 position of the aglycone, C-1""-OH of D-glucose was linked to C-4"'-OH of D-xylose and C-1"'-OH of D-xylose interlinkage (1→4) was found between D-glucose and D-xylose which was further confirmed by ^{13}C-NMR spectra (see in experimental section).

Enzymatic hydrolysis of compound B(35 mg) with takadiastase enzyme liberated L- rhamnose(R_f 0.38) and proaglycone identified as 3, 5, 7, 8, 4' pentahydroxy 3' methoxy flavone-7-O-*β*-D-glucopyranosyl-(1→4)-O-*β*-D-xylopyranoside showed the presence of α linkage between L- rhamnose and proaglycone. Proaglycone on further hydrolysis with almond emulsin enzyme liberated D- glucose (R_f 0.15) first followed by D- xylose (R_f 0.15) and aglycone suggesting the presence of β linkage between D- glucose and D- xylose as well as D- xylose and aglycone. On the basis of above evidences the structure of compound B was characterized as 3,5,7,8,4' pentahydroxy 3' methoxy flavone-3-O-*α*-L-rhamnopyranosyl-7-O-*β*-D-glucopyranosyl-(1→4)- O-*β*-D-xylopyranoside.

Conclusions

The present study has been undertaken to evaluate antimicrobial activity of compound A and compound B of plant *Galinsoga purviflora* Cav. stems at the concentration of 10, 20,40, 60, and 80 µg/ml against some pathogenic bacterial strains namely; *Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Staphylococcus aureus, Bacillus subtilis.* The antimicrobial screening showed concentration dependent zone of inhibition compared with that of standard drugs.

From table 1 and 2 it was concluded that compound A and B were screened for antibacterial activity against various gram (+ve) and gram (-ve) bacteria The resuld reported in table no 1 and 2 showed that compound A was found to be highest activity against *S. aureus* at highest concentration even at compound should less activity against *E. coli,* and *S. subtilis* at minimum concentration. Compound B showed highest activity against *E. coli* at highest concentration even at compound B should less activity against *P. aeruginosa,* and *S. aureus* at minimum concentration.

Acknowledgement

Aurthors are thankfull to the Director, CDRI Lucknow (U.P.) for recording various spectral data and Head, Department of Chemistry, Dr. H.S. Gour Central University, Sagar (M.P.) for providing necessary laboratory facilities.

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Publication History
Received 10th Jan 2015
Revised 13th Feb 2015
Accepted 06th Mar 2015
Online 30th Mar 2015