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A New Antiviral Flavone Glycoside from *Vernonia cinerea* Less.

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A new flavone glycoside (**A**) m.p. 258-260 ºC, m.f. $C_{33}H_{40}O_{19}$, $[M]^+$ 740 (FABMS), has been isolated from roots of *Vernonia cinerea* Less. It was characterized as 5,7,4'-trihydroxy 3'-methoxy flavone-4'-O-α-L-rhamnopyranosyl-(1→4)-O-α-L-arabino-pyranosyl-(1→3)-O-β-D-galactopyranoside by various chemical degradations and spectral analysis which showed antiviral activity alongwith two known compounds luteolin (**B**) and taxifolin (**C**).

Key Words: *Vernonia cinerea* Less., Compositae, Roots, Flavone glycoside.

INTRODUCTION

Vernonia cinerea Less.[1,2] belongs to family Compositae which is commonly known as "Sahadevi" in Hindi. It is found throughout in India. It is an annual, erect, 15-75 cm high, stem stiff, cylindric, striate, more or less pubscent, slightly branched. Its leaves are petioled, 2.5-5 by 2.3-8 cm the upper leaves, variable in shape, irregularly toothed or shallowly create-serrate, petioles variable, 6-13 mm long. Heads small, about 20-floalered, 6 mm. diameter, in lax divaricate terminal corymbs, with a minute linear bract beneath each head of flowers and with small bracts in the forks of the peduncles; flowers pinkish violet. In-volueral bract linear-lanceolate, awned, silky on the back, pappus white. Ayurvedic system of medicines describes that its flowers cures fevers, asthma and bronchitis. Its roots are used as alexipharmic and anthelmintic. The whole plant is given as a remedy for spasm of the bladder and strangury. Earlier workers[3-5] have reported the presence of various constituents from this plant. In the present paper, we report the isolation and structural elucidation of a new flavone glycoside 5,7,4'-trihydroxy-3'-methoxyflavone-4'-O-α-L-rhamnopyranosyl-(1→ 4)-O-α-L-arabinopyranosyl-(1→3)-O-β-D-galactopyranoside by several colour reactions, chemical degradations and spectral analysis alongwith two known compounds luteolin (**B**) and taxifolin (**C**).

EXPERIMENTAL

All the melting points were determined by soft capillary tube in thermoelectric m.p. apparatus and are uncorrected. The IR spectra were recorded on Shimadzu FTIR-8400s spectro-photometer. ^{1}H NMR spectra were recorded on Bruker DRX 300 NMR spectrometer and ^{13}C NMR spectra were recorded at 75 MHz using ($CDCl_3$) as solvent. Mass spectra on a JEOL-AccuTOF JMS-T100LC mass spectrometer.

The roots of *Vernonia cinerea* Less. were collected around Sagar region and were taxonomically authenticated by the 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, Dr. H. S. Gour Central University, Sagar (M.P.).

Extraction and isolation: Air dried and powdered roots (3.5 kg) of the plant were extracted with ethanol in a Soxhlet apparatus for 7 days. The ethanolic extract of the roots of the plant was successively extracted with $CH_3COOC_2H_5$, CH_3COCH_3 and MeOH. The methanolic fraction of the plant was concentrated and subjected to TLC examination showed three spots indicating it to be a mixture of three compounds **A**, **B** and **C**. These compounds were separated by TLC and purified by column chromatography over silica-gel and studied separately.

Study of compound A: It has m.p. 258-260 ºC, m.f. $C_{33}H_{40}O_{19}$, $[M]^+$ 740 (FABMS); Found (%): C 52.96, H 5.10, calcd. for m.f. $C_{33}H_{40}O_{19}$, C 53.03, H 4.97; IR (KBr, ν_{max}, cm^{-1}); 3349, 2872, 1621, 1559, 1344, 867, 796 and 763; ^{1}H NMR (300 MHz, $CDCl_3$): δ, 10.96 (1H, s, 5-OH), 9.89 (1H, s, 7-OH), 4.24 (3H, s, 3'-OCH_3), 6.71 (1H, s, H-3), 6.65 (1H, d, *J* 2.3 Hz, H-6), 6.52 (1H, d, *J* 2.3 Hz, H-8), 7. 68 (1H, d, *J* 2.7 Hz, H-2'), 7.48 (1H, d, *J* 8.5 Hz, H-5'), 7.72 (1H, dd, *J* 8.2, 2.1 Hz, H-6'), 5.56 (1H, d, *J* 8.1 Hz, H-1''), 3.77-4.20 (5H, m, H-2'', H-3'', H- 4'', H-5'', H-6''), 5.14 (1H, d, *J* 7.3 Hz, H-1'''), 4.44-5.07 (5H, m, H-2''', H-3''', H- 4''', H-5'''), 5.36 (1H, d, *J* 2.3 Hz, H-1''''), 4.12-4.23 (5H, m, H-2'''', H- 3'''', H-4'''',

H-5""), 1.19 (3H, m, 6""-Me); ^{13}C NMR (75 MHz, $CDCl_3$): δ, 160.3 (C-2), 108.2 (C-3), 182.1 (C-4), 162.4 (C-5), 102.3 (C-6), 163.9 (C-7), 103.2 (C-8), 162.3 (C-9), 105.8 (C-10), 121.3 (C-1'), 127.4 (C-2'), 142.9 (C-3'), 158.7 (C-4'), 116.3 (C-5'), 129.8 (C-6'), 56.9 (OCH_3 at 3'), 105.22 (C-1''), 72.20 (C-2''), 75.43 (C-3''), 68.94 (C-4''), 60.84 (C-5''), 66.5 (C-6''), 102.9 (C-1'''), 74.2 (C-2'''), 73.9 (C-3'''), 71.9 (C-4'''), 69.8 (C-5'''), 107.50 (C-1""), 72.2 (C-2""), 72.11 (C-3""), 73.42 (C-4""), 69.51 (C-5""), 18.2 (C-6"").

Acid hydrolysis of compound A: 95 mg of compound **A** was dissolved in ethanol (35 mL) and refluxed with 20 mL of 10 % H_2SO_4 on water bath for 8-10 h. The reaction mixture was concentrated and allowed to cool and residue was extracted with diethyl ether. The ether layer was washed with water and the residue was chromatographed over silica gel using $CHCl_3$:MeOH (2:4) to give compound **A_1** which was identified as 5,7,4'-trihydroxy 3'-methoxy flavone by comparison of its known spectral data. The aqueous hydrolysate was neutralized with $BaCO_3$ and $BaSO_4$ filtered off. The filtrate was concentrated and subjected to paper chromatography examination using *n*-BAW (4:1:5) as solvent and aniline hydrogen phthalate as detecting agent, confirmed the presence of D-galactose (R_f 0.19), L-arabinose (R_f 0.22) and L-rhamnose (R_f 0.36) by (Co-Pc).

Study of compound A_1: It has m.p. 325-327 ºC, m.f. $C_{16}H_{12}O_6$, $[M]^+$ 300, IR (KBr, ν_{max}, cm^{-1}): 3298, 2876, 1635, 1562, 1347, 871, 798 and 765 ; ^{1}H NMR (300 MHz, $CDCl_3$): δ, 10.98 (1H, s, 5-OH), 9.94 (1H, s, 7-OH), 9.12 (1H, s, 4'-OH), 4.18 (3H, s, 3'-OCH_3), 6.69 (1H, s, H-3), 6.61 (1H, d, *J* 2.2 Hz, H-6), 6.49 (1H, d, *J* 2.2 Hz, H-8), 7.64 (1H, d, *J* 2.8 Hz, H-2'), 7.39 (1H, d, *J* 8.6 Hz, H-5'), 7.69 (1H, dd, *J* 8.3, 2.2 Hz, H-6'), ^{13}C NMR (75 MHz, $CDCl_3$): δ, 159.2 (C-2), 109.3 (C-3), 182.2 (C-4), 161.2 (C-5), 101.9 (C-6), 164.2 (C-7), 102.9 (C-8), 161.1 (C-9), 106.2 (C-10), 120.4 (C-1'), 129.7 (C-2'), 143.2 (C-3'), 159.8 (C-4'), 115.6 (C-5'), 130.7 (C-6'), 57.3 (OCH_3 at C-3').

Permethylation of compound A: Compound **A** (45 mg) was refluxed with MeI (5 mL) and Ag_2O (5 mg) in DMF (40 mg) for one day and then filtered. The filtrate was hydrolyzed with 10 % ethanolic H_2SO_4 for 7-8 h to yield methylated aglycone identified as 5,7,3'-trimethoxy 4'-hydroxy flavone and methylated sugars which were identified as 2,3,4-*tri*-O-methyl-L-rhamnose, 2,3-di-O-methyl-L-arabinose and 2,4,6-*tri*-O-methyl-D-galactose.

Enzymetic hydrolysis of compound A: The compound **A** (30 mg) was dissolved in ethanol (30 mL) and hydrolyzed with an equal volume of takadiastase at room temperature in a 150 mL round bottomed flask fitted with air condenser. The contents were left for 2 days and filtered to yield L-rhamnose (R_f 0.36) first then L-arabinose (R_f 0.22) and proaglycone confirming the presence of α-linkage between L-arabinose and L-rhamnose as well as between L-arabinose and proaglycone. Proaglycone on further hydrolysis with almond emulsin liberated D-galactose (R_f 0.17) and aglycone, confirming the presence of β-linkage between D-galactose and aglycone.

Study of compound (B): It has m.p. 330-331 ºC, m.f. $C_{15}H_{10}O_6$, $[M]^+$ 286, IR (KBr, ν_{max}, cm^{-1}), 3450, 3300, 1659, 1612, 1585; ^{1}H NMR (300 MHz, $CDCl_3$): δ, 10.88 (1H, s, 5-OH), 9.92 (1H, s, 7-OH), 9.05 (1H, s, 3'-OH), 9.16 (1H, s, 4'-OH), 6.72 (1H, s, H-3), 6.46 (1H, d, *J* 2.1 Hz, H-6), 6.82 (1H, d, *J* 2.2 Hz, H-8), 7.45 (1H, d, *J* 2.2 Hz, H-2'), 6.88 (1H, d, *J* 8.4 Hz, H-5'), 7.42 (1H, dd, *J* 8.4, 2.3 Hz, H-6'); ^{13}C NMR (75 MHz, $CDCl_3$): δ, 164.2 (C-2), 102.8 (C-3), 182.2 (C-4), 161.2 (C-5), 89.8 (C-6), 163.4 (C-7), 94.5 (C-8), 156.8 (C-9), 104.6 (C-10), 121.6 (C-1'), 113.2 (C-2'), 145.7 (C-3'), 150.4 (C-4'), 117.3 (C-5'), 119.4 (C-6').

Study of compound (C): It has m.p. 228-230 ºC, m.f. $C_{15}H_{12}O_7$, $[M]^+$ 304, ^{1}H NMR (300 MHz, $CDCl_3$): δ, 9.98 (1H, s, 3-OH), 10.95 (1H, s, 5-OH), 9.79 (1H, s, 7-OH), 9.12 (1H, s, 3'-OH), 9.23 (1H, s, 4'-OH), 4.92 (1H, d, *J* 11.4 Hz, H-2), 4.42 (1H, d, *J* 11.2 Hz, H-3), 5.83 (1H, d, *J* 2.3 Hz, H-6), 5.89 (1H, d, *J* 2.1 Hz, H-8), 6.86 (1H, s, H-2'), 6.69 (1H, d, 8.1 Hz, H-5'), 6.72 (1H, d, *J* 8.1 Hz, H-6'); ^{13}C NMR (75 MHz, $CDCl_3$): δ, 86.6 (C-2), 72.8 (C-3), 196.9 (C-4), 162.9 (C-5), 95.5 (C-6), 166.9 (C-7), 94.6 (C-8), 163.2 (C-9), 101.2 (C-10), 125.7 (C-1'), 114.7 (C-2'), 145.9 (C-3'), 144.9 (C-4'), 115.9 (C-5'), 118.6 (C-6').

Antiviral activity of compound A: Compound **A** was tested for antiviral activity against *Japanese Encephalitis Virus in vitro* (Vero cells). The results showed that compound A exhibited 50 % antiviral activity. Thus compound **A** may be used as antiviral agent diseases caused by these viruses.

RESULTS AND DISCUSSION

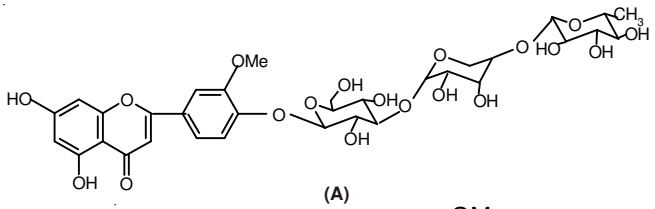
The ethanol soluble fraction of the roots of the plant afforded a new compound **A**, m.p. 258-260 ºC, m.f. $C_{33}H_{40}O_{19}$, $[M]^+$ 740 (FABMS). It responded Molisch[6] and Shinoda tests[7] showing its flavonoidal glycosidic nature. Its IR spectra showed absorption bands at 3349, 2872, 1621, 1559, 1344, 867, 796 and 763 cm^{-1}. In ^{1}H NMR spectrum, a double doublet at δ 7.72 showed both ortho (*J* 2.1 Hz) and meta (*J* 8.2 Hz) coupling for H-6', a doublet of one proton intensity at δ 7.68 (*J* 2.7 Hz) for H-2' proton and doublet of one proton intensity at δ 7.48 (*J* 8.5 Hz) for H-5' proton. Three singlets at δ 10.98, 9.94 and 9.12 were assigned for OH groups at C-5, C-7 and C-4'. Two doublets at δ 6.65 and 6.52, were assigned to H-6 and H-8 protons respectively. Three doublets at δ 5.54 (*J* 7.9 Hz), 5.14 (*J* 7.3 Hz) and 5.36 (*J* 2.3 Hz), each of one proton intensity, were assigned for the anomeric proton of D-galactose, L-arabinose and L-rhamnose. In ^{13}C NMR spectrum, the chemical shifts at δ 160.3 and 108.2 were assigned for C-2 and C-3 positions and a shift at δ 182.1 revealed the presence of carbonyl group at C-4 position, A chemical shift at δ 56.9 confirmed the presence of methoxy group at C-3' position.

Acid hydrolysis of compound **A** with l0 % ethanolic H_2SO_4 gave aglycone **A_1** m.p. 325-327 ºC, m.f. $C_{16}H_{12}O_6$, $[M]^+$ 300 (FABMS). It was identified as 5,7,4'-trihydroxy 3'-methoxy flavone by comparison of its spectral data with reported literature values[8]. The aqueous hydrolysate, after the removal of the aglycone, was neutralized with $BaCO_3$ and the $BaSO_4$ was filtered off. The filtrate was then concentrated and subjected to paper chromatography examination showed the presence L-rhamnose (R_f 0.36), D-galactose (R_f 0.17), and D-arabinose (R_f 0.27), Periodate oxidation[9] of compound A confirmed that all the sugars were present in the pyranose forms.

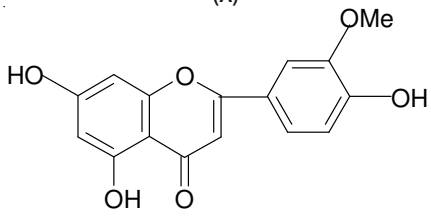
The position of sugar moieties in compound A was determined by methylation[10] followed by acid hydrolysis which yielded methylated aglycone identified as 4'-hydroxy-5,7,3'-trimethoxy flavone which confirmed that glycosidation was involved at C-4' position of -OH group. The methylated sugars were identified as 2,3,4-*tri*-*O*-methyl-L-rhamnose (R_G 1.07), 2,3-di-*O*-methyl-L-arabinose (R_G 0.68) and 2,4,6-*tri*-*O*-methyl-D-galactose (R_G 0.69) by (Co-Pc)[11]. Enzymatic hydrolysis of A with takadiastase liberated L-rhamnose first followed by L-arabinose and proaglycone confirming the presence of α-linkage between L-arabinose and L-rhamnose as well as between L-arabinose and proaglycone. Proaglycone on further hydrolysis with almond emulsin liberated D-galactose and aglycone, confirming the presence of β-linkage between D-galactose and aglycone.

Thus on the basis of above evidences, the structure of compound **A** was characterized as 5,7,4'-*tri*-hydroxy-3'-methoxy flavone-4'-*O*-α-L-rhamnopyranosyl -(1→4)-*O*-α-L-arabinopyranosyl-(1→3)-*O*-β-D-galactopyranoside. Compound A was tested against *Japanese Encephalitis* virus which showed 50 % antiviral activity.

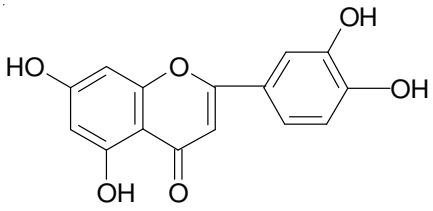
Compound (**B**) was analyzed for m.p. 330-331 ºC, m.f. $C_{15}H_{10}O_6$, $[M]^+$ 286, It was identified as luteolin by comparison of its spectral data with reported literature values[12]. Compound (**C**) was analyzed for m.p. 228-230 ºC, m.f. $C_{15}H_{12}O_7$, $[M]^+$ 304, It was identified as taxifolin by comparison of its spectral data with reported literature values[13].



(A)



(A$_1$)



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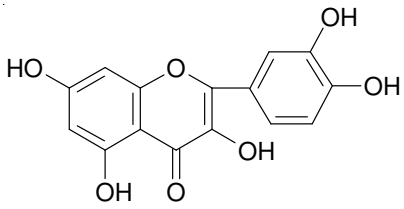

(C)

Structures of **A**, **A$_1$**, **B**, **C**

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