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ISOLATION AND CHARACTERISATION OF A NEW ALLELOCHEMICAL FROM *PARTHENIUM HYSTEROPHORUS* L.

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ABSTRACT

Parthenium hysterophorus L. is commonly known as “Gajar ghas" in Hindi and belongs to family Composite. It is an annual herb erect up to 1.5 m in hight. Its stems is branched and covered with trichomes. Its leaves are pale green, branched and covered with soft fine hairs. In Homoeopathy system, allergies caused by *Parthenium* can be treated by a drug prepared from *Parthenium*. Root decoction is useful in dysentery. In the present paper, we report the isolation and structurel elucidation of a new allelochemical identified (**I**) as 3 , 5, 7, 4’ tetrahydroxy-3'-methoxyflavone-3-O-β-L-galactopyranosyl-(1→3)-O-β-D-arabinopyranosyl-7-O-α-L-rhamnopyranoside alongwith two known compounds Lutexin (**II**) and Cirsilineol (**III**) from methanolic extract of the stems of this plant by several colour reactions, chemical degradations and spectral analysis.

INTRODUCTION: *Parthenium hysterophorus* L.[1-3] is commonly known as “Gajar ghas" in Hindi which belongs to family Composite. It is an annual herb erect up to 1.5 m in hight. Its stems is branched and covered with trichomes. Its leaves are pale green, branched and covered with soft fine hairs. The small white flowers have five distinct corners and grow on the stem tips. It is considered a highly invasive weed. It releases chemicals that inhibit the germination and growth of pasture grasses and other plants. This weed is common throughout the World. In Homoeopathy system, allergies caused by *Parthenium* can be treated by a drug prepared from *Parthenium*. Root decoction is useful in dysentery.

Earlier workers [4-8] have reported the presence of various constituents from this plant. In the present paper, we report the isolation and structure elucidation of a new allelochemical **(I)** identified as 3 , 5, 7, 4’ tetrahydroxy-3'-methoxyflavone-3-O-β-L-galactopyranosyl-(1→3)-O-β-D-arabinopyranosyl-7-O-α-L-rhamnopyranoside by several colour reactions, chemical degradations and spectral analysis.

EXPERIMENTAL SECTION:

General experimental procedure: All the m.p. were determined on a thermoelectrically melting point apparatus and are uncorrected. The IR spectra were recorded in KBr disc; ^{1}H-NMR spectra at 300 MHz in $CDCl_3$ using TMS as internal standard; ^{13}C-NMR spectra were recorded at 300 MHz using $CDCl_3$ as solvent; UV spectra were determined in MeOH and Mass spectra on a Jeol D-300 mass spectrometer.

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Plant material: The leaves of the plant were collected locally around Sagar region and were taxonomically authenticated by the Department of Botany, Dr.H.S.Gour 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.) India.

Isolation of the Compound: Air-dried and powdered leaves (5 kg) of the plant were extracted with 95% ethanol in a Soxhlet apparatus for five days. The ethanolic extract of the leaves of this plant was concentrated under reduced pressure to give light brown viscous mass which was successively partitioned with pet-ether (40-60^{o}C), chloroform, ethyl acetate, acetone and methanol. The methanol soluble fraction was concentrated under reduced pressure to yield light brown mass (1.24 gm), which was subjected to TLC examination showed three spots indicating it to be mixture of three compounds. These compounds were separated and purified by column chromatography over silica gel G and studied separately.

Study of Compound (I): It has m.p. 246-248^{o}C, $[M]^{+}$ 756 (FABMS); Found (%): C 52.98, H 5.12, calcd. for m.f. $C_{33}H_{40}O_{20}$ C 53.01, H 5.14; UV (MeOH) λ_{max} nm: 254, 366; IR (KBr) ν_{max} (cm-^{1}); 3346, 2923, 1653, 1625, 1245, 1063, 868, and 835; ^{1}H-NMR: (300 MHz, $CDCl_3$) δ (ppm); 12.14 (1H, s, 5- OH), 10.16 (1H, s, 4'-OH), 3.81 (3H, s, 3'-OMe), 6.19 (1H, d, *J* 1.83 Hz, H-6), 6.64 (1H, d, *J* 1.84 Hz, H-8), 7.18 (1H, d, *J* 8.7 Hz, H-2'), 6.91 (1H, d, *J* 8.3 Hz, H-5'), 7.12(1H, d, *J* 8.1 Hz, H-6'), 5.46 (1H, d, *J* 7.1 Hz, H-1''), 4.41 (1H, d, *J* 3.8 Hz H-2"), 4.12 (1H,dd, *J* 3.7, 10.3 Hz H-3"), 4.15 (1H, m, H- 4''), 4.39 (2H, d, *J* 8.4 Hz H-5''), 5.12 (1H, d *J* 7.5 Hz H-1'''), 3.80 (1H, dd *J* 3.6, 9.97 Hz H-2'''), 3.78 (1H, dd, *J* 3.7,9.98 Hz H-3'''), 3.54 (1H, dd, *J* 3.7, 9.98 Hz H-4'''), 4.01 (1H, m H-5'''), 5.36 (1H, d, *J* 1.5 Hz, H-1''''), 4.80 (1H, dd *J* 3.7, 9.99 Hz H-2"''), 4.76 (1H, dd, *J* 3.6, 9.97 Hz H-3"''), 4.58 (1H, dd, *J* 3.6, 10. Hz H-4"''), 4.31 (1H, m, H-5"''), 1.19 (3H, m, 6''''-Me); ^{13}C-NMR: (300 MHz, $CDCl_3$) δ (ppm); 158.3 (C-2), 135.7 (C-3), 177.4 (C-4), 163.4 (C-5), 99.9 (C-6), 164.8 (C-7), 95.01 (C-8), 157.89 (C-9), 105.0 (C-10), 123.4 (C-1'), 132.1 (C-2'), 115.4 (C-3'), 160.5 (C-4'), 116.4 (C-5'), 130.8(C-6'), 100.21 (C-1''), 74.2 (C-2''), 73.1 (C-3''), 70.5 (C-4''), 69.4 (C-5''), 104.3 (C-1'''), 72.08 (C-2'''), 72.0 (C-3'''), 69.5 (C-4'''), 62.8 (C-5'''), 65.23 (C-6'''). 107.2 (C-1''''), 80.3 (C-2''''), 72.0(C-3''''), 74.40(C-4''''), 67.2(C-5''''), 18.2(C-6''''); $[M]^{+}$ 756 (FABMS).

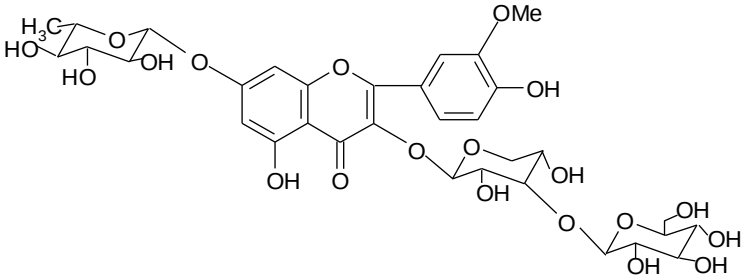

FIG. 1: COMPOUND (I)

Acid hydrolysis of Compound (I): The compound A (650 mg) was dissolved in ethanol (30ml) and refluxed with 20 ml of 10% H_2SO_4 on water bath for 72 hrs. 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 3, 5, 7, 4'-tetrahydroxy 3'-methoxy flavones 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)

Permethylation of Compound (I): Compound A (25 mg) was refluxed with MeI (5 ml) and Ag_2O (5 mg) in DMF (15 mg) for one day and then filtered. The filtrate was hydrolyzed with 10% ethanolic H_2SO_4 for 72 hrs to yield methylated aglycone identified as 3, 7-dihydroxy-5, 3', 4'-trimethoxy flavone and methylated sugars which were identified as 2,3,4-tri-O-methyl-L-rhamnose (R_G 1.07), 2,3,4,6-tetra-O-methyl-D-glactoes (R_G 1.02) and 2,4-di-O- methyl- D-arabinoes (R_G 0.67).

Enzymatic hydrolysis of Compound (I): The compound A (25 mg) was dissolved in MeOH (30 ml) and hydrolysed 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 two days and filtered to yield L-rhamnose (R_f 0.36) and proaglycone, confirming the presence of α-linkage between L-rhamnose and proaglycone.

Proaglycone on further hydrolysis with almond emulsin liberated D-glactoes (R_f 0.17) first, then D-arabinoes (R_f 0.27) and aglycone, which confirmed the presence of the β-linkage between D-glactoes and D-arabinoes as well as between D-arabinoes and aglycone.

Study of Compound A-1: It was crystalysed to acetone to yield 950 mg. It has m.p. 309-310°C, m.f. $C_{16}H_{12}O_7$, M^+ 316, (EIMS) Found (%) C, 63.57; H, 3.97, Calcd for m.f. $C_{16}H_{12}O_7$ C, 63.57; H, 3.97. UV: (MeOH) λ_{max} (nm) 255, 274, 362, 372; IR: ν^{KBr}_{max} (cm^{-1}); 3405, 2890, 1731, 1650, 1602, 1567, 1447, 1383, 1365, 1301, 1204, 1072, 1025 cm^{-1}. ^{1}H-NMR: (300 MHz, $CDCl_3$) δ (ppm); 10.40 (1H, s, 3-OH), 12.01 (1H, s, 5-OH), 9.79 (1H, s, 7-OH), 9.42 (1H, s, 3′-OH), 9.29 (1H, s, 4′-OH), 6.20 (1H, d, 2.2 Hz, H-6), 6.60 (1H, d, *J* 2.4 Hz, H-8), 7.62 (1H, d, *J* 2.1 Hz, H-2′), 6.82 (1H, d, *J* 8.4 Hz, H-5′), 7 (1H, d, *J* 8.0 Hz H-6′), ^{13}C-NMR: (300 MHz, $CDCl_3$) δ (ppm); 162.9 (C-2), 104.2 (C-3), 180.4 (C-4), 160.4 (C-5), 101.2 (C-6), 163.0 (C-7), 95.8 (C-8), 157.89 (C-9), 105.0 (C-10), 130.0 (C-1′), 117.2 (C-2′), 128.87 (C-3′), 132.5 (C-4′), 116.0 (C-5′), 124.0 (C-6′).

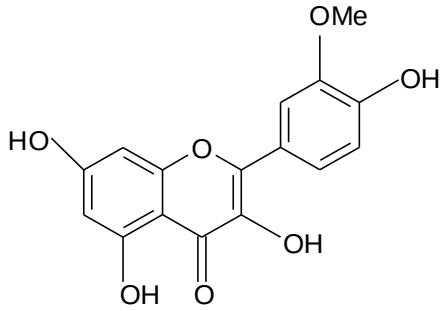

FIG. 2: COMPOUND (A-1)

Compound (II): It has m.p. 261-262°C, $[M]^+$ 448 (FABMS); found (%): C 54.95, H 4.47, calcd.for m. f. $C_{21}H_{20}O_{11}$ C 54.82, H 4.55; UV (MeOH) λ_{max} nm:, 256, 268, 351, (+$AlCl_3$) 277, 335, 418; (+$AlCl_3$–HCl) 265, 274, 311, 355; (+NaOMe) 267, 276, 403; (+NaOAc) 268, 275, 326; (+NaOAc–H_3BO_3) 265, 302, 374; IR (KBr) ν_{max} (cm^{-1}); 3420, 1658, 1610; ^{1}H-NMR: (300 MHz, DMSO-d_6) δ (ppm); 13.14 (1H, s, 5- OH), 9.14-10.79 (3H, s, 3′, 4′, 7′-OH), 7.50 (1H, d, *J* 2.2 Hz, H-2′,6′), 6.89 (1H, d, J 8.3 Hz, H-5′), 6.63 (1H, s, H-3), 6.54 (1H, s, H-6), 5.02 (1H, d, J 7.0 Hz)3.32-3.91 (sugar protons, m); ^{13}C-NMR: (300 MHz, DMSO-d_6) δ (ppm); 164.14 (C-2), 102.45 (C-3), 181.01 (C-4), 161.45 (C-5), 98.34 (C-6), 162.83 (C-7), 104.68 (C-8), 156.10 (C-9), 104.20 (C-10), 121.95 (C-1′),114.05 (C-2′), 145.93 (C-3′), 149.87 (C-4′), 115.75 (C-5′), 119.43 (C-6′), 73.49 (C-1"), 70.89 (C-2"), 78.86 (C-3"), 70. 82 (C-4"), 82.01 (C-5"), 61.72 (C-6"); (ESIMS): 448 [M] $^+$, 430 [M-H_2O] $^+$, 314 [M-$C_8H_6O_2$] $^+$, 299 [M-aglycone-CH_2] $^+$, 286 [M-162] $^+$, 134 [M-314]$^+$, 69 [MM-379] $^+$: Thus, it was identified as Lutexin by comparison of its spectral data with reported literature value.

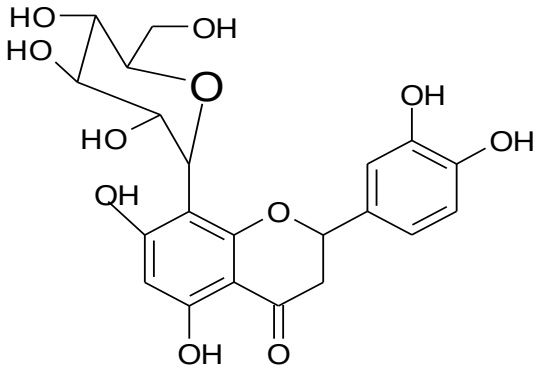

FIG. 3: COMPOUND (II)

Compound (III): It has m.p. 194-195°C, $[M]^+$ 344 (EIMS); Found (%): C 62.79, H 4.65, calcd. for m.f. $C_{18}H_{16}O_7$, C 62.56, H 4. 60; UV (MeOH) λ_{max} nm: 282, 325; ($AlCl_3$); 342, 376 IR (KBr) ν_{max} (cm^{-1});1690, 1595, 1351, 1203, 1115, 1026. ^{1}H-NMR: (300 MHz, $CDCl_3$) δ (ppm); 3.98 (3H, s, 6-OCH_3), 3.82 (3H, s, 7-OCH_3), 3.80 (3H, s, 3′-OCH_3), 7 (1H, s, 3-H), 6.86 (1H, s, 8-H), 7.62 (1H, s, 2′-H), 7.30 (1H, d, J 8.81 Hz, 5′-H), 7.65 (1H, d, J 8.84 Hz, 6′-H); ^{13}C-NMR: (300 MHz, $CDCl_3$) δ (ppm); 55.80 (3′-OCH_3), 56.20 (7-OCH_3), 60.39 (6-OCH_3), 164.95 (C-2), 103.91 (C-3), 183.31(C-4), 153.74 (C-5), 133.16 (C-6), 159.43 (C-7), 91.57 (C-8), 153.63 (C-9), 106.32 (C-10), 122.47 (C-1′), 112 (C-2′), 149.10 (C-3′), 152.65 (C-4′), 117.05 (C-5′), 121.41 (C-6′). Thus it was identified as Cirsilineol by comparison of its spectral data with reported literature value.

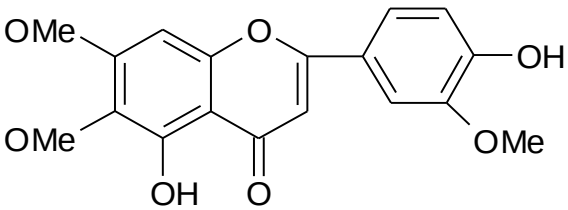

FIG. 4: COMPOUND (III)

Antiviral activity of Compound (I): Compound (I) was tested for antiviral activity against Japanese Encephalitis Virus *in vitro* (Vero cells) and result is given in following **Table 1**. Result shown in Table 1 showed that no antiviral activity was found.

TABLE 1: ANTIVIRAL ACTIVITY OF COMPOUND (I)

Code No.	Concentration µg/ml	% Antiviral Activity
Compound (I)	500-4	0

RESULTS AND DISCUSSION: The methanol soluble fraction of the leaves of the plant afforded a new compound (**I**) (**Fig. 1**), m.p. 246-248°C, m.f. $C_{33}H_{40}O_{20}$ $[M]^+$ 756 (FABMS). It responded Molisch and Shinoda tests [9] showing its flavonoidal glycosidic nature. Its IR spectrum showed absorption bands at 3346, 2923, 1653, 1625, 1245, 1063, 868, and 835 cm^{-1}. In ^1H NMR spectrum, a singlet at δ 12.24 confirmed the presence of -OH group at C-5 position. A singlet at δ 3.81 integrating three proton intently confirmed the presence of methoxy group at H-3' position. A doublet at δ 7.98 was assigned for H-2' and H-6'. A doublet at δ 6.95 was assigned for H-5'. A singlet at δ 10.16 was assigned for –OH group at C-4' position. The ortho coupled doublets at 6.18 (d, 1H, J 1.85 Hz) and 6.44 (d, 1H, J 1.85 Hz) were assigned at C-6 and C-8 positions respectively. The anomeric proton signals at δ 5.10 (1H, d, J 7.2 Hz), δ 5.12 (1H, d, J 7.5 Hz) and δ 5.36 (1H, d, J 1.5 Hz) were assigned to H-1'', H-1''' and H-1'''' of D-arabinose (R_f 0.19), D-galactose (R_f 0.25) and L-rhamnose ((R_f 0.37) respectively [10]. In ^{13}C-NMR spectrum, a singlet at δ 58.5 showed the presence of methoxy group at C-3' position.

Acid hydrolysis of compound (**I**) with 10% ethanolic H_2SO_4 yielded aglycone 1-A, m. p. 268-270°C, m.f. $C_{16}H_{12}O_7$, $[M]^+$ 316 (FABMS), identified as 3,5,7,4'-tetrahydroxy 3'-methoxy flavone (see ^{1}HNMR and ^{13}CNMR data in experimental section) [11-12]. The aqueous hydrolysate obtained after hydrolysis of the glycoside was neutralized with $BaCO_3$ and the $BaSO_4$ was filtered off. The filtrate was concentrated under reduced pressure and subjected to paper chromatography examination showed the presence of L-rhamnose (R_f 0.37), D-galactose (R_f 0.25) and L-arabinose (R_f 0.19). Quantitative estimation of sugars carried out by the procedure of Mishra and Rao, showed that all three sugars were present in equimolar ratio (1:1:1). Periodate oxidation of compound (**I**), confirmed that all the sugars were present in the pyranose form [13].

The position of sugar moiety (ies) in compound (**I**) was determined by permethylation [14] followed by acid hydrolysis yielded methylated aglycone identified as 3,7-dihydroxy-5, 3', 4'-trimethoxy flavone which confirmed that glycosidation was involved at C-3 –(OH) and C-7 –(OH) position of the alycone. The methylated sugars were identified as 2, 3, 4-tri-O-methyl-L-rhamnose (R_G 1.07), 2, 3, 4, 6- tetra-O-methyl-D-galactose (R_G 1.02) and 2, 4-di-O-methyl-D-arabinose (R_G 0.67) [15].

Therefore it was concluded that C-1'''-OH of D-galactose was linked with C-3'''-OH of D-arabinose and C-1''-OH of D-arabinose attached with –OH group at C-3 position of aglycone. The interglycosidic linkage (1→3) was found between D-galactose and L-arabinose [16].

Enzymatic hydrolysis of compound (**I)** with takadiastase liberated L-rhamnose and proaglycone confirming the presence of α-linkage between L-rhamnose and 3, 5, 7, 4'-tetra hydroxy 3'-methoxy flavone-3-O-β-D-glactopyranosyl (1→3)-O-β-D-arabino pyranoside as proaglycone. Proaglycone on further hydrolysis with almond emulsin liberated D-galactose first followed by D-arabinose and aglycone, confirming the presence of β-linkage between D-galactose and D-arabinose as well as between D-arabinose and aglycone. On the basis of above evidence, the compound A was characterized as 3, 5, 7, 4' tetrahydroxy-3'-methoxyflavone-3-O-β-D-galacto pyranosyl-(1→3)-O-β-D-arabinopyranosyl-7-O-α-L-rhamnopyranoside.

Compound (**I)** was tested for antiviral activity against Japanese Encephalitis Virus *in vitro* (Vero cells) but no antiviral activity was shown by compound (**I)**.

Compound (**II**) (**Fig. 3**), was analyzed for m. f. $C_{21}H_{20}O_{11}$, m. p. 261-262°C, $[M]^+$ 448 (EIMS) It was identified as Lutexin by comparison of its spectral data with reported literature values [17].

Compound (**III**) (**Fig. 4**), was analyzed for m. f. $C_{18}H_{16}O_7$, m. p. 194-195°C, $[M]^+$ 344 (EIMS). It was identified as Cirsilineol by comparison of its spectral data with reported literature values [18].

CONCLUSION: *Parthenium hysterophorus* L. belongs to composite family we have isolated a new allelochemical along with two known compound Lutexin, Cirsilineol from the stem of

Parthenium hysterophorus L . A new allechemical have show no antiviral activity. The secondary plant metabolites are called an allechemical, These chemicals include flavonoids, alkaloids, tannins etc.

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