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CHEMICAL EXAMINATION OF A NEW ALLELOCHEMICAL FROM STEMS OF *GLOSSOCARDIA BOSVALLIA* DC.

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ABSTRACT

Glossocardia bosvallia DC.[1-3] belongs to family Composite which is commonly known as "Patthar-suva or seri" in Hindi. It is found almost throughout in India and Deccan Peninsula. It is a small annual herb, 10-20 cm tall. The tribal inhabitants of western Maharashtra use a decoction of the plant as febrifuge. It has a bitter taste and fennel like odor. Besides serving as a medicinal plant Pithari plant is also used in culinary purposes. In the present paper, we report the isolation and structural elucidation of a new allelochemical 5,6,7,4′, tetrahydroxy 3-methoxy flavone -7-*O*-β-D xylopyranosyl (1→4)-O-β-D- glucopyranoside (A) which showed antiviral activity , alongwith two known compounds 6, 4′-dimethoxy-5, 7-dihydroxy-flavone (B) and Isoorientin (C) from methanolic extract of the stems of this plant.

INTRODUCTION: *Glossocardia bosvallia* DC. [1-3] belongs to family Composite which is commonly known as "Patthar-suva or seri" in Hindi. It is found almost throughout in India and Deccan Peninsula. It is a small annual herb, 10-20 cm tall. The plant is branched from the base and the branches are grooved. Alternately arranged leaves. Yellow flowers are borne in heads carried on slender stalks, in leaf axils or at the end of branches.

The tribal inhabitants of western Maharashtra use a decoction of the plant as febrifuge. A paste of the fresh Pithari plant is applied to promote healing of sores and wounds. It has a bitter taste and fennel like odor. Besides serving as a medicinal plant Pithari plant is also used in culinary purposes. Earlier workers [4-6] have reported various constituents from this plant. In the present paper, we report the isolation and structural elucidation of a new allelochemical 5, 6,7, 4′, tetrahydroxy-3-methoxyflavone-7-*O*-β-D-xylopyranosyl -(1→4)-O-β-D- glucopyranoside (A) (**Fig. 1**) which showed antiviral activity, alongwith two known compounds 6, 4′-dimethoxy-5, 7-dihydroxy-flavone (B) (**Fig. 3**) and Isoorientin (C) (**Fig. 4**) from methanolic extract of the stems of this plant.

EXPERIMENTAL SECTION:

General Experimental Procedure: All the melting points were determined on a thermoelectrically melting point apparatus and uncorrected. UV Spectra were determined in MeOH and Mass Spectra on a Jeol SX-102 (FABMS) mass spectrometer. IR Spectra were obtained on a Shimadzu FTIR-8400 spectrometer. ^{1}H-NMR Spectra were recorded at 300 MHz spectrometer in $CDCl_3$ using TMS as internal standard.

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^{13}C-NMR Spectra were recorded at 300 MHz spectrometer using $CDCl_3$. Thin Layer Chromatography on silica gel G and column chromatography on silica gel were used.

Plant Material: The stems of *Glossocardia bosvallia* DC. were collected around Sagar region and were taxonomically authenticated by the Department of Botany, Dr. H. S. Gour Central University, Sagar (M.P.), India.

Isolation of the Compound: Air dried and powdered stems (5 kg) of the plant were extracted with methanol in Soxhlet extractor for five days. The MeOH soluble fraction of the plant was concentrated under reduced pressure to yield a light brown viscous mass (2.35 gm) which was subjected to TLC examination showed three spots indicating it to be mixture of three compounds A, B and C. These compounds were separated and purified by column chromatography over silica gel-G using $CHCl_3$: MeOH in various proportions. After removal of the solvent and crystallization from ether above eluates yielded compound A (1.62 gm), compound B (0.67 gm) and C (0. 45 gm) respectively.

STUDY OF COMPOUND A: It had m. p. 219-220^{o}C, m. f. $C_{27}H_{32}O_{16}$, [M^+] 612 (FABMS) found (%); C 52.94 H 5.22, calcd for m.f. $C_{27}H_{32}O_{16}$ (%); C 53.02, H 5.07, UV: (MeOH) λ_{max} (nm) 287, 369, (+$AlCl_3$), 277, 324, 383, (+$AlCl_3$/HCl), 279, 315, 380; IR: ν_{max}^{KBr} (cm^{-1}); 3409, 2949, 1730, 1678, 1582, 1464, 1383, 1264, 1188, 1071, 843, 823cm^{-1}. ^{1}H-NMR: (300 MHz, $CDCl_3$) δ (ppm); 3.70 (3H, s, 3- OCH_3), 12.14 (1H, s, 5-OH), 4.24 (1H, s, 4'-OH), 6.74 (1H, d, *J* 2.3 Hz, H-8), 7.16 (2H, d, *J* 2.5 Hz, H-2′,6′), 6.79 (2H, d, *J* 8.3 Hz, H-5′,3′), 5.21 (1H, d, *J* 7.1 Hz, H-1''), 3.15-3.65 (4H, m, H-2'', H-3'', H- 4'', H-5''), 3.70 (1H, dd, *J* 11.90, 2.01 Hz, H-6''), 4.88 (1H, d, *J* 7.01 Hz, H-1'''), 2.98 (1H, m, H-2'''), 3.21 (1H, m, H-3'''), 3.25 (1H, m, H-4'''), 3.15 (2H, m, H-5'''); ^{13}C-NMR (75 MHz, $CDCl_3$), δ 162.6 (C-2), δ 107.6 (C-3), δ 175.9(C-4), δ 95.0 (C-5), δ 133.1 (C-6), δ 162.7 (C-7), δ 159.5 (C-8), δ 154.4 (C-9), δ 103.6 (C-10), δ 120.9 (C-1′), δ 128.6(C-2′), δ 115.3 (C-3′), δ 162.9 (C-4′), δ 117.0 (C-5′), δ 128.5 (C-6′), δ 102.01 (C-1′′), δ 84.6 (C-2′′), δ 78.5 (C-3′′), δ 70.8 (C-4′′), δ 76.2 (C-5′′), δ 65.3 (C-6′′), δ 105.9 (C-1′′′), δ 76.8 (C-2′′′), δ 78.2 (C-3′′′), δ 73.5 (C-4′′′), δ 75.3 (C-5′′′).

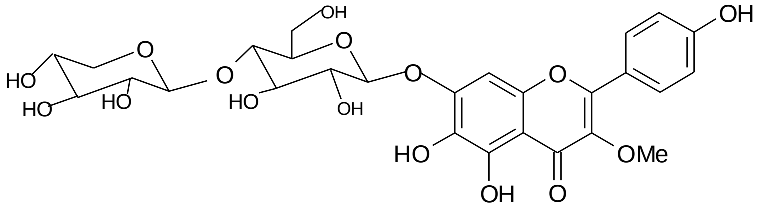


FIG. 1: COMPOUND A

Acid hydrolysis of Compound A: Compound A (75 mg) was dissolved in ethanol (20 ml) and refluxed with 10 ml of 10% H_2SO_4 on water bath for 7 hrs. The contents were 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 as solvent to give compound (A-1) which was identified as 5, 6, 7, 4′-tetrahydroxy 3- methoxy flavone. The aqueous hydrolysate obtained after hydrolysis was neutralized with $BaCO_3$ and the $BaSO_4$ was filtered off. The filtrate was concentrated and subjected to paper chromatography examination using nBAW (4:1:5) solvent system and sugars were identified as D-glucose (R_f 0.19) and D-xylose (R_f 0.26).

Permethylation of Compound A: Compound A (40 mg) was refluxed with MeI (5 ml) and Ag_2O (20 mg) in DMF (20 ml) for 4 hrs and then filtered. The filtrate was hydrolysed with 10% ethanolic H_2SO_4 for 6 hrs to give methylated aglycone, identified as 7-hydroxy- 5, 6, 4′-trimethoxy flavone and methylated sugars which were identified as 2, 3, 4-tri-O-methyl-D-xylose and 2, 3, 6-tri-O-methyl-D-glucose.

Enzymatic hydrolysis of Compound A: The compound A (25 mg) was dissolved in MeOH (10 ml) and hydrolysed with equal volume of almond emulsin. The reaction mixture was allowed to stay at room temperature for two days and filtered. The aglycone and hydrolysate were studied separately.

The hydrolysate was concentrated and subjected to paper chromatography examination using nBAW (4:1:5) as solvent systems and aniline hydrogen phthalate as spraying reagent, showed the presence of D-glucose (R_f 0.26) and D-xylose (R_f 0.29) and aglycone identified as 5, 6, 7, 4′ tetrahydroxy 3- methoxy flavone.

Study of Compound A-1: It had m. f. $C_{16}H_{12}O_7$, m. p. 265-266^{o}C, [M^+] 316 (FABMS), found (%); C 60.75, H. 3.79, calcd for m.f. $C_{16}H_{12}O_7$, (%) C 60.56, H 4.10. UV

(MeOH) λ_{max} ; 243, 291, 231, 345nm; NaOMe : 221, 314, 349, 387; $AlCl_3$: 291, 311, 343, 359, 369, 409; $AlCl_3$/HCl : 291, 301, 343, 395. ^{1}H-NMR (300 MHz, $CDCl_3$); δ 6.03 (1H, s, H-3), 6.78 (1H, s, H-8), 7.38 (2H, d, J 8.4 Hz, H-2′, 6′), 6.65 (2H, d, J 8.32, Hz, H-3′, 5′); ^{13}C-NMR (300 MHz, $CDCl_3$); δ 163 (C-2), δ 101 (C-3), δ 175.9(C-4), δ 157.1(C-5) δ 143.08 (C-6), δ 161.03 (C-7), δ 97.43 (C-8), δ 160 (C-9), δ 103.9 (C-10), δ 120.4 (C-1′), δ 128.6 (C-2′),δ 118.0 (C-3′), δ 154.0 (C-4′) , δ 116.1 (C-5′), δ 128.7 (C-6′). Thus, it was identified 5, 6, 7, 4′ - tetrahydroxy 3-methoxy flavone.

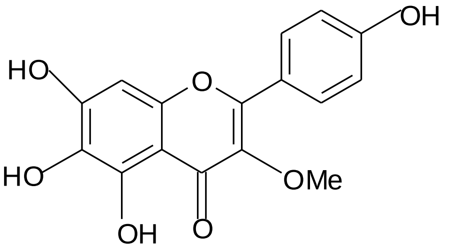
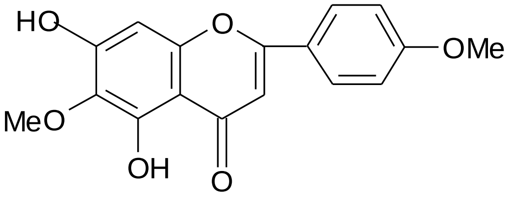


FIG. 2: COMPOUND A-1

Compound B: It had m. f. $C_{17}H_{14}O_6$, m. p. 265-266^{0}C, [M^+] 314 (FABMS), found (%); C 61.83, H. 4.26, calcd for m. f. $C_{17}H_{14}O_6$, (%) C 61.81, H 4.24. UV MeOH λ_{max} ; 280, 362, (+$AlCl_3$); 308, 352, (+$AlCl_3$ / HCl); 285, 355, (+NaOAc); 248, 286, (+NaOMe); 283, 334 nm. IR (KBr) ν_{max} ; 3250, 2955, 1714, 1635, 1600, 1540, 1428, 1370, 1255, 1082, 810 cm^{-1}. ^{1}H-NMR (300 MHz, $CDCl_3$); δ 7.24 (1H, s, H-3), 7.38 (2H, d, J 8.5 Hz, H-2′, 6′), 7.10 (2H, d, J 8.5, Hz, H-3′, 5′), 12.64 (s, OH-5), 3.90 (3H, s, OMe-6), 4.12 (3H, s, OMe-4′). ^{13}C-NMR (300 MHz, $CDCl_3$); δ 161.4 (C-2), 106.8 (C-3), 176.5(C-4), 158.2 (C-5), 130.6 (C-6), 162.3 (C-7), 94.6 (C-8), 154.5 (C-9), 103.9 (C-10), 120.4 (C-1′), 128.6 (C-2′), 117.0 (C-3′), 152.0 (C-4′), 116.1 (C-5′), 128.7(C-6′).Thus it was identified as6, 4′-dimethoxy-5, 7-dihydroxy-flavone.



COMPOUND B (FIG. 3)

Compound C: It had m. f. $C_{21}H_{20}O_{11}$, m. p. 245-246^{0}C, [M^+] 448 (FABMS), found (%); C 56.25, H. 4.46, calcd for m. f. $C_{21}H_{20}O_{11}$, (%) C 56.81, H 4.24. UV MeOH λ_{max} ; 252, 271, 345: IR (KBr) ν_{max} ; 3393 , 1652 , 1451 , 1267, 1087 cm^{-1}. 1H NMR (300MHz, CD_3OD): δ 6.55 (1H, *s*, H-3), 6.43 (1H, *s*, H-8), 7.31 (1H, *d*, *J* 1.2 Hz, H-2’), 7.01 (1H, *d*, *J* 8.89 Hz, H-5’), 7.30 (1H, *dd*, *J* 8.81 ,1.2 Hz, H-6’), , 4.88 (1H, *d*, *J* 9. 1 Hz, H-1’’), 4.15 (1H, *d*, *J* 9.23 Hz, H-2’’), 3.43 (3H, *m*, H-3’’, H-4’’, H-5’’), 3.72 (1H, *dd*, *J* 12.7 , 5.4 Hz, H-6’’); 13C NMR (300 MHz, CD_3OD): δ 164.1 (C-2), 102.7 (C-3), 182.1 (C-4), 160.2 (C-5), 107.0 (C-6), 162.7 (C-7), 94.2 (C-8), 155.2 (C-9), 103.5 (C-10), 121.0 (C-1’), 112.8 (C-2’), 145.0 (C-3’), 151.0 (C- 4’), 116.8 (C-5’), 122.9 (C-6’), 72.5 (C-1’’), 73.6 (C-2’’), 79.3 (C-3’’), 71.0 (C-4’’), 80.5 (C-5’’), 62.2 (C-6’’). Thus it was identify as Isoorientin.

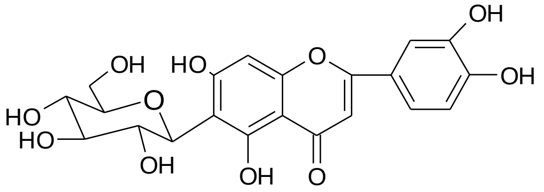


FIG. 4: COMPOUND C

RESULT AND DISCUSSION: Compound A (**Fig. 1**) had m.f. $C_{27}H_{32}O_{16}$, m.p. 219-220^{o}C, [M] $^+$ 612 (FABMS). It gave Molisch [7] and Shinoda test [8] showing its flavonoidal glycosidic nature. It also responded to neutral ferric chloride test. Its UV spectrum showed absorption band at 267 and 372 indicating it to be flavone. Its IR spectrum showed strong absorptions at 3446, 2890, 1655, 1585. Two bathochromic shifts of 25 nm and 45 nm in band I on addition of $AlCl_3$ and $AlCl_3$ + HCl relative to methanol confirmed the presence of -OH groups at C-5 and C-6 position [9-10].

A bathochromic shift of 16 nm in band I with NaOMe showed the presence of -OH group at C-4' position in compound A. In ^{1}H-NMR spectrum of compound A, two singlets at δ 7.16 and δ 6.79 were assigned to H-2′, 6′ and H-3′, 5′ respectively. A singlet at δ 3.70 confirmed the presence OMe group at C-3 position. The anomeric proton signals at δ 5.21 (1H, d, J 7.1 Hz) and δ 4.94 (1H, d, J 7.3 Hz) were assigned to H-1′′ of D-glucose H-1′′′ and D-xylose.

Acid hydrolysis of compound A with 10% ethanolic H_2SO_4 yielded aglycone **A-1** (FIG.2), m.p. 265-266^{o}C, m. f. $C_{16}H_{12}O_7$, [M^+] 316 (FABMS) which was identified as 5,6,7, 4′-tetrahydroxy- 3-methoxy flavone by comparison its spectral data with reported literature values [11]. The aqueous hydrolysate after the removal of aglycone, was neutralized with $BaCO_3$ and the $BaSO_4$ was filtered off.

The filtrate was concentrated and subjected to paper chromatography examination [12-13] and the sugars were identified as D-glucose (R_f 0.19) and D-xylose (R_f 0.26), (Co-PC, Co-TLC). Periodate oxidation of compound A confirmed that all sugars were present in the pyranose form [14].

The position of sugar moieties in the compound A were determined by permethylation[15] followed by acid hydrolysis which yielded methylated aglycone identified as 7-hydroxy- 5, 6, 4′-trimethoxy-flavone which confirmed that hydroxy group at C-7 position of the aglycone was involved in glycosidation. The methylated sugars which were identified as 2, 3, 6-tri-O-methyl-D-glucose and 2, 3,4- tri-O-methyl-D-xylose according to Petek [16] indicating that the C-1′′′ of D-xylose was linked to C-4′′ position of D-glucose, C-1′′ of D-glucose was attached to the C-7 position of aglycone and also showed the interlinkage (1→4) between D-xylose and D-glucose, which was further confirmed by their ^{13}C-NMR spectral data. (See in experimental section).

Enzymatic hydrolysis [17] of compound A with almond emulsin liberated D-xylose, first and D-glucose suggesting the presence of β-linkage between D-xylose and D-glucose and between D-glucose and aglycone. On the basis of above evidences, the structure of compound A was characterized as 5, 6, 7, 4′, tetrahydroxy 3-methoxy flavone -7-*O*-β-D xylopyranosyl (1→4)-O-β-D- glucopyranoside. Compound A was tested against *Japanese Encephalitis Virus* which showed 50% antiviral activity.

Compound B: It was crystallized for acetone to yield yellow powder mass. It was analyzed for m.p. 265-266°C, m. f. $C_{17}H_{14}O_6$, $[M^+]$ 314 (FABMS) which was identified as 6, 4′-dimethoxy-5, 7-dihydroxy-flavone by comparison its spectral data with reported literature values [18].

Compound C: It was crystallized for acetone to yielded yellow amorphous powder. It was analyzed for m. p. 244-246°C, m. f. $C_{21}H_{20}O_{11}$, M^+ 585 (EIMS) and identified as Luteolin 6-*C*-glucoside by comparison of its spectral data with reported literature values [19].

Antiviral Activity of Compound A: Compound A was tested for antiviral activity against ***Japanese Encephalitis* Virus** *in vitro* (Vero cells). The results recorded in the following **Table 1** suggests that compound A showed 50% antiviral activity. Thus compound A may be used as antiviral agent diseases caused by these viruses.

TABLE 1: ANTIVIRAL ACTIVITY

Code No.	Concentration µg/ml	% Antiviral Activity
Compound A	67.2	50%

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