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NEW POTENTIAL ALLELOCHEMICALS FROM AERIAL PARTS OF *INDIGOFERA TRIFOLIATA* LINN.

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ABSTRACT: Two new potential allelochemicals were isolated from ethanolic extract of the aerial parts of *Indigofera trifoliata* Linn. Along with three known compounds Tricin (**3**), Taraxerol (**4**) and 5-hydroxy-3, 4′, 7-trimethoxyflavone (**5**). The structures of new allelochemicals were characterized as 6, 3′, 4′-trihydroxy-5, 7, 8-trimethoxyflavanone-4′-O-α-L-rhamnopyranosyl-(1→4)-O-β-D-xylopyranosyl-(1→3)-O-β-D-galacto pyranoside (**1**) and 3, 5, 7-trihydroxy-3′,4′-dimethoxyflavone-3-O-β-D-galactopyranosyl-(1→4)-O-β-D-arabinopyranosyl-7-O-α-L-rhamno pyranoside (**2**) by various color reactions, spectral analysis and chemical degradations. Allelo chemicals can be defined as secondary plant metabolites, produced by plants, algae, bacteria, fungi and viruses that influence the growth and development of agricultural and biological systems (excluding animals).

INTRODUCTION: *Indigofera trifoliata* Linn. [1-3] belongs to family Leguminosae. It is commonly known as "Ganglimethi" in Hindi. It is found throughout India, Ceylon-Java. The seeds of this plant are used with other mucilaginous drugs as a restorative. Its Seeds are used in rheumatism and leucorrhoea. Earlier workers [4-6] have reported various chemical constituents from this plant. In present paper we report the isolation and structural elucidation of two new allelochemicals 6, 3′, 4′-trihydroxy-5,7,8-trimethoxyflavanone-4′-O-α-L-rhamnopyranosyl-(1→4)-O-β-D-xylopyranosyl-(1→3)-O-β-D-galactopyranoside (**1**) and 3, 5, 7-trihydroxy-3′, 4′-dimethoxyflavone-3-O-β-D-galactopyranosyl-(1→4)-O-β-D-arabinopyranosyl-7-O-α-L-rhamno pyranoside (**2**) from ethanolic extract of the aerial parts of this plant.

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EXPERIMENTAL SECTION:

General procedure: All of the melting points were determined on a thermoelectrical melting point apparatus and are uncorrected.

The IR spectra were recorded in KBr disc on Perkin Elimer spectrum RX1 (4000-450 cm^{-1}). ^{1}H NMR and ^{13}C NMR spectra were recorded at 90 MHz using solvent DMSO-d_6 and TMS as internal standard on Bruker DRX-300 spectrometer.

UV spectra were recorded in MeOH (Shimadzu UV 1800 spectrophotometer) and mass spectra on a Jeol SX- 300 mass spectrometer.

Plant material: The aerial parts of the plant 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 aerial parts (3.5kg) of the plant were extracted with ethanol in a Soxhlet apparatus for 74 hr. The ethanolic extract of aerial parts of the plant was concentrated under reduced pressure and successively partitioned with chloroform, ethyl acetate and acetone.

The methanol soluble fraction was further concentrated under reduced pressure to yield brown viscous mass (2.65gm), which was subjected to TLC examination using nBAW (4:1:5) as solvent and I_2 vapors as visualizing agent. It gave five spots indicating, it to be mixture of five compounds **1**, **2, 3, 4** and **5**. These compounds were separated by TLC and purified by column chromatography over silica gel using $CHCl_3$: MeOH (4:8) as eluent and studied separately.

Study of compound (1): It was crystalysed from acetone to yield 1.35g. It had m.p. 148-150°C, m.f. $C_{35}H_{46}O_{21}$, $[M]^+$ 802 (FABMS); found (%): C 52.20, H 5.16, calcd.(%) for m.f. $C_{35}H_{46}O_{21}$: C 52.37, H 5.74; UV λ_{max} MeOH (nm) : 286, 326; IR (KBr) ν^{max} (cm^{-1}), 3410, 2968, 2950, 2832, 1678, 1592, 1482, 1460, 1424; ^{1}H NMR (90 MHz, DMSO-d_6), δ (ppm): 5.38 (1H, dd, *J* = 13.1, 3.2 Hz, H-2), 2.82 (1H, dd, *J* = 16.6, 3.1 Hz, H-3a), 3.01 (1H, dd, *J* = 16.6, 13.2 Hz, H-3b), 5.52 (1H, s, 6-OH), 3.90 (3H, s, 5-OCH_3), 4.09 (3H, s, 7-OCH_3), 3.85 (3H, s, 8-OCH_3), 7.42 (2H, dd, *J*=7.3, 1.5 Hz, H-2′, H-6′), 7.37 (1H, d, *J*=7.3 Hz, H-5′), 8.91 (1H, s, 3′-OH), 9.0 (1H, s, 4′-OH), 5.06 (1H, d, *J* =7.7 Hz, H-1″), 3.32-3.73 (4H, m, H-2″, H-3″, H-4″, H-5″), 3.82 (1H, dd, *J* =11.2, 7.1 Hz, H-6a″), 4.23 (1H, dd, *J* =11.2, 4.2 Hz, H-6b″), 4.74 (1H, d, *J* = 7.3 Hz, H-1‴), 3.20-3.34 (4H, m, H-2‴, H-3‴, H-4‴, H-5‴), 5.18 (1H, d, *J* = 3.6 Hz, H-1⁗), 4.21-4.82 (4H, m, H-2⁗, H-3⁗, H-4⁗, H-5⁗), 1.37 (3H, d, *J* = 5.8, CH_3-6⁗). ^{13}C NMR (90 MHz, DMSO-d_6), δ (ppm): 80.1 (C-2), 44.8 (C-3), 190.4 (C-4), 147.8 (C-5), 136.5 (C-6), 142.3 (C-7), 137.5 (C-8), 149.6 (C-9), 110.4 (C-10), 132.6 (C-1′), 114.2 (C-2′), 146.1 (C-3′), 145.6 (C-4′), 116.2 (C-5′), 117.9 (C-6′), 61.8 (5-OCH_3), 62.3 (7-OCH_3), 61.2 (8-OCH_3), 104.6 (C-1″), 78.4 (C-2″), 73.0 (C-3″), 71.6 (C-4″), 75.4 (C-5″), 61.6 (C-6″), 103.2 (C-1‴), 76.5 (C-2‴), 73.6 (C-3‴), 69.1(C-4‴), 66.6(C-5‴), 100.4 (C-1⁗), 70.6 (C-2⁗), 71.5 (C-3⁗), 72.5 (C-4⁗), 67.3 (C-5⁗), 17.8 (C-6⁗) and $[M]^+$ 802 (FABMS).

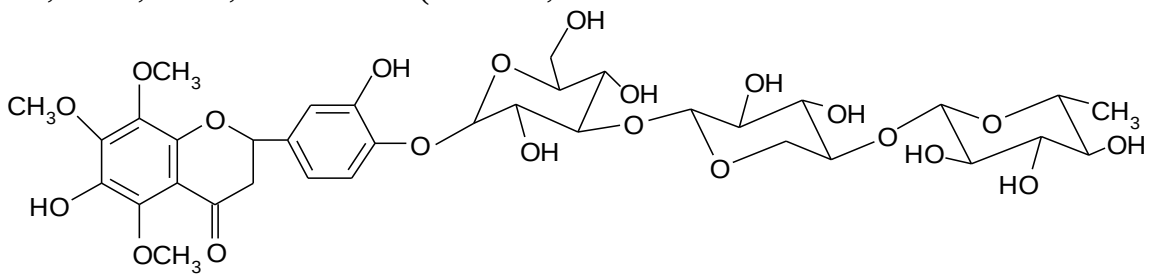

FIG. 1: COMPOUND (1)

Acid hydrolysis of compound (1): Compound **1** (400 mg) was dissolved in ethanol (15 ml) and refluxed with 20 ml of H_2SO_4 on water bath for 7-8 hr. 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 (3:6) to give compound **1-A**, identified as 6, 3′, 4′-trihydroxy-5, 7, 8-trimethoxy flavanone. The aqueous hydrolysate 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 and aniline hydrogen phthalate as spraying reagent, showed the presence of D-galactose (R_f 0.17), D-xylose (R_f 0.29) and L-rhamnose (R_f 0.36) (Co-PC).

Permethylation of compound (1): Compound **1** (35 mg) was refluxed with MeI (10 ml) and Ag_2O (20 ml) in DMF (25 mg) for two days and then filtered. The filtrate was hydrolyzed with 10% ethanolic H_2SO_4 for 6-7 hr, to give methylated aglycone, identified as 4'-hydroxy-5, 6, 7, 8, 3'-pentamethoxy flavanone and methylated sugars, which were identified as 2, 3, 4-tri-O-methyl-L-rhamnose (R_G 1.03), 2, 3-di-O-methyl-D-xylose (R_G 0.75) and 2, 4, 6- tri-O-methyl-D-galactose (R_G 0.68).

Enzymatic hydrolysis of compound (1): Compound **1** (40 mg) was dissolved in MeOH (20 ml) and hydrolyzed with equal volume of takadiastase enzyme. The reaction mixture was allowed to stay at room temperature for two days and filtered. The proaglycone and hydrolysate were studied separately. 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) (Co-PC). The proaglycone was dissolved in MeOH (25 ml) and further hydrolyzed with equal volume of almond emulsin enzyme at room temperature as usual procedure yielded aglycone, identified as 6, 3', 4'-trihydroxy-5, 7, 8-trimethoxy flavanone and sugars were identified as D-xylose (R_f 0.29) and D-galactose (R_f 0.17)(Co-PC).

Study of compound (1-A): It had m.f. $C_{18}H_{18}O_8$, m.p. 175-177°C, $[M]^+$ 362 (EIMS); found (%): C 59.48, H 4.73, calcd. (%) for m.f. $C_{18}H_{18}O_8$, C 59.67, H 4.97; UV: λ_{max} (nm): (MeOH) 282, 356; IR (KBr) ν^{max} (cm^{-1}): 3404, 2962, 2946, 2835, 1676, 1598, 1486, 1464, 1428; ^{1}H NMR (90 MHz, DMSO-d_6), δ (ppm): 5.40 (1H, dd, *J*=13.2, 3.0 Hz, H-2), 2.85 (1H, dd, *J*=16.7, 3.2 Hz, H-3a), 3.04 (1H, dd, *J*=16.7, 13.2 Hz, H-3b), 5.56 (1H, s, 6-OH), 3.94 (3H, s, 5-OCH_3), 4.12 (3H, s, 7-OCH_3), 3.89 (3H, s, 8-OCH_3), 7.46 (2H, dd, *J*=7.4, 1.6 Hz, H-2′, H-6′), 7.40 (2H, d, *J*=7.2 Hz, H-5′), 8.94 (1H, s, 3′-OH), 9.03 (1H, s, 4′-OH). ^{13}C NMR (90 MHz, DMSO-d_6), δ (ppm): 79.5 (C-2), 45.5 (C-3), 189.6 (C-4), 147.6 (C-5), 136.7 (C-6), 142.5 (C-7), 137.3 (C-8), 149.8 (C-9), 110.7 (C-10), 129.8 (C-1′), 114.6 (C-2′), 145.6 (C-3′), 145.9 (C-4′), 115.7 (C-5′), 117.6 (C-6′), 61.5 (5-OCH_3), 62.0 (7-OCH_3), 61.7 (8-OCH_3).

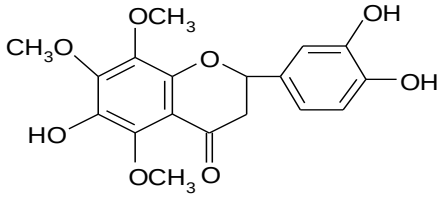

FIG. 2: COMPOUND (1-A)

Study of compound (2): It was crystalysed from acetone to yield 1.30 g. It had m.p. 256-258°C, m.f. $C_{34}H_{42}O_{20}$, $[M]^+$ 770 (FABMS); found (%): C 52.36, H 5.24, calcd.(%) for m.f. $C_{34}H_{42}O_{20}$:C 52.99, H 5.45; UV: λ_{max} (nm): (MeOH) 252, 365; (+$AlCl_3$) 262, 424; (+$AlCl_3$ / HCl) 250, 364; (+NaOAc) 276, 380; IR: (KBr) ν^{max} (cm^{-1}): 3448, 3018, 2884, 1650, 1619, 1268 and 850; ^{1}H NMR (90 MHz, DMSO-d_6), δ_H (ppm); 6.14 (1H, d, *J*=1.6 Hz, H-6), 6.50 (1H, d, *J*=1.8 Hz, H-8), 9.31 (1H, s, 3-OH), 12.97 (1H, s, 5-OH), 10.80 (1H, s, 7-OH), 7.54 (1H, d, *J*=2.1 Hz, H-2′), 7.65 (1H, dd, *J*=7.6, 2.0 Hz, H-6′), 3.81 (6H, s, 3′-OCH_3, 4′-OCH_3), 6.95 (1H, d, *J*=7.7 Hz, H-5′), 3.92 (1H, d, *J*=6.82 Hz, H-1″), 3.10-3.53 (4H, m, H-2″, H-3″, H-4″, H-5″), 5.41 (1H, d, *J*=7.6 Hz, H-1‴), 3.21-3.78 (5H, m, H-2‴, H-3‴, H-4‴, H-5‴, H-6‴), 5.53 (1H, s, H-1⁗), 3.1-5.4 (4H, m, H-2⁗, H-3⁗, H-4⁗, H-5⁗), 1.14 (3H, d, *J*=6.2 Hz, CH_3 -6⁗). ^{13}C NMR (90 MHz, DMSO-d_6), δ_C (ppm): 173.8 (C-2), 140.4 (C-3), 183.7 (C-4), 163.2 (C-5), 100.3 (C-6), 162.7 (C-7), 95.1 (C-8), 158.2 (C-9), 104.5 (C-10), 126.8 (C-1′), 113.2 (C-2′), 150.4 (C-3′) , 149.4 (C-4′),111.6 (C-5′), 124.48 (C-6′), 56.9 (3′-OCH_3, 4′-OCH_3), 102.56 (C-1″), 72.38 (C-2″), 70.34 (C-3″), 67.14 (C-4″), 64.72 (C-5″), 104.5 (C-1‴), 75.36 (C-2‴), 76.92 (C-3‴), 71.24 (C-4‴), 77.84 (C-5‴), 62.58 (C-6‴), 99.8 (C-1⁗), 70.2 (C-2⁗), 70.5 (C-3⁗), 71.6 (C-4⁗), 69.8 (C-5⁗), 18.3 (C-6⁗) and $[M]^+$ 770 (FABMS).

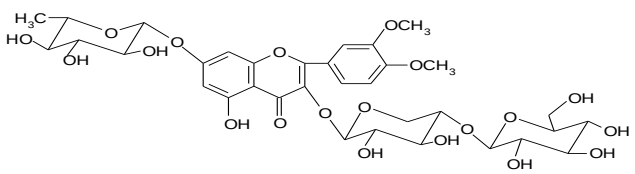

FIG. 3: COMPOUND (2)

Acid hydrolysis of compound (2): Compound **2** (300 mg) was dissolved in ethanol (15 ml) and refluxed with 25 ml of H_2SO_4 on water bath for 7-8 hr. 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 (3:6) to give compound **2-A**, identified as 3, 5, 7-trihydroxy-3′, 4′-dimethoxy flavone by comparison of its spectral data with reported literature values. The aqueous hydrolysate was neutralized with $BaCO_3$ and the $BaSO_4$ 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), D-galactose (R_f 0.17) and d-arabinose (R_f 0.22) (Co-PC).

Permethylation of compound (2): Compound **2** (35 mg) was refluxed with MeI (10ml) and Ag_2O (20 ml) in DMF (25 mg) for two days and then filtered. The filtrate was hydrolyzed with 10% ethanolic H_2SO_4 for 6-7 hr to give 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 1.02), 2, 3,4, 6 -tetra-O-methyl-D-galactose (R_G 0.89) and 2, 3-di-O-methyl-D-arabinose (R_G 0.63).

Enzymatic hydrolysis of compound (2): Compound **2** (25 mg) was dissolved in MeOH (20 ml) and hydrolyzed with equal volume of takadiastase enzyme. The reaction mixture was allowed to stay at room temperature for 48 hr and filtered. The proaglycone and hydrolysate were studied separately. 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) (Co-PC). The proaglycone was dissolved in MeOH (25 ml) and further hydrolyzed with equal volume of almond emulsin enzyme at room temperature as usual procedure yielded aglycone, identified as 3, 5, 7-trihydroxy-3′, 4'-dimethoxyflavone and sugars were identified as D-galactose (R_f 0.17) and D-arabinose (R_f 0.22) (Co-PC).

Study of compound (2-A): It has m.f. $C_{17}H_{14}O_7$, m.p. 289-291°C, $[M]^+$ 330 (EIMS); found(%): C 61.34, H 4.12, calcd (%) for m.f. $C_{17}H_{14}O_7$, C 61.82, H 4.24; UV: λ_{max} (nm): (MeOH) 256, 368; (+$AlCl_3$**)** 265, 422; **(**+$AlCl_3$ / HCl) 258, 368; (+NaOAc) 278, 384; IR (KBr) ν^{max} (cm^{-1}): 3454, 3015, 2887, 1654, 1616, 1264 and 846; ^{1}H NMR (90 MHz, DMSO-d_6), δ_H (ppm): 6.17 (1H, d, *J*=1.5 Hz, H-6), 6.53 (1H, d, *J*=1.7 Hz, H-8), 9.34 (1H, s, 3-OH), 12.94 (1H, s, 5-OH), 10.83 (1H, s, 7-OH), 7.56 (1H, d, *J* 2.1 Hz, H-2′), 7.62 (1H, dd, *J* 7.7, 2.1 Hz, H-6′), 3.84 (6H, s, 3′-OCH_3, 4′-OCH_3), 6.98 (1H, d, *J* 7.8 Hz, H-5′). ^{13}C NMR (90 MHz, DMSO-d_6), δ_C (ppm): 164.3 (C-2), 139.6 (C-3), 181.9 (C-4), 161.6 (C-5), 98.5 (C-6), 163.3 (C-7), 94.3 (C-8), 157.5 (C-9), 103.4 (C-10), 126.6 (C-1′), 113.4 (C-2′), 150.6 (C-3′) , 149.7 (C-4′),111.9 (C-5′), 124.5 (C-6′), 56.6 (3′-OCH_3, 4′-OCH_3).

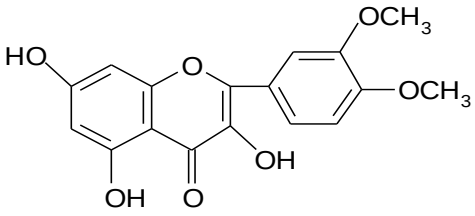

FIG. 4: COMPOUND (2-A)

Antimicrobial activity of compounds 1 and 2: The antibacterial activity of compounds (**1**) and (**2**) was determined by Filter Paper Disc Diffusion method[25]. The various Gram (+ve) and Gram (-ve) bacterial species were first incubated at 48°C for 42 hr. The sterile filter paper discs (6 mm) were soaked with standard antibacterial agent and various test samples and were dried at 50°C. The discs were then placed on soft nutrient agar (2%) petri plates previously seeded with suspension of each bacterial species. The diameters of zone of inhibition were measured at 35±1°C after 24 hr. The results are recorded in **Table 1**.

The antifungal activity of compounds was measured by PDA (Potato Dextrose Agar) with 4% agar for the preparation of plates and incubated with spores and mycelium suspension of fungi obtained from one week old culture. The diameters of zone of inhibition were measured at 26±1°C after 46 hr. The results are recorded in **Table 2**.

TABLE 1: ANTIBACTERIAL ACTIVITY OF COMPOUND 1 AND 2.

Sl. No.	Bacterial species	Diameters of zone of inhibition (mm)*								Std,**
		Concentration of compound 1 (%)				Concentration of compound 2 (%)				
		100	80	60	40	100	80	60	40	
1.	*Escherichia coli*	-	-	-	-	-	-	4.58	-	11.25
2.	*Staphylococcus aureus*	-	7.55	-	-	-	-	-	-	34.33
3.	*Bacilius subtilis*	-	-	-	-	-	-	-	5.64	30.25
4.	*Micrococcus luteus*	-	-	-	-	-	-	-	-	10.5

*The zone of inhibition (mm) taken as average of four determination direction. **Ampicillin (10 mg/mL) used as standard antibacterial agent.

TABLE 2: ANTIFUNGAL ACTIVITY OF COMPOUND 1 AND 2

Sl. No.	Fungal species	Diameters of zone of inhibition (mm)*								
		Concentration of compound 1 (%)				Concentration of compound 2 (%)				Std.***
		100	80	60	40	100	80	60	40	
1.	*Candida albicans*	-	5.66	-	-	-	-	-	-	13.00
2.	*Rhizopus oryzae*	-	-	-	-	-	-	-	-	32.25
3.	*Aspergillus niger*	-	-	-	-	-	-	-	4.57	13.75
4.	*Mucor indicus*	-	-	-	-	-	-	3.84	-	10.00

*The zone of inhibition (mm) taken as average of four determination direction. ***Ketocozole (100 mg/mL) used as standard antifungal agent.

Compound (3): It was crystalysed from acetone to yield 950 mg. It had m.f. $C_{17}H_{14}O_7$, m.p. 283-285°C, $[M]^+$ 330 (EIMS); found(%), C 61.56, H 4.53, calcd (%) for m.f. $C_{17}H_{14}O_7$, C 61.82, H 4.24; UV: λ_{max} (nm): (MeOH) 266, 356; IR (KBr) ν^{max} (cm^{-1}): 3413, 2944, 2842, 2364, 1662, 1614, 1510, 1465, 1260, 1116, 1032, 834. ^{1}H NMR (90 MHz, DMSO-d_6), δ (ppm): 6.95 (1H, s, H-3), 6.21 (1H, d, *J*=2.1 Hz, H-6), 6.58 (1H, d, *J*=2.1 Hz, H-8), 12.93 (1H, s, 5-OH), 10.84 (1H, s, 7-OH), 7.36 (2H, s, H-2′, H-6′), 9.34 (1H, s, 4′-OH), 3.85 (6H, s, 3′-OCH_3, 5′-OCH_3). ^{13}C NMR (90 MHz, DMSO-d_6), δ (ppm): 164.8 (C-2), 104.3 (C-3), 182.7 (C-4), 157.5 (C-5), 99.7 (C-6), 164.5 (C-7), 94.4 (C-8), 161.5 (C-9), 104.6 (C-10), 120.6 (C-1′), 104.4 (C-2′, C-6′), 148.3 (C-3′, C-5′), 140.7 (C-4′), 56.5 (OCH_3-3′, 5′).

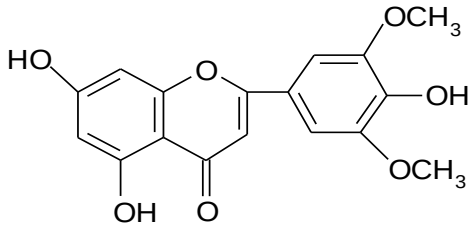

FIG. 5: COMPOUND (3)

Compound (4): It was crystalysed from acetone to yield 1.10 gm. It had m.f. $C_{30}H_{50}O$, m.p. 280-282°C, $[M]^+$ 426 (EIMS); found (%), C 84.20, H 11.25, calcd (%) for m.f. $C_{30}H_{50}O$, C 84.51, H 11.74; IR (KBr) ν^{max} (cm^{-1}): 3482, 3055, 2994, 2935, 2858, 1643, 1474, 1383, 1378; ^{1}H NMR (90 MHz, DMSO-d_6), δ (ppm): 5.55 (1H, dd, *J* =8.1, 3.1 Hz, H-15), 3.26 (1H, dd, *J*=11.2, 4.8 Hz, H-3), 2.06 (1H, dd, *J*=12.7, 3.2 Hz, H-7a), 1.95 (1H, dd, *J*=14.8, 3.2 Hz, H-16a), 0.95 (3H, s, H-23), 0.83 (3H, s, H-24), 0.91 (3H, s, H-25), 1.07 (3H, s, H-26), 0.94 (3H, s, H-27), 0.85 (3H, s, H-28), 0.92 (3H, s, H-29), 0.87 (3H, s, H-30).^{13}C NMR (90 MHz, DMSO-d_6) : δ (ppm): 38.3 (C-1), 27.4 (C-2), 79.3 (C-3), 39.2 (C-4), 55.8 (C-5), 18.6 (C-6), 35.4 (C-7), 38.3 (C-8), 48.9 (C-9), 37.8 (C-10), 17.2 (C-11), 35.6 (C-12), 37.9 (C-13), 158.4 (C-14), 116.6 (C-15), 36.9 (C-16), 37.4 (C-17), 49.5 (C-18), 41.6 (C-19), 28.5 (C-20), 33.9 (C-21), 33.3 (C-22), 28.3 (C-23), 15.7(C-24), 15.7 (C-25), 29.6 (C-26), 25.7 (C-27), 30.1 (C-28), 33.6 (C-29), 21.7 (C-30).

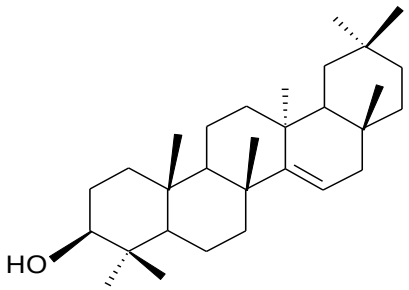

FIG. 6: COMPOUND (4)

Compound (5): It was crystalysed from acetone to yield 750mg. It has m.f. $C_{18}H_{16}O_6$, m.p. 146-148°C, $[M]^+$ 328 (EIMS); found (%), C 65.16, H 4.80, calcd (%) for m.f. $C_{18}H_{16}O_6$, C 65.85, H 4.88; UV: λ_{max} (nm): (MeOH) 276, 358; (+$AlCl_3$) 278, 348; (+$AlCl_3$ / HCl) 277, 352; (+NaOAc) 272, 344; ^{1}H NMR (90 MHz, DMSO-d_6), δ (ppm): 6.28 (1H, d, *J*=2.1 Hz, H-6), 6.45 (1H, d, *J*=2.1 Hz, H-8), 12.57 (1H, s, 5-OH), 8.27 (2H, d, *J*=8.8 Hz, H-2′, H-6′), 7.06 (2H, d, *J*=8.8 Hz, H-3′,H-5′), 3.85 (1H, s, 3-OCH_3), 3.94 (1H, s, 7-OCH_3), 3.82 (1H, s, 4′-OCH_3). ^{13}C NMR (90 MHz, DMSO-d_6), δ (ppm): 156.5 (C-2), 139.4 (C-3), 179.3 (C-4), 161.7 (C-5), 98.4 (C-6), 165.2 (C-7), 92.8 (C-8), 157.3 (C-9), 106.7 (C-10), 123.4 (C-1′), 130.7 (C-2′, C-6′), 114.7 (C-3′, C-5′), 162.6 (C-4′), 60.3 (3-OCH_3), 56.4 (7-OCH_3), 55.9 (4′-OCH_3).

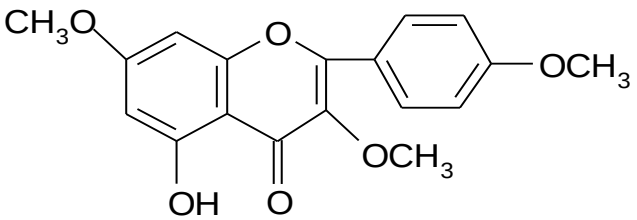

FIG. 7: COMPOUND (5)

RESULTS AND DISCUSSIONS: Compound (**1**) (**FIG.1**) had m. f. $C_{35}H_{46}O_{21}$, m.p. 148-150°C, $[M]^+$ 802 (FABMS). It gave Molisch's and Shinoda tests [7] suggesting its flavonoidal glycosidic nature. Its IR spectra showed strong absorption bands at 3410, 2968, 2950, 2832, 1678, 1592, 1482, 1460 and 1424 cm^{-1}. In UV spectrum two bands at 286 nm and 326 nm showed its flavonoidal skeleton. In ^{1}H NMR spectrum, a singlet at δ 5.52 showed the presence of –OH group at C-6 position. Three singlets at δ 3.90, 4.09 and 3.85 confirmed the presence of –OMe groups at C-5, C-7 and C-8 positions. Two singlets at δ 8.91 and 9.0 confirmed the presence of –OH groups at C-3' and C-4' positions.

In ^{1}H NMR spectrum, a double doublet at δ 5.38 (1H, dd, *J* = 13.1, 3.2 Hz) was assigned to H-2. Two doublets at δ 2.82 (1H, dd, *J* = 16.6, 3.1 Hz) and 3.01 (1H, dd, *J*= 16.6, 13.2 Hz) were assigned to H-2, H-3a and H-3b of **C** ring in the compound (**1**). A double doublet at δ 7.42 (2H, dd, *J* = 7.3, 1.5 Hz) was assigned to H-2' and H-6'. A doublet at δ 7.37 (1H, d, *J* = 7.3 Hz) was assigned to H-5' in ring **B**. The anomeric proton signals at δ 5.06 (1H, d, *J* = 7.7 Hz), 4.74 (1H, d, *J* = 7.3 Hz) and 5.18 (1H, d, *J* = 3.6 Hz) were assigned for H-1", H-1"' and H-1"" for D-galactose, D-xylose and L-rhamnose respectively.

In the mass spectrum of the compound (**1**), characteristic ion peaks at *m/z* 802 $[M]^+$, 656 [M^+-L-rhamnose], 524 [M^+-D-xylose] and 362 [M^+-D-galactose, aglycone] were found by subsequent losses from the molecular ion of each molecule of L-rhamnose, D-xylose and D-galactose showing L-rhamnose as terminal sugar, D-xylose as middle sugar and D-galactose was directly attached to –OH group at C-4' position of aglycone.

Acid hydrolysis of compound (**1**) with ethanol and 10% H_2SO_4 gave aglycone (**1-A**), m.p. 175-177°C, m.f. $C_{18}H_{18}O_8$, $[M]^+$ 362 (EIMS) and sugar moiety(ies). These were separated and studied separately. The aglycone (**1-A**) (**Fig. 2**) was identified as 6, 3', 4'-trihydroxy-5, 7, 8-trimethoxy flavanone (See details in experimental section).The aqueous hydrolysate after the removal of aglycone was neutralized with $BaCO_3$ and $BaSO_4$ filtered off.

The filtrate was concentrated and subjected to paper chromatography examination and sugars were identified as L-rhamnose (R_f 0.36), D-xylose (R_f 0.29) and D-galactose (R_f 0.17) (Co-PC) [8]. Periodate oxidation of compound (**1**), confirmed that all the sugars were present in the pyranose form [9].

The positions of sugar moieties in compound (**1**) were determined by permethylation[10] followed by acid hydrolysis, yielded methylated aglycone identified as 4′-hydroxy-5, 6, 7, 8, 3′-pentamethoxy flavanone showed that glycosylation was involved at C-4′ position of the flavanone and methylated sugars were identified as 2, 3, 4 -tri-O-methyl-L-rhamnose (R_G 1.03), 2, 3-di-O-methyl-D-xylose (R_G 0.75) and 2, 4, 6-tri-O-methyl-D-galactose (R_G 0.68) indicating that C-1""-OH of L-rhamnose was linked to C-4"'-OH of D-xylose, C-1"'-OH of D-xylose was attached to C-3"-OH of D-galactose and C-1"-OH of D-galactose was attached with C-4' position of the aglycone. Therefore it was concluded that interlinkages (1→4) between L-rhamnose and D-xylose and (1→3) between D-xylose and D-galactose were found which were further confirmed by ^{13}C NMR spectra. (See in experimental section)

Enzymatic hydrolysis of compound (**1**) with takadiastase enzyme liberated L-rhamnose (R_f 0.36) and proaglycone identified as 6, 3′, 4′-trihydroxy-5, 7, 8-trimethoxyflavanone-4′-O-β-D-xylopyranosyl-(1→3)-O-β-D-galactopyranoside showed the presence of α-linkage between L-rhamnose and proaglycone. Proaglycone on further hydrolysis with almond emulsin enzyme liberated D-xylose (R_f 0.29) followed by D- galactose (R_f 0.17) and aglycone suggesting the presence of β-linkage between D-xylose and D-galactose as well as D-galactose and aglycone.

On the basis of above deleberations, the structure of compound (**1**) was characterized as 6, 3′, 4′-trihydroxy-5, 7, 8-trimethoxyflavanone-4′-O-α-L-rhamnopyranosyl-(1→4)-O-β-D-xylopyranosyl-(1→3)-O-β-D-galactopyranoside.

Compound (**2**) (**Fig. 3**) had m. f. $C_{34}H_{42}O_{20}$, m.p. 256-258°C, $[M]^+$ 770 (FABMS). It gave Molisch's and Shinoda tests [7] showing its flavonoidal glycosidic nature.

Its IR spectra showed strong absorption bands at 3448, 3018, 2884, 1650, 1619, 1268 and 850 cm^{-1}. In UV spectrum two bands at 252 nm and 365 nm showed its flavonoidal skeleton. In ^1H NMR spectrum three singlets at δ 9.31, 12.97 and 10.80 confirmed the presence of – OH groups at C-3, C-5 and C-7 positions. Two singlets at δ 3.81 confirmed the presence of – OMe groups at C-3′ and C-4' positions. In ^1H NMR spectrum, doublets at δ 6.14 (1H, d, *J* = 1.6 Hz) and 6.50 (1H, d, *J* = 1.8 Hz) were assigned to H-6 and H-8 of **A** ring in the compound (**2**).

A doublet at δ 7.54 (1H, d, *J* = 2.1 Hz) was assigned to H-2' and a double doublet at δ7.65 (1H, dd, *J* = 7.6, 2.0 Hz) was assigned to H-6' in ring **B**. A doublet at δ 6.95 (1H, d, *J* = 7.7 Hz) was assigned to H-5' in ring **B**. The anomeric proton signals at δ 3.92 (1H, d, *J* = 6.82 Hz, H-1″), 5.41 (1H, d, *J* = 7.6 Hz, H-1‴) and 5.53 (1H, s, H-1⁗) were assigned for H-1", H-1‴ and H-1"" for D-arabinose, D-galactose and L-rhamnose respectively.

In the mass spectrum of the compound (**2**), characteristic ion peaks at *m/z* 770 $[M]^+$, 608 $[M^+$-d-galactose], 476 $[M^+$-D-arabinose] and 330 $[M^+$-L-rhamnose, aglycone] were found by subsequent losses from the molecular ion of each molecule of D-galactose, D-arabinose and L-rhamnose revealing D-galactose as terminal sugar, D-arabinose was linked to aglycone at C-3 position and L-rhamnose was attached at C-7 position of aglycone.

Acid hydrolysis of compound (**2**) with ethanol and 10 % H_2SO_4 gave aglycone (**2-A**), m.p. 289-291°C, m.f. $C_{17}H_{14}O_7$, $[M]^+$ 330 (EIMS) and sugar moiety (ies). These were separated and studied separately. The aglycone (**2-A**) (**Fig. 4**) was identified as 3, 5, 7-trihydroxy-3′, 4'-dimethoxy flavone by comparision of its spectral data with reported literature values [11-13].

The aqueous hydrolysate after the removal of aglycone was neutralized with $BaCO_3$ and $BaSO_4$ filtered off. The filtrate was concentrated and subjected to paper chromatography examination and sugars were identified As L-rhamnose (R_f 0.36), D-galactose (R_f 0.17) and D-arabinose (R_f 0.22) (Co-PC) [8].

Periodate oxidation of compound **2**, confirmed that all the sugars were present in the pyranose form [9]. The positions of sugar moieties in compound **2** were determined by permethylation [10] followed by acid hydrolysis, yielded methylated aglycone identified as 3, 7-dihydroxy-5, 3′, 4'-trimethoxy flavone showed that glycosylation was involved at C-3 and C-7 positions of the flavone and methylated sugars were identified as 2, 3, 4-tri-O-methyl-L-rhamnose (R_G 1.02), 2, 3, 4, 6-tetra-O-methyl-D-galactose (R_G 0.89) and 2, 3-di-O-methyl-D-arabinose (R_G 0.63) indicating that C-1""-OH of L-rhamnose was linked at C-7 position of the aglycone, C-1'"-OH of D-galactose was linked to C-4"-OH of D-arabinose and C-1"-OH of D-arabinose was attached with C-3 position of the aglycone.

Therefore, it was concluded that interlinkage (1→4) was found between D-galactose and D-arabinose, which was further confirmed by ^{13}C NMR spectra (see in experimental section).

Enzymatic hydrolysis of compound **2** with takadiastase enzyme liberated L-rhamnose (R_f 0.36) and proaglycone identified as 3, 5, 7-trihydroxy-3', 4′-dimethoxyflavone-3-O-β-D-galactopyranosyl-(1→4)-O-β-D-arabinopyranoside showed the presence of α-linkage between L-rhamnose and proaglycone. Proaglycone on further hydrolysis with almond emulsin enzyme liberated D-galactose (R_f 0.17) followed by D-arabinose (R_f 0.22) and aglycone suggesting the presence of β-linkage between D-galactose and D-arabinose as well as D-arabinose and aglycone.

On the basis of above discussions the structure of compound **2** was characterized as 3, 5, 7-trihydroxy-3′,4′-dimethoxyflavone-3-O-β-D-galactopyranosyl-(1→4)-O-β-D-arabinopyranosyl-7-O-α-L-rhamnopyranoside.

Compound **3 (Fig. 5)** had m.p. 283-285°C, m.f. $C_{17}H_{14}O_7$, $[M]^+$ 330 (EIMS). It was characterized as Tricin by comparision of its spectral data with reported literature values[14-15].

Compound **4** (**Fig. 6**) had m.p. 280-282°C, m.f. $C_{30}H_{50}O$, $[M]^+$ 426 (EIMS). It was identified as Taraxerol by comparison of its spectral data with reported literature values [16-17].

Compound **5 (Fig. 7**) had m.p.146-148°C, m.f. $C_{18}H_{16}O_6$, $[M]^+$ 328 (EIMS). It was identified as 5-hydroxy-3, 7, 4′-trimethoxy flavone by comparison of its spectral data with reported literature values [18-20].

Compounds (**1**) and (**2**) were screened for antibacterial and antifungal activity against various gram (+ve) gram (-ve) bacteria and fungi.

The results reported in **Table 1,** showed that compound (**1**) was found to be active against bacteria *Staphyloccus aureus* (+ve) and no activity was found against *E. coli*(-ve), *Micrococcus luteus* (+ve) and *Bacilius subtilis* (+ve). Compound (**2**) had also shown less activity against bacteria *E. coli* (–ve) and *Bacilius subtilis* (+ve).

The results reported in **Table 2**, showed that compound (**1**) showed activity against *Candida albicans* and no activity was found against *Rhizopus oryzae, Aspergillus niger* and *Mucor indicus*. Compound (**2**) showed less activity against *Aspergillus niger* and *Mucor indicus* at lower concentrations.

CONCLUSION: The phytochemical analysis of ethanolic extract of *Indigofera trifoliata* Linn. showed the presence of two new allelochemicals. These allelochemicals showed antibacterial and antifungal activity against various gram positive and gram negative bacteria and fungi. The results also assigned the medicinal values of the plant. The plant could serve as useful sources for new antimicrobial agents.

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REFERENCES:

1. Kirtikar KR and Basu BD: Indian Medicinal Plants. 2nd ed, Vol. 2, Allahabad 1975:711.
2. Chopra RN, Nayar SL and Chopra IC: Glossary of Indian Medicinal Plants. CSIR, New Delhi 1956:141.
3. The Wealth of India: Dictionary of Indian Raw Materials and Industrial Products. Vol.V (H-K).CSIR New Delhi 1959:183.
4. Bhalla NP and Dakwake RN: Chemotaxonomy of *Indigofera trifoliata* Linn. J Indian Bot Soc1978; 57:180-5.
5. Dheng RM and Itankar PR: Anti-inflammatory and antioxidant potential of ethanolic extract of *Indigofera trifoliata* Linn. Indian Journal of Natural Products 2008; 24(1): 3-7.
6. Bharathi RV, Vamsadhara C, Sumathi G and Rajendran K: An identity based pharmacognostical profile of *Indigofera trifoliata* leaf. International Journal of Phytopharmacy Research 2012; 3(2):87-92.
7. Shinoda J: J Pharm Soc Jpn 1928; 48: 214.
8. Lederer E and Lederer M: Chromatography. Elsevier Publishing Company, New York: 1957; 1: 247.
9. Hakomoni S: J Biochem 1965; 66: 205-7.
10. Petek F: Bull Soc Chem Fr 1965; 68: 263-68.
11. Yin JG, Yuan CS and Jia Z: A new iridoid and other chemical constituents from *Pedicularis kansuensis* forma *albiflora* Li. J Arch Pharm Res 2007; 30: 431-35.
12. Muhit MA, Tareq SM, Apu AS, Basak D and Islam MS: Isolation and identification of compounds from the leaf extract of *Dillenia indica* Linn. Bangladesh Pharmaceutical Journal 2010; 13: 49-53.
13. El-Mousallami AMD, Hussein MAS and Nawwar MAM: Polyphenolic metabolites of the flowers of *Tamarix tetragyna*. Natural Product Sciences. 2000; 6: 193-98.
14. Zhang J, Wang Y, Zhang XQ, Zhang QW and Ye WC: Chemical constituents from the leaves of *Lophatherum gracile*. Chin J Nat Med 2009; 7: 428-31.
15. Jiao J, Zhang Y, Liu C, Liu J, Wu X and Zhang Y: Separation and purification of tricin from an antioxidant product derived from Bamboo leaves. J Agric Food Chem 2007; 55:10086-92.
16. Deng YR, Song AX and Wang HQ: Chemical components of *Seriphidium santolium* Poljak. Journal of the Chinese Chemical Society 2004; 51: 629-36.
17. Ignacio HC, Luis WTT, Paulino SP, Roberto CR, Rosa MP and Sergio RPS: Antigirardial activity of *Cupania dentata* bark and its constituents. J Mex Chem Soc 2012; 56, 105-8.
18. Lefahal M, Benahmed M, Louaar S, Zallagui A, Duddecck H, Medjroubi K and Akkal S: Antimicrobial activity of *Tamarix gallica* L. extracts and isolated flavonoids. Advances in Natural and Applied Sciences 2010; 4, 289-92.
19. Paula VF and Cruz MP: Chemical constituents of *Bombacopsis glabra*. Quim Nova 2006; 29, 213-15.
20. Ronaldo NDA, Jose MBF, Victor MR and Raimundo BF : Botocudorol, a new bisabolene type nor-sesquiterpenoid from *Sparattanthelium botocudorum*. J Braz Chem Soc 1991; 2, 71-73.
21. Casida LE: Industrial Microbiology. John Willey and Sons Ltd.1961, New York.

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