

**ANTIOXIDANT AND ANTIMICROBIAL ACTIVITY OF A NEW
ISOFLAVONE GLYCOSIDE FROM THE ROOTS OF *Prosopis Spicigera*****Linn****R. N. Yadava* and Prakrati Yadav**

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ABSTRACT

A new Isoflavone glycoside 5, 7, 4' trihydroxy-6-methoxyisoflavone-7-O- α -L-rhamnopyranosyl(1 \rightarrow 4)O- α -L-arabinopyranosyl-4'-O- β -D-xylopyranoside (A) was isolated from the roots of *prosopis spicigera* Linn. together with two known compounds Wogonin (B) and Diosmetin (C). The structure A was elucidated on the basis of various chemical degradations, color reactions and spectroscopic analysis viz. UV, IR, ^1H NMR and ^{13}C NMR. Compound A exhibited higher radical scavenging activity in the 1,1-diphenyl-2-picryl-hydrazyl (DPPH) assay system and also screened against various bacteria and fungi.

KEY WORDS: *Prosopis spicigera* Linn, Leguminosae, Isoflavone glycoside, Antioxidant activity, Antimicrobial activity.

INTRODUCTION

Prosopis spicigera Linn.^[1] commonly known as Jhand in Hindi. It is found in Punjab, Rajputana, Bundelkhand, Gujarat, Sind and Baluchistan. Its bark is used as remedy in rheumatism and scorpion sting. Its flowers are pounded and mixed with sugar eaten by women during pregnancy as a safeguard against miscarriage. Its ashes are rubbed over the skin to remove hair. This plant is xerophytic and draught resistant plant, it can survive far long. Tribal people use this plant as fodder and source of wood. Seeds are dark brown in color packed in brown pulp. Seeds contain fixed oil, those are major part of cattle feed.^[2-5]

Earlier workers^[6-9] have been reported various constituents from this plant. In the present study, we report the isolation and structure elucidation of a new compound A from the methanolic extract of the roots of this plant. The structure of this compound has been characterized as 5, 7, 4' trihydroxy-6-methoxyisoflavone-7-O- α -L-rhamnopyranosyl(1 \rightarrow 4)O- α -L-arabinopyranosyl-4'-O- β -D-xylopyranoside.

RESULTS AND DISCUSSION

2.1 Study of Compound A

Compound A was isolated as yellow amorphous powder. Flavonoidal glycosidic nature of compound was confirmed by Molisch and Shinoda tests.^[10-11] UV spectrum of compound exhibits absorption bands at 250, 265 and 320 nm, indicating isoflavone skeleton. Presence of hydroxyl (3430 cm⁻¹), α - β unsaturated carbonyl (1630 cm⁻¹) and aromatic ring (1620, 1560, 1510 cm⁻¹) were confirmed by IR spectroscopy. The ¹H-NMR spectrum of compound showed doublet at δ_H 7.40 (1H, d, J = 8.4 Hz) assigned for H-2' and 6' in Ring B respectively. Another doublet at δ_H 6.83 (1H, d, J = 8.4 Hz) assigned for H-3' and H-5' in Ring B. Two singlets at δ_H 8.43 and δ_H 6.88 assigned for at H-2 and C-8 positions in ring C and ring A respectively. The anomeric proton signals at δ_H 5.62 (1H, d, J = 5.8 Hz, H-1''), δ_H 5.80 (1H, d, J = 1.9 Hz, H-1''') and δ_H 4.52 (1H, d, J = 7.5 Hz, H-1''') were assigned to H-1'' of L-arabinose, H-1''' of L-rhamnose and H-1'''' of D-xylose respectively. Two coupling constants at (J = 5.8 Hz) of H-1'' and (J = 1.9 Hz) of H-1''' confirmed the α -anomeric configuration for the L-arabinose and L-rhamnose. A coupling constant at (J = 7.5 Hz) of H-1'''' confirmed the β -anomeric configuration for the D-xylose respectively.^[12-13] In the mass spectrum of the compound A, characteristic ion peaks at m/z 710 [M]⁺, 564 [M⁺-L-rhamnose], 432 [M⁺-L-arabinose] and 300 [M⁺-D-xylose] were found by subsequent losses from the molecular ion of each molecule of L-rhamnose, L-arabinose, D-xylose and revealing L-rhamnose and L-arabinose were attached to aglycone at C-7 position and D-xylose was attached to aglycone at C-4' position.

Acid hydrolysis of compound A with 10% ethanolic H₂SO₄ gave aglycone A-1, m.p 210-211°C, m.f. C₁₆H₁₂O₆ [M]⁺ 300 (FABMS) which was identified as 5, 7, 4'-trihydroxy-6-methoxyisoflavone (see in experimental section). The aqueous hydrolysate obtained was neutralized with BaCO₃ and the BaSO₄ was filtered off. The filtrate was concentrated and subjected to Paper chromatography examination on Whatman filter Paper No. 1 and showed

the presence of L-rhamnose (R_f 0.37), D-xylose (R_f 0.28) and L-arabinose (R_f 0.21). Quantitative estimation of sugars revealed that all the three sugars were present in equimolar ratio 1:1:1.^[14] Periodate oxidation of compound **A** confirmed that all sugars were present in the pyranose form.^[15]

The position of the sugar moieties in compound **A** were determined by permethylation followed by acid hydrolysis, yielded methylated aglycone and methylated sugars. The methylated aglycone was identified as 5-hydroxy-6, 7, 4'-trimethoxyisoflavone which confirmed that glycosidation was involved at C-7-OH and C-4'-OH of aglycone. The methylated sugars were identified as 2, 3, 4-tri-O-methyl-L-rhamnose (R_G 1.01), 2, 3, 4-tri-O-methyl-D-xylose (R_G 0.94) and 2, 3, -di-O-methyl-L-arabinose (R_G 0.64) by paper chromatography with authentic samples.

Therefore it was concluded that C-1'''-OH of L-rhamnose was linked with C-4'' of L-arabinose, C-1''-OH of L-arabinose was attached with C-7-OH position and C-1'''' of D-xylose was attached with C-4'-OH position of aglycone. The interglycosidic linkages (1→4) was found between L-rhamnose and L-arabinose. Enzymatic hydrolysis^[16] of compound **A** with almond emulsin enzyme liberated D-xylose indicating the presence of β linkage between D-xylose and as 5, 7, 4' trihydroxy-6-methoxyisoflavone-7-O- α -L-rhamnopyranosyl(1→4)-O- α -L-arabinopyranoside as proaglycone. Proaglycone on further hydrolysis with takadiastase enzyme liberated L-rhamnose first followed by L-arabinose and aglycone. Thus compound **A** was identified as 5, 7, 4' trihydroxy-6-methoxyisoflavone-7-O- α -L-rhamnopyranosyl (1→4)-O- α -L-arabinopyranosyl-4'-O- β -D-xylopyranoside.

3.0 Experimental

All the melting points were determined on a thermoelectrically melting point apparatus and are uncorrected. The IR Spectra were recorded in KBr disc. UV Spectra were determined on Shimadzu-120 double beam spectrometer in MeOH. The IR spectra were recorded on Shimadzu FT-IR 8400S in KBr disc. ¹H-NMR Spectra were recorded on Bruker DRX 300 MHz spectrometer in DMSO-d₆ using TMS as internal standard. ¹³C-NMR Spectra were recorded on Bruker DRX 75 MHz spectrometer using DMSO-d₆. The chemical shift values are reported in ppm (δ) units and coupling constant (J) in Hz. The FAB mass spectra were recorded on a Jeol-SX (102) Mass spectrometer. The stems of *prosopis spicigera* Linn. were procured from Sagar region and were taxonomically authenticated by the Department of

Botany, Dr. H. S. Gour University sagar. A voucher specimen has been deposited in the Natural Products Laboratory, Department of Chemistry, Dr. H. S. Gour University, Sagar (M.P.) INDIA.

Air dried and Powdered roots (6.5 kg) of the plant were extracted with petroleum ether (40-60°C) in Soxhlet apparatus for 6 days. The roots of the plant were successively extracted with methanol for four days. The MeOH soluble fraction of the plant was concentrated under reduced pressure, to yield a light brown viscous mass (4.80 g) which was subjected to TLC examination over silica gel-G using n BAW (4:1:5) as solvent and I₂ vapors as visualizing agent, showed three spots, indicating it to be a mixture of three compounds **A**, **B** and **C**. These compounds were separated and purified by column chromatography over silica gel using CHCl₃: MeOH in various proportions (2:6, 3:5, 4:4). After removal of the solvent and crystallization from ether, above eluates yielded compound **A** (1.70 g), compound **B** (1.49 g) and compound **C** (1.60 g) respectively.

3.1. Study of Compound A:

It was analyzed for m.f. C₃₂H₃₈O₁₈, m.p. 267-268°C, [M]⁺ 710 found (%) C 64.05, H 4.07, O 32.04, Calcd for m.f. C₃₃H₄₀O₂₀ (%) C 64.00, H 4.00, O 32.00, UV (MeOH) λ_{max} nm 250, 265 and 320. IR (KBr) ν_{max} (cm⁻¹); 3430, 1630, 1620, 1560, 1510. ¹H NMR (300 MHz, DMSO-d₆); 8.44 (1H, s, H-2), 6.89 (1H, s, H-8), 7.40 (1H, d, *J* 8.4 Hz, H-2'-6'), 6.83 (1H, d, *J* 8.4 Hz, H-3'-5'), 5.62 (1H, d, *J* 5.8 Hz, H-1''), 4.06 (1H, dd, *J* 6.6, 5.8 Hz, H-2''), 3.80 (1H, m, H-3''), 3.78 (1H, m, H-4''), 3.42 (1H, dd, *J* 9.5, 4.0 Hz, H-5''_a), 3.82 (1H, m, H-5''_b), 5.80 (1H, d, *J* 1.9, H-1'''), 3.93 (1H, m, H-2'''), 3.69 (1H, dd, *J* 9.5, 3.1, H-3'''), 3.32 (1H, t, *J* 9.5, H-4'''), 3.90 (1H, m, H-5'''), 0.98 (1H, d, *J* 6.1 Hz, H-6'''), 4.52 (1H, d, *J* 7.5 Hz, H-1'''), 3.00 (1H, dd, *J* 7.5, 8.9, H-2'''), 3.14 (1H, dd, *J* 8.9, 8.4 Hz, H-3'''), 3.24 (1H, m, H-4'''), 3.58 (1H, dd, *J* 5.2, 11.3 Hz, H-5'''). ¹³C NMR (75 MHz, DMSO-d₆); δ 154.80 (C-2), 122.20 (C-3), 180.90 (C-4), 152.80 (C-5), 132.70 (C-6), 157.50 (C-7), 94.10 (C-8), 152.50 (C-9), 106.60 (C-10), 121.20 (C-1'), 130.20 (C-2'-6'), 115.20 (C-3'-5'), 157.60 (C-4'), 101.10 (C-1''), 77.50 (C-2''), 73.35 (C-3''), 68.92 (C-4''), 65.70 (C-5''), 102.40 (C-1'''), 72.32 (C-2'''), 72.40 (C-3'''), 73.90 (C-4'''), 70.00 (C-5'''), 0.98 (C-6'''), 104.60 (C-1'''), 74.20 (C-2'''), 76.10 (C-3'''), 70.00 (C-4'''), 65.70 (C-5''').

3.2 Study of Compound A-1

It was analyzed for m.f. $C_{16}H_{12}O_6$, m.p. 210-211°C, $[M]^+$ 300 found (%) C 64.05, H 4.07, O 32.04, Calcd for m.f. $C_{16}H_{12}O_6$ (%) C 64.00 H 4.00 O 32.00. UV (MeOH) λ_{max} nm 250, 265 and 320. IR (KBr) ν_{max} (cm⁻¹); 3430, 1630, 1620, 1560, 1510. ¹H NMR (300 MHz, DMSO-d₆); 8.44 (1H, s, H-2), 6.89 (1H, s, H-8), 7.40 (1H, d, *J* 8.4 Hz, H-2'-6'), 6.83 (1H, d, *J* 8.4 Hz, H-3'-5'). ¹³C NMR (75 MHz, DMSO-d₆); δ 154.80 (C-2), 122.20 (C-3), 180.90 (C-4), 152.80 (C-5), 132.70 (C-6), 157.50 (C-7), 94.10 (C-8), 152.50 (C-9), 106.60 (C-10), 121.20 (C-1'), 130.20 (C-2'-6'), 115.20 (C-3'-5'), 157.60 (C-4').

3.3. Acid hydrolysis of compound A

Compound A (74 mg) was dissolved in ethanol (15 ml) and refluxed with 10% H₂SO₄ on water bath for 6 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 CHCl₃ : MeOH as solvent to give aglycone A-1, which was identified as 5,7,4'-trihydroxy-6-methoxyisoflavone. The aqueous hydrolysate was neutralized with BaCO₃ and the BaSO₄ 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 L-rhamnose (R_f 0.37), D-xylose (R_f 0.28) and L-arabinose (R_f 0.21) (Co-PC and Co-TLC).

3.4. Permethylation of compound A

Compound A (44 mg) was dissolved in DMF (35 ml) and treated with MeI (5 ml) and Ag₂O (20 mg) in a 150 ml round bottomed flask fitted with air condenser and refluxed for 2 days. The reaction mixture was filtered and washed with DMF. The filtrate was concentrated under reduced pressure and hydrolysed with 10% H₂SO₄ to give methylated aglycone 5-hydroxy-6, 7, 4'-trimethoxy isoflavone. The aqueous hydrolysate obtained after the removal of aglycone was neutralized with BaCO₃ and the BaSO₄ was filtered off. The filtrate was concentrated under reduced pressure and subjected to Paper chromatography examination using nBAW (4:1:5) as solvent system and aniline hydrogen phthalate as spraying agent. The methylated sugars were identified as 2,3,4-tri-O-methyl-L-rhamnose (R_G 1.01), 2,3 -di-O-methyl-L-arabinose (R_G 0.64) and 2,3, 4-tri-O-methyl-D-xylose (R_G 0.94) (by m.m.p and Co-PC).

3.5. Enzymatic hydrolysis of compound A

Compound A (32 mg) was dissolved in MeOH (25 ml) and hydrolyzed with equal volume of almond emulsion enzyme. The contents were allowed to stay at room temperature for 3 days

and filtered. The hydrolysate was concentrated and subjected to paper chromatography examination using nBAW (4:1:5) as solvent system and aniline hydrogen phthalate as a spraying reagent which showed the presence of D-xylose (R_f 0.28). The proaglycone was dissolved in MeOH (20 ml) and further hydrolyzed with equal volume of takadiastase enzyme at room temperature as usual procedure yielded aglycone identified as 5, 7, 4' trihydroxy-6-methoxyisoflavone sugars were identified as L-rhamnose (R_f 0.37) and L-arabinose (R_f 0.21).

3.6. Study of compound B (Wogonin)

It was analyzed for m.f. $C_{16}H_{12}O_5$, $[M]^+$ 284 found (%) C 67.70, H 4.27, O 28.17, Calcd for m.f. $C_{16}H_{12}O_5$ (%) C 67.60, H 4.22, O 28.16, UV(MeOH) λ_{max} nm 321, 276 and 216. IR (kBr) ν_{max} (cm^{-1}); 3445, 1668, 1610, 1545, 1508. 1H NMR (300 MHz, DMSO- d_6); 6.69 (1H, s, H-3), 98.9 (1H, s, H-6), 7.93 (1H, d, J 6.89 Hz, H-2'), 7.57 (1H, m, H-3'), 7.57 (1H, m, H-4'), 7.57 (1H, m, H-5'), 7.92 (1H, d, J 6.87, H-6'), ^{13}C NMR (75 MHz, DMSO- d_6); δ 163.50 (C-2), 105.90 (C-3), 182.45 (C-4), 157.75 (C-5), 98.85 (C-6), 148.80 (C-7), 126.80 (C-8), 155.2 (C-9), 105.40 (C-10), 131.10 (C-1'), 126.20 (C-2'), 129.20 (C-3'), 131.90 (C-4'), 129.20 (C-5'), 126.20 (C-6').

3.7. Study of compound C (Diosmetin)

It was analyzed for m.f. $C_{16}H_{12}O_6$, $[M]^+$ 300 found (%)C 64.00 H 4.00 O 32.00, Calcd for m.f. $C_{16}H_{12}O_6$ (%) C 64.10, H 4.10, O 32.10, UV(MeOH) λ_{max} nm 226, 253 and 346. IR (kBr) ν_{max} (cm^{-1}); 3408, 2924, 1722, 1655, 1607. 1H NMR (300 MHz, DMSO- d_6); 6.85 (1H, s, H-3), 6.37 (1H, d, J 2.1 Hz, H-6), 6.81 (1H, d, J 2.1 Hz, H-8), 7.84 (1H, d, J 2.1 Hz, H-2'), 6.99 (1H, d, J 8.7 Hz, H-5'), 7.45 (1H, dd, J 8.7, 2.1 H-6'), ^{13}C NMR (75 MHz, DMSO- d_6); δ 165.10 (C-2), 103.90 (C-3), 182.25 (C-4), 161.50 (C-5), 100.50 (C-6), 163.20 (C-7), 94.20 (C-8), 157.50 (C-9), 105.90 (C-10), 122.90 (C-1'), 113.40 (C-2'), 151.70 (C-3'), 148.80 (C-4'), 112.30 (C-5'), 118.10 (C-6').

4.0 Antioxidant activity

4.1. DPPH radical scavenging assay method: DPPH quenching ability of extract was measured according to Christudas S. and Ignacimuthu S., 100 mg of dried extract were dissolved in 100 ml methanol. Various aliquotes of concentration 10-100 μ g/ml were prepared with methanol. 2 ml of methanolic solution of DPPH (0.1mM) was mixed with 3 ml of extracts (10-100 μ g/ml) and allowed to incubate for 30 min at dark place. After 30 minute

decrease in absorbance of DPPH solution was measure at 517 nm against control sample. The antiradical activity was expressed as IC_{50} ($\mu\text{g/ml}$), (the antiradical dose required to cause a 50% inhibition). Vitamin C was used as standard. The ability to scavenge the DPPH radical was calculated using the following equation:

$$\text{DPPH scavenging effect (\%)} = (A_0 - A_1 / A_0) \times 100$$

Where A_0 is the absorbance of the control at 30 min, and A_1 is the absorbance of the sample at 30 min.

(Table-I) Absorbance of 0.1mM DPPH (A_0) = 2.076

S. No	Vitamin C			Plant Extract		
	Conc.	Test	% Inhibition	Conc.	Test	% Inhibition
1	10	1.524	25.72	10	1.598	23.02
2	20	1.012	51.25	20	1.017	51.01
3	40	0.655	68.44	40	0.741	64.16
4	60	0.322	84.48	60	0.351	83.09
5	80	0.268	87.09	80	0.299	85.39
6	100	0.236	88.61	100	0.254	87.76
IC_{50} ($\mu\text{g/ml}$)			24.65	IC_{50} ($\mu\text{g/ml}$)		27.91

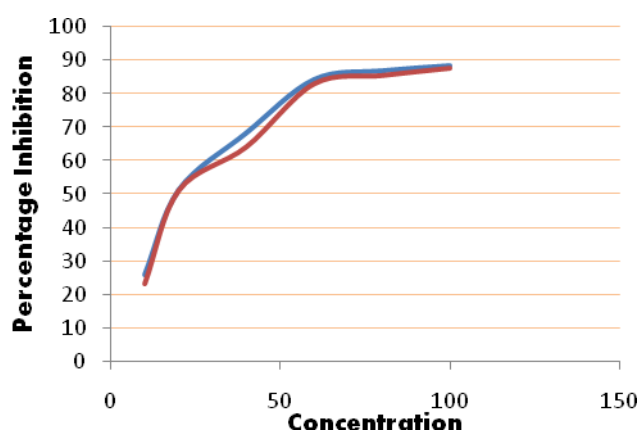


Figure- 1

5.0. Antimicrobial activity - The antimicrobial activities of compound was determined by Kirby Bauer method^[17-18]. The various bacterial and fungal species were first incubated at 37°C for 24 hrs for the proper growth of microbes then prepared agar plate (Muller Hinton Agar Media for the bacteria and Potato Dextrose Agar media for the fungus). Antibacterial

and Antifungal activities of compound was determined with Ciprofloxacin and Fluconazole as standard. Anti microbial and antifungal activities of Compound A are represented in figure 2 and 3.

Table- II. Zone of Inhibition (mm)

Bacterial species		Compound A		Fungal species		Compound A	
1.	<i>S. aureus</i>	14	18	1.	<i>C. albicans</i>	15	18
2.	<i>B. subtilis</i>	18	21	2.	<i>A. niger</i>	17	20

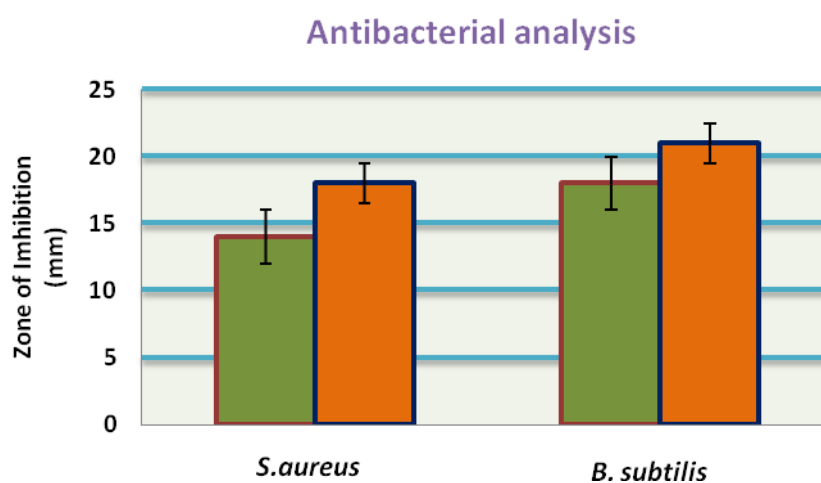


Figure- 2

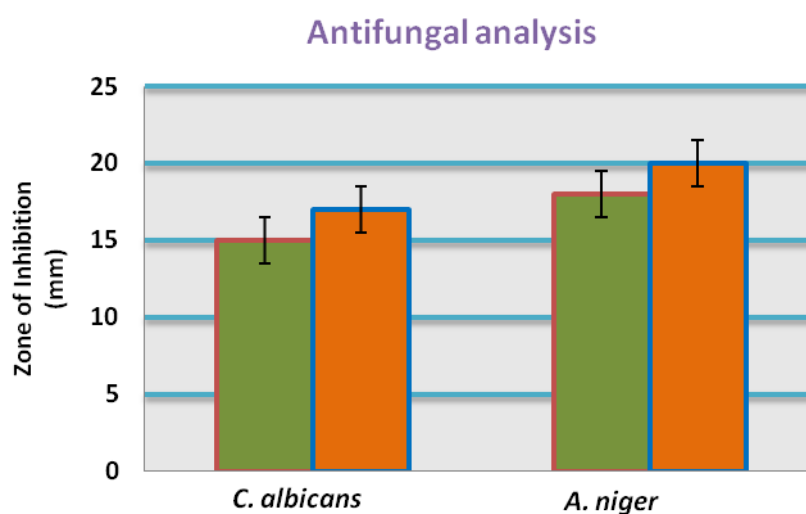
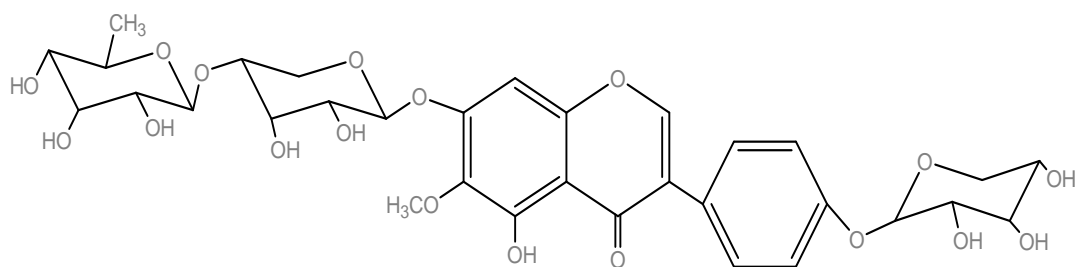
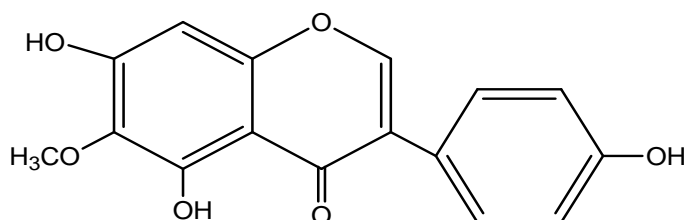
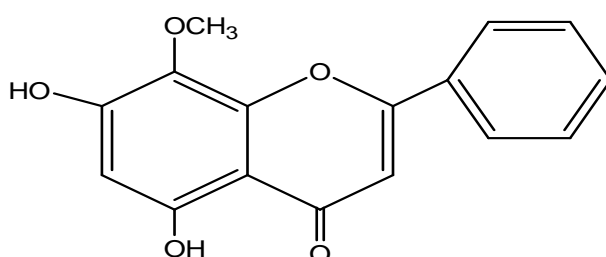
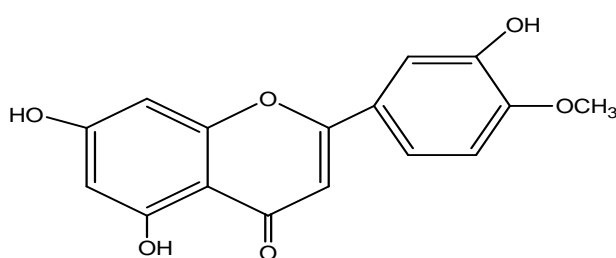


Figure- 3

**Compound A****Compound A-1****Compound B****Compound C****REFERENCES**

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