

## NEW ANTIBACTERIAL ISOFLAVONE GLYCOSIDE FROM FLOWERS OF ARTEMISIA ABSINTHIUM LINN

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### ABSTRACT

Chemical investigation of flowers of *Artemisia absinthium* Linn., resulted in the isolation of a new isoflavone glycoside 7, 4'-dihydroxy-6-methoxyisoflavone-7-O- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 4)-O- $\beta$ -D-xylopyranosyl-4'-O- $\beta$ -D-glucopyranoside (1) alongwith four known compounds  $\alpha$ -spinasterol (2), oleanolic acid (3), apigenin-7-O-glucoside (4) and hispidulin (5). Structure of the new compound was elucidated by various colour reactions, spectral analysis and chemical degradations. Compound 1 showed significant antibacterial activity.

**KEYWORDS:** *Artemisia absinthium* Linn., Asteraceae, isoflavone glycoside, antibacterial activity.

### INTRODUCTION

*Artemisia absinthium* Linn.<sup>[1-3]</sup> belongs to family Asteraceae, which is commonly known as “*Vilayatiafsantin*” in hindi, “*Apsinthion*” in Greek and “*Wormwood*” in english. It grows in Europe, North Africa, parts of Asia and North and South America. In Kashmir, it is found at altitude of 5000-7000 feet. It is a perennial hoary, silky herbaceous very aromatic plant of 100 cm in height and occurs throughout the year. Flowering and fruiting take place from July to September. Stems are 0.3-0.9 meter Erect, angular, hoary and ribbed. Leaves are Ovate to obovate, unequally 2-3 pinnatifidly, cut into spreading linear or lanceolate-obtuse segments, hoary on both surfaces. External colour of flowers is white but internally it is yellow. There is a small bulb at the base of flower which is filled with small seeds. The seeds are bitter in taste. The whole plant is an aromatic tonic and formerly enjoyed a high reputation in debility of the digestive organs. It was also regarded as an anthelmintic. It is prescribed in form of a poultice or fomentation as an antiseptic. By distillation, it yields dark green or yellow oil, having a strong odour of the plant and an acrid taste. It is therapeutically used in various diseases such as anaemia, amenorrhoea, anal fissure, anorexia, ascitis, chronic fever, deficient quality and quantity of gastric juice, diphtheria, dyspepsia, epilepsy, helminthiasis, hysteria, hepatitis, inflammation of uterus and stomach, jaundice, loss of appetite,

menstrual disorder, mental exhaustion and nervous depression, paralysis, renal calculus and skin diseases.<sup>[4-6]</sup> Earlier workers<sup>[7-13]</sup> have reported various chemical constituents from this plant. In the present paper we report the isolation and structural elucidation of a new isoflavone glycoside 7, 4'-dihydroxy-6-methoxyisoflavone-7-O- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 4)-O- $\beta$ -D-xylopyranosyl-4'-O- $\beta$ -D-glucopyranoside (1) alongwith four known compounds  $\alpha$ -spinasterol (2), oleanolic acid (3), apigenin-7-O-glucoside (4) and hispidulin (5) from ethanolic extract of the flowers of this plant.

### Experimental Section

#### Plant material

The flowers of the plant were collected locally around sidhi region and were taxonomically authenticated by taxonomist, Department of Botany, Govt S G S College Sidhi (M.P.) India. A voucher specimen has been deposited in the Laboratory, Department of Chemistry of this college.

#### General experimental 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 FT-IR spectrometer Shimadzu 8201 PC (4000-400  $\text{cm}^{-1}$ ).  $^1\text{H-NMR}$  and  $^{13}\text{C-NMR}$  spectra were recorded

using solvent  $\text{CDCl}_3$  and TMS as internal standard on Bruker DRX-300 spectrometer.

#### Extraction and isolation

Air dried powdered plant flowers (2.5kg) were extracted with 90% ethanol (55-60°C) in a Soxhlet apparatus for 72 hours. The ethanolic extract was further exhaustively partitioned with chloroform, ethyl acetate, acetone and methanol. The methanol soluble fraction was further concentrated under reduced pressure to yield brown viscous mass (3.60g), which was subjected to TLC examination using nBAW (4:1:5) as eluent and  $\text{I}_2$  vapours as visualizing agent. It gave five spots indicating it to be a mixture of five compounds **1**, **2**, **3**, **4** and **5**.

**Table I:**  $^1\text{H-NMR}$  (300 MHz,  $\text{CDCl}_3$ ) of compound **1**.

Position	$\delta_{\text{H}}$ (ppm)	Position	$\delta_{\text{H}}$ (ppm)
2	8.19 (1H, s)	3''',4''''	3.77-3.81(2H,m)
5	7.36 (1H, s)	5 <sub>a</sub> ''''	3.40(1H, dd, <i>J</i> 9.5, 4.2 Hz)
8	6.88 (1H, s)	5 <sub>b</sub> ''''	3.81(1H, dd, <i>J</i> 10.6, 5.2Hz)
6	3.84 (3H, s, 6-OCH <sub>3</sub> )	Rha-1''''	5.78 (1H, d, <i>J</i> 1.9Hz)
2'	7.36 (1H, d, <i>J</i> 8.3 Hz)	2''', 3''', 4''', 5''''	3.32-3.92 (4H, m)
3'	6.80 (1H, d, <i>J</i> 8.3 Hz)	6''-CH <sub>3</sub>	0.98 (1H, d, <i>J</i> 6.2Hz)
5'	6.80(1H, d, <i>J</i> 8.3 Hz)	Glu-1''''	5.70 (1H, d, <i>J</i> 7.6 Hz)
6'	7.36 (1H, d, <i>J</i> 8.3 Hz)	2''''', 3''''', 4''''', 5''''''	4.16-4.43(4H,m)
Xyl-1''''	5.03 (1H, d, <i>J</i> 6.7Hz)	6 <sub>a</sub> ''''	4.56(1H, d, <i>J</i> 12.3 Hz)
2''''	4.04 (1H, dd, <i>J</i> 6.6, 5.9Hz)	6 <sub>b</sub> ''''	4.32(1H, m)

**Table II:**  $^{13}\text{C-NMR}$  (125 MHz,  $\text{CDCl}_3$ ) of compound **1**.

C	$\delta$ (ppm)	C	$\delta$ (ppm)
2	151.5	2''	75.5
3	123.0	3''	77.18
4	164.6	4''	70.66
5	104.2	5''	66.70
6	147.9	1''''	100.46
7	174.1	2''''	70.23
8	102.4	3''''	69.31
9	151.5	4''''	71.92
10	113.8	5''''	68.19
1'	122.1	6''''	17.62
2'	129.3	1'''''	101.5
3'	115.08	2'''''	73.8
4'	145.9	3'''''	76.3
5'	115.08	4'''''	70.3
6'	130.01	5'''''	77.8
6-OCH <sub>3</sub>	55.9	6'''''	60.5
1''''	106.6		

#### Acid hydrolysis of compound **1**

Compound **1** (400 mg) was dissolved in ethanol (18 ml) and refluxed with 20 ml of  $\text{H}_2\text{SO}_4$  on water bath for 6 hr. The reaction mixture was concentrated and allowed to cool and residue was extracted with diethyl ether ( $\text{Et}_2\text{O}$ ). The ether layer was washed with water and evaporated to dryness. The residue was subjected to column chromatography over silica gel column using  $\text{CHCl}_3$ :  $\text{MeOH}$  (5:7) to give compound **1-A**, identified as 7, 4'-dihydroxy- 6-methoxyisoflavone by comparison of its spectral data with reported literature values. The aqueous

These compounds were separated by TLC and purified by column chromatography over silica gel using  $\text{CHCl}_3$ :  $\text{MeOH}$  (6:4) as eluent and studied separately.

#### Study of compound **1**

It was crystallised from acetone to yield 1.35g. It has m.p. 258-259°C, m.f.  $\text{C}_{33}\text{H}_{40}\text{O}_{18}$ ,  $[\text{M}]^+$  724 (FABMS); found(%): C 53.42, H 5.13, O 41.45 calcd.(%) for m.f.  $\text{C}_{33}\text{H}_{40}\text{O}_{18}$  : C 54.70, H 5.52, O 39.78; UV:  $\lambda_{\text{max}}$  nm: (MeOH) 252, 318; IR:  $\nu_{\text{max}}^{\text{KBr}}$  ( $\text{cm}^{-1}$ ), 3408, 3062, 1690, 1615, 1576, 1520, 1414, 1368, 1273, 1204, 1172, 1044, 837. For  $^1\text{H-NMR}$  and  $^{13}\text{C-NMR}$  data see tables I and II respectively.

hydrolysate was neutralized with  $\text{BaCO}_3$  and the  $\text{BaSO}_4$  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 D-xylose ( $R_f$  0.27), D-glucose ( $R_f$  0.19) and L-rhamnose ( $R_f$  0.37), (Co-PC and Co-TLC).

#### Study of compound **1-A**

It has m.f.  $\text{C}_{16}\text{H}_{12}\text{O}_5$ , m.p. 302-304°C,  $[\text{M}]^+$  284 (EIMS); found(%): C 66.84, H 4.75, O 28.41, calcd.(%) for m.f.  $\text{C}_{16}\text{H}_{12}\text{O}_5$ , C 67.60, H 4.23, O 28.17; UV:  $\lambda_{\text{max}}$  nm: (MeOH) 258, 320; IR:  $\nu_{\text{max}}^{\text{KBr}}$  ( $\text{cm}^{-1}$ ), 3410, 3069, 1693, 1618, 1580, 1523, 1416, 1372, 1276, 1207, 1175, 1048, 840;  $^1\text{H-NMR}$  (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  (ppm): 8.20 (1H, s, H-2), 7.38 (1H, s, H-5), 6.90 (1H, s, H-8), 7.38 (1H, d, *J* 8.2 Hz, H-2', H-6'), 6.82(1H, d, *J* 8.2 Hz, H-3', H-5'), 5.50(1H, s, 7-OH), 3.85 (3H, s, 6-OCH<sub>3</sub>), 3.18 (1H, s, 4'-OH).  $^{13}\text{C-NMR}$  (125 MHz,  $\text{CDCl}_3$ ):  $\delta$ (ppm) 151.8(C-2), 123.3(C-3), 168.2(C-4), 104.5(C-5), 148.2(C-6), 152.8(C-7), 102.7(C-8), 151.8 (C-9), 114.0(C-10), 122.4(C-1'), 129.6(C-2'), 115.13(C-3'), 157.28(C-4'), 115.14(C-5'), 130.07 (C-6'), 55.4(6-OCH<sub>3</sub>).

#### Permetylation of compound **1**

Compound **1** (30mg) was refluxed with  $\text{MeI}$  (15ml) and  $\text{Ag}_2\text{O}$  (20ml) in  $\text{DMF}$  (20mg) for three days. The reaction mixture was filtered and washed with  $\text{DMF}$ . The filtrate was concentrated under reduced pressure and treated with  $\text{CHCl}_3$  (20ml) and washed with water. After removal of solvent a syrupy mass was obtained which

was hydrolyzed with 10% ethanolic  $H_2SO_4$  (10ml) for 6-7 hrs to give aglycone, identified as 7, 4'-dihydroxy-6-methoxyisoflavone. 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 on Whatmann filter paper No.1 using n-butanol:ethanol:water (6:1:3) solvent and aniline hydrogen phthalate as spraying agent. The methylated sugars were identified as 2, 3, 4-tri-O-methyl-L-rhamnose ( $R_G$  1.04), 2, 3-di-O-methyl-D-xylose ( $R_G$  0.72) and 2, 3, 4, 6-tetra-O-methyl-D-glucose ( $R_G$  1.01).

### Enzymatic hydrolysis of compound 1

Compound **1** (24 mg) was dissolved in MeOH (16 ml) and hydrolysed with equal volume of takadiastase enzyme. The reaction mixture was allowed to stay at room temperature for 38 hrs 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 and aniline hydrogen phthalate as spraying reagent, which showed the presence of sugar L-rhamnose ( $R_f$  0.37). The proaglycone was dissolved in MeOH (24ml) and further hydrolysed with equal volume of almond emulsion enzyme at room temperature as usual procedure yielded aglycone identified as 7,4'-dihydroxy-6-methoxyisoflavone and sugars were identified as D-xylose ( $R_f$  0.27) and D-glucose ( $R_f$  0.19).

### Study of compound 2

It has m.f.  $C_{29}H_{48}O$ , m.p. 167-168°C,  $[M]^+$  412 (EIMS); found(%), C 84.86, H 10.32, O 4.82, calcd.(%) for m.f.  $C_{29}H_{48}O$ , C 84.47, H 11.65, O 3.88; UV:  $\lambda_{max}$  nm: (MeOH) 206; IR:  $\nu_{max}^{KBr}$  ( $cm^{-1}$ ), 3400, 3372, 2939, 2870, 1645, 1440, 1387, 1043, 966.  $^1H$ -NMR (300 MHz,  $CDCl_3$ ):  $\delta$ (ppm) 3.57 (1H, tt,  $J$  10.8, 4.2 Hz, H-3), 5.10 (1H, m, H-7), 1.22 (2H, s, H-16), 0.54 (3H, s, H-18), 0.73 (3H, s, H-19), 0.90 (1H, d,  $J$  6.6 Hz, H-20), 1.06 (1H, d,  $J$  6.8 Hz, H-21), 5.17 (1H, dd,  $J$  7.8, 2.1 Hz, H-22), 5.02 (1H, dd,  $J$  7.8, 2.1 Hz, H-23), 0.85 (3H, d,  $J$  6.8 Hz, H-26), 0.86 (3H, d,  $J$  6.8 Hz, H-27).  $^{13}C$ -NMR (100 MHz,  $CDCl_3$ ):  $\delta$ (ppm) 37.20(C-1), 31.68(C-2), 71.25(C-3), 38.15(C-4), 40.36(C-5), 29.88(C-6), 17.56(C-7), 139.68(C-8), 49.65(C-9), 34.28(C-10), 21.52(C-11), 39.57(C-12), 43.32(C-13), 55.18(C-14), 23.42(C-15), 28.50(C-16), 55.78(C-17), 12.25(C-18), 13.10(C-19), 40.86(C-20), 21.39(C-21), 138.35(C-22), 129.57(C-23), 51.38(C-24), 31.57(C-25), 21.18(C-26), 19.15(C-27), 25.26(C-28), 12.44 (C-29).

### Study of compound 3

It has m.f.  $C_{30}H_{48}O_3$ , m.p. 285-286°C,  $[M]^+$  456 (EIMS); found(%), C 78.20, H 10.04, O 11.76, calcd.(%) for m.f.  $C_{30}H_{48}O_3$ , C 78.95, H 10.53, O 10.52; UV:  $\lambda_{max}$  nm: (MeOH) 208; IR:  $\nu_{max}^{KBr}$  ( $cm^{-1}$ ), 3406, 3026, 2925, 1690, 1606, 1466, 1370, 1212, 786.  $^1H$ -NMR (300 MHz,  $CDCl_3$ ):  $\delta$ (ppm) 3.25(1H, dd,  $J$  11.2, 4.6 Hz, H-3), 5.30(1H, t,  $J$  3.6 Hz, H-12), 2.84(1H, dd,  $J$  3.7, 13.1 Hz,

H-18), 0.74, 0.76, 0.88, 0.90, 0.94, 1.04, 1.16 (each 3H, s,  $M \times 7$ ).  $^{13}C$ -NMR (75 MHz,  $CDCl_3$ ):  $\delta$ (ppm) :  $\delta$  38.6(C-1), 23.6(C-2), 81.2(C-3), 38.6(C-4), 55.2(C-5), 18.3(C-6), 32.4(C-7), 39.5(C-8), 47.7(C-9), 37.1(C-10), 23.5(C-11), 123.3(C-12), 144.4(C-13), 41.4(C-14), 28.5(C-15), 23.3(C-16), 46.6(C-17), 41.7(C-18), 46.5(C-19), 31.4(C-20), 34.7(C-21), 32.5(C-22), 28.8(C-23), 17.2(C-24), 15.9(C-25), 17.4(C-26), 26.3(C-27), 180.2(C-28), 33.5(C-29), 23.9(C-30).

### Study of compound 4

It has m.f.  $C_{21}H_{20}O_{10}$ , m.p. 158-159°C,  $[M]^+$  432 (EIMS); found(%), C 57.86, H 4.92, O 37.22, calcd.(%) for m.f.  $C_{21}H_{20}O_{10}$ , C 58.33, H 4.63, O 37.04; UV:  $\lambda_{max}$  nm: (MeOH) 265, 334; IR:  $\nu_{max}^{KBr}$  ( $cm^{-1}$ ), 3426, 3134, 2928, 1650, 1600, 1586, 1508, 1498, 1456, 1418, 1373, 1272, 1104, 1084, 1033, 912, 830, 774.  $^1H$ -NMR (300 MHz,  $CDCl_3$ ):  $\delta$ (ppm) 6.84(1H, s, H-3), 6.45(1H, d,  $J$  2.2 Hz, H-6), 6.80(1H, d,  $J$  2.2 Hz, H-8), 8.02(2H, d,  $J$  8.8 Hz, H-2', H-6'), 6.96(d,  $J$  8.8 Hz, H-3', H-5'), 12.92(1H, s, 5-OH), 10.55(1H, s, 4'-OH), 5.42(1H, d,  $J$  7.4 Hz, H-1''), 3.74(1H, d,  $J$  10.2 Hz, H-2''), 3.24-3.43(5H, m, sugar protons).  $^{13}C$ -NMR (75 MHz,  $CDCl_3$ ):  $\delta$ (ppm) 164.8(C-2), 104.1(C-3), 182.2(C-4), 163.7(C-5), 95.2(C-6), 163.2(C-7), 95.8(C-8), 157.3(C-9), 104.9(C-10), 123.7(C-1'), 121.3(C-2'), 115.7(C-3'), 161.6(C-4'), 115.7(C-5'), 129.5(C-6'), 100.6(C-1''), 73.2(C-2''), 77.3(C-3''), 70.5(C-4''), 77.3(C-5''), 61.6 (C-6'').

### Study of compound 5

It has m.f.  $C_{16}H_{12}O_6$ , m.p. 280-290°C,  $[M]^+$  300 (EIMS); found(%), C 62.88, H 3.88, O 33.24, calcd.(%) for m.f.  $C_{16}H_{12}O_6$ , C 64.00, H 4.00, O 32.00; UV:  $\lambda_{max}$  nm: (MeOH) 274, 340; IR:  $\nu_{max}^{KBr}$  ( $cm^{-1}$ ), 3412, 3328, 1656.  $^1H$ -NMR (300 MHz,  $CDCl_3$ ):  $\delta$ (ppm) 6.62(1H, s, H-3), 12.36(1H, s, 5-OH), 6.58(1H, s, H-8), 7.83(2H, d,  $J$  8.8, H-2', H-6'), 6.94(2H, d,  $J$  8.8, H-3', H-5'), 3.90(3H, s, 6-OMe).  $^{13}C$ -NMR (75 MHz,  $CDCl_3$ ):  $\delta$ (ppm) 164.2(C-2), 103.8(C-3), 183.2(C-4), 153.4(C-5), 131.0(C-6), 157.6(C-7), 94.8(C-8), 154.5(C-9), 105.6(C-10), 123.8(C-1'), 130.7(C-2'/6'), 116.3(C-3'/5'), 162.2(C-4'), 60.6(6-OCH<sub>3</sub>).

### Antibacterial activity of compound 1

The antibacterial activity of compound **1** was measured by Filter Paper Disc Diffusion method.<sup>[32]</sup> The various Gram (+ve) and Gram (-ve) bacterial species were first incubated at 46°C for 40 hrs. The sterile filter paper discs (6 mm) were soaked with standard antibacterial agent and various test samples and were dried at 52°C. The discs were then placed on soft nutrient agar (3%) petri plates previously seeded with suspension of each bacterial species. The diameters of zone of inhibition were measured at 36±1°C after 25 hrs. The results are recorded in **Table III**.

**Table III: Antibacterial activity of compound 1.**

Bacterial species	Diameters of zone of inhibition (mm)*					Std.**	
	Concentration of compound 1 (%)						
	100	80	60	40	20		
<i>Escherichia Coli</i>	12.38	9.7	-	-	-	12.32	
<i>Staphylococcus aureus</i>	-	-	-	-	-	25.5	
<i>Bacillus subtilis</i>	-	5.4	3.3	-	-	14.65	
<i>Micrococcus luteus</i>	-	-	-	-	2.8	10.8	

\*The zone of inhibition (mm) taken as average of four determination direction.

\*\*Ampicillin (100 mg/mL) used as standard antibacterial agent.

## RESULTS AND DISCUSSION

Chemical examination of ethanolic extract of flowers of *Artemisia absinthium* Linn., yielded a new compound **1**. It has molecular formula,  $C_{33}H_{40}O_{18}$ , m. p. 258-259°C,  $[M]^+$  724 (FABMS). Positive results with Molisch and Shinoda tests<sup>[14]</sup> showed its flavonoidal glycosidic nature. The IR absorptions at 3408  $\text{cm}^{-1}$  (-OH), 1690  $\text{cm}^{-1}$  (C=O), 1576  $\text{cm}^{-1}$  (aromatic C=C) and a broad stretching band in the region 1044-1272  $\text{cm}^{-1}$  suggested glycosidic nature of the compound. UV spectrum of compound **1** showed the  $\lambda_{\text{max}}$  absorption at 318 nm and 252 nm suggestive of flavonoid moiety. In  $^1\text{H-NMR}$  spectrum, a singlet at  $\delta$  3.84 showed the presence of methoxy group at C-6 position. Singlets at  $\delta$  8.19, 87.36 and 86.88 were assigned as H-2, H-5 and H-8. Doublets at  $\delta$  7.36 and  $\delta$  6.80 were assigned to H-2'/H-6' and H-3'/H-5' respectively. In the  $^{13}\text{C-NMR}$  spectrum a very downfield signal at  $\delta$  164.6 represents to carbonyl C-4. The downfield shifts at  $\delta$  174.1 and  $\delta$  145.9 suggested that compound **1** was an isoflavone glycoside with glycosylation at C-7 and C-4'.

The anomeric proton signals at  $\delta$  5.03 (1H, d,  $J$  6.7Hz, H-1''), 55.78 (1H, d,  $J$  1.9Hz, H-1'') and 55.70 (1H, d,  $J$  7.6Hz, H-1'') were assigned for H-1'', H-1'' and H-1''' of D-xylose, L-rhamnose and D-glucose respectively.

In the mass spectrum of the compound **1**, characteristic ion peaks at m/z 724 [ $M^+$ ], 578 [ $M^+$ - L-rhamnose], 446 [ $M^+$ -D-xylose] and 284 [ $M^+$ -D-glucose, aglycone] were found by subsequent losses from the molecular ion of each molecule of L-rhamnose, D-xylose and D-glucose revealing L-rhamnose as terminal sugar at C-7 position, D-xylose was linked to aglycone at C-7 position and D-glucose was attached at C-4' position of aglycone.

Acid hydrolysis of compound **1** with 10% ethanolic  $\text{H}_2\text{SO}_4$  gave aglycone **1-A**, m.p. 302-304°C, m.f.  $C_{16}H_{12}O_5$ ,  $[M]^+$  284 (EIMS). It was identified as 7, 4'-dihydroxy-6-methoxyisoflavone by comparision of its spectral data with reported literature values.<sup>[15-17]</sup>

The aqueous hydrolysate after the removal of aglycone was neutralized with  $\text{BaCO}_3$  and the  $\text{BaSO}_4$  filtered off. The filtrate was concentrated under reduced pressure and subjected to paper chromatography examination and sugars were identified as L-rhamnose ( $R_f$  0.37), D-xylose ( $R_f$  0.27) and D-glucose ( $R_f$  0.19) (Co-PC and Co-

TLC).<sup>[18]</sup> Periodate oxidation of compound **1**, confirmed that all the sugars were present in the pyranose form.<sup>[19]</sup>

Permetylation<sup>[20]</sup> followed by acid hydrolysis of compound **1** yielded aglycone identified as 7, 4'-dihydroxy- 6-methoxyisoflavone showed that glycosylation was involved at C-7 and C-4' positions and methylated sugars were identified as 2, 3, 4-tri-O-methyl-L-rhamnose ( $R_f$  1.04), 2, 3-di-O-methyl-D-xylose ( $R_f$  0.72) and 2, 3, 4, 6-tetra-O-methyl-D-glucose ( $R_f$  1.01) indicating that C-1'''-OH of D-glucose was linked to C-4' position of the aglycone, C-1'''-OH of L-rhamnose was linked to C-4''-OH of D-xylose and C-1''-OH of D-xylose was linked with C-7 position of the aglycone. Therefore interlinkage (1→4) between L-rhamnose and D-xylose was confirmed. The linkage was further confirmed by spectral data of  $^{13}\text{C-NMR}$ .

Enzymatic hydrolysis<sup>[21]</sup> of compound **1** with enzyme takadiastase liberated L-rhamnose ( $R_f$  0.37) and proaglycone identified as 7, 4'-dihydroxy-6-methoxyisoflavone-7-O- $\beta$ -D-xylopyranosyl-4'-O- $\beta$ -D-glucopyranoside suggesting the presence  $\alpha$ -linkage between L-rhamnose and D-xylose. Proaglycone on further hydrolysis with enzyme almond emulsion liberated D-xylose ( $R_f$  0.27) followed by D-glucose ( $R_f$  0.19) suggesting the presence of  $\beta$ -linkage between D-xylose and aglycone as well as between D-glucose and aglycone.

On the basis of above evidences, the structure of compound **1** was characterized as 7, 4'-dihydroxy- 6-methoxyisoflavone-7-O- $\alpha$ -L-rhamnopyranosyl-(1→4)-O- $\beta$ -D-xylopyranosyl-4'-O- $\beta$ -D-glucopyranoside.

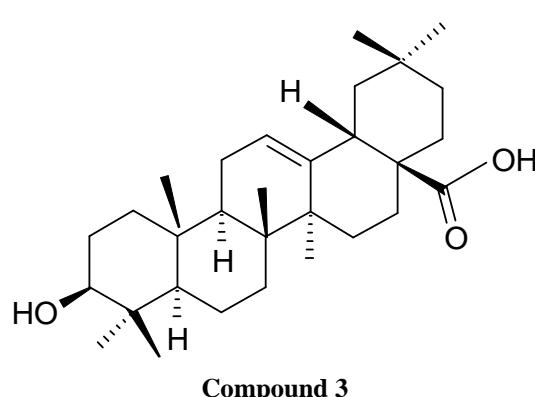
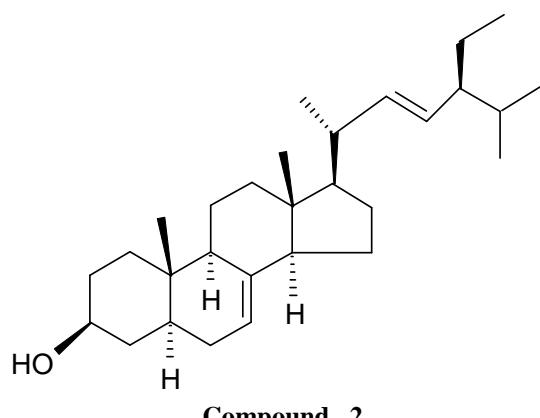
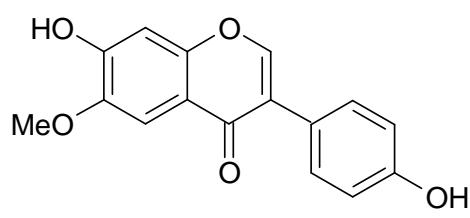
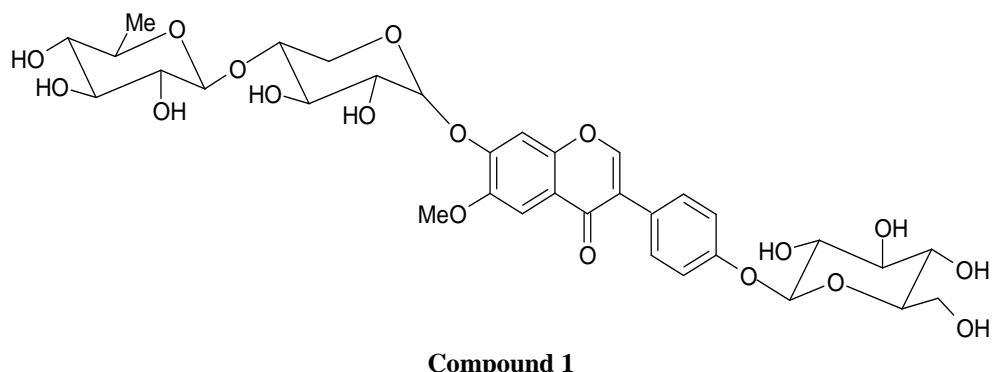
Compound 2, has m.p. 167-168°C, m.f.  $C_{29}H_{48}O$ ,  $[M]^+$  412 (EIMS). It was characterized as  $\alpha$ -spinasterol by comparision of its spectral data with reported literature values.<sup>[22-24]</sup>

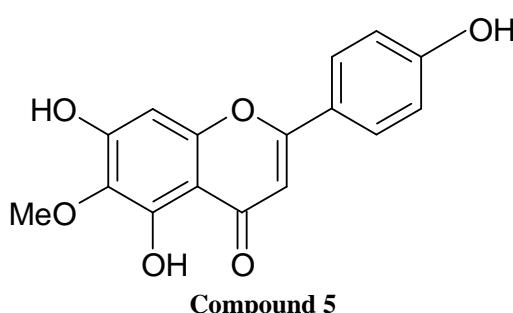
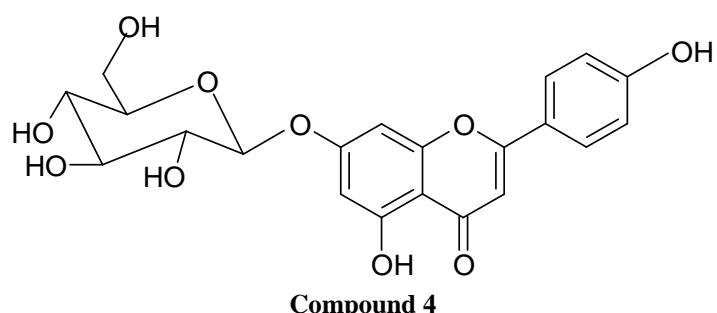
Compound 3, has m.p. 285-286°C, m.f.  $C_{30}H_{48}O_3$ ,  $[M]^+$  456 (EIMS). It was identified as oleanolic acid by comparision of its spectral data with reported literature values.<sup>[25-27]</sup>

Compound 4, has m.p. 158-159°C, m.f.  $C_{21}H_{20}O_{10}$ ,  $[M]^+$  432 (EIMS). It was identified as apigenin-7-O- $\beta$ -D-glucopyranoside by comparision of its spectral data with reported literature values.<sup>[28-29]</sup>

Compound 5, has m.p. 280-290°C, m.f.  $C_{16}H_{12}O_6$ ,  $[M]^+$  300 (EIMS). It was identified as hispidulin by comparision of its spectral data with reported literature values.<sup>[30-31]</sup>

Compound **1** was screened for antibacterial activity against various bacteria. The results obtained in **Table III** showed that antibacterial activity of compound **1** is fairly good against *Escherichia coli* and *Bacillus subtilis*.





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