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Antibacterial activity of a triterpenoid saponin from the stems of *Caesalpinia pulcherrima* Linn.

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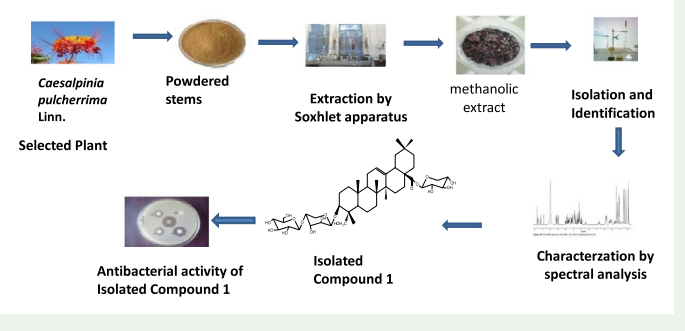
ABSTRACT

A new compound **1** was isolated from the methanolic extract of the stems of the *Caesalpinia pulcherrima* Linn. along with a reported compound (**2**) 3-O-*β*-D-glucopyranosyl-(1→4)-*β*-D-xylopyranosyl-(1→3)-*α*-L-rhamnopyranosyl-(1→2)-*α*-L-arabinopyranosyl hederagenin 28-O-*β*-D-glucopyranosyl-(1→6)-*β*-D-glucopyranosyl ester. The new compound **1** has m.p. 272–274°C, m.f. $C_{46}H_{74}O_{17}$, $[M]^+$ *m/z* 898. It was characterised as 3-O-*β*-D-glucopyranosyl-(1→4)-α-L-arabinopyranosyl hederagenin 28-O-*β*-D- xylopyranosyl ester by various colour reactions, chemical degradations and spectral analyses. Antibacterial activity of compound **1** was screened against various Gram-positive and Gram-negative bacteria and showed significant results.



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Caesalpinia pulcherrima Linn.; fabaceae; triterpenoid saponin; bidesmosidic; antibacterial activity

1. Introduction

Caesalpinia pulcherrima Linn**.** (Chatterjee & Pakrashi 1992) belongs to the family Fabaceae, which is commonly known as '*Gulutora*' in Hindi. It is small sized tree which grows about 3.7–4.3 m high, its barks are grey. Its leaves are abruptly bipinnate and 1.3–1.9 cm long. Its flowers are red or yellow in colour. Its Pods are flat, thin, straight, strap-shaped, 5.1–7.6 cm long. Its leaves are used as emmenagogue, purgative and stimulant. Its barks are used as an abortifacient and flowers used as pectoral and febrifuge. It is also used in the treatment of bronchitis, asthma and malarial fevers. Earlier workers (Sivasankari et al. 2010; Pawar et al.

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2011; Pulipati et al. 2012; Kumar et al. 2014; Osayemwenre et al. 2014; Zhao et al. 2014) have reported various active constituents from this plant. The paper deals with the isolation, structural elucidation and antibacterial activity of a new triterpenoid saponin **1**, 3-O-*β*-D-glucopyranosyl-(1→4)-*α*-L-arabinopyranosyl hederagenin 28-O-*β*-D- xylopyranosyl ester from the methanolic extract of the stems of the plant along with a known compound **2**.

2. Results and discussion

The methanolic extract of the stems of the plant was concentrated under reduced pressure to give compound **1**, m.p. 272–274°C, m.f. $C_{46}H_{74}O_{17}$, $[M]^+$ *m/z* 898 (FABMS). It gave positive results for Liebermann–Burchard test which ensure the presence of triterpenoid saponin. (Liebermann & Burchard 1885; Burchard1890) In the IR spectrum, two peaks at 3475, 1669 cm^{-1} were assigned for hydroxyl group and double bond, respectively. In ^1H NMR spectrum (300 MHz, CD_3OD) singlets at δ 0.80, 1.12, 0.95, 1.24, 1.31 and 1.09, (each 3H, s, Me × 6) showed the presence of six singlet methyl of an olean-12-ene-type triterpene. A signal at δ 5.29 (1H, *t*, 3.5 Hz, H-12), showed the presence of trisubstituted olefinic proton, A double doublets at 3.49(1H, dd, *J* 11.2,3.8 Hz) was assigned to H-3 and two doublets at 3.52 (1H, d, *J* 11.5 Hz, H_a-23), 3.23 (1H, d, *J* 11.5 Hz, H_b-23) were assigned for H-23. The anomeric proton signal at δ 4.82 (1H, d, *J* 6.3 Hz, H-1′), 4.42 (1H, d, *J* 7.2 Hz, H-1″) and 4.52 (1H, d, *J* 7.2 Hz, H-1‴), were assigned to H-1′, H-1″ and H-1‴. In ^{13}C NMR spectrum (75 MHz, CD_3OD), two signals at δ 123.2 (C-12), and 147.4 (C-13), showed the presence of double bond between C-12 and C-13. In ^{13}C NMR spectrum a downfield signal at δ 177.2 showed the presence of carboxylic group at C-28. Two signal at δ 80.3 (C-3) and 65.2 (C-23) suggested the presence of hydroxyl group at C-3 and C-23.^{13}C NMR spectrum of compound **1** exhibited 46 signals, out of them 30 signals could be assigned to the aglycone and 16 signals to the sugar moieties, suggested the nature of compound **1** as bidesmosidic.

In the mass spectrum of the compound **1**, characteristic ion peaks at $[M]^+$ m/z 898, 736, 604 and 472 were found by subsequent losses from the molecular ion of each molecule of D-xylose, L-arabinose and D-glucose showing D-xylose as terminal sugar, L-arabinose was linked at C-3 position of aglycone.

Acid hydrolysis of compound **(1)** (Figure 1) with ethanol and 10% H_2SO_4 gave compound **1a** (Figure 2). Compound **1a** has m.p. 231–233°C, molecular formula $C_{30}H_{48}O_4$ $[M]^+$ *m/z* 472 (FABMS). It responded to all the characteristic colour reactions of triterpenoid (Barton 1951; Fieser & Fieser 1966). Thus it was identified as hederagenin. The aqueous hydrolysate obtained after acid hydrolysis of the compound **1** was neutralised with $BaCO_3$ and the $BaSO_4$ was filtered off. The filtrate was concentrated under reduced pressure and subjected to paper chromatography examination using *n*BAW (4 : 1 : 5) as solvent and aniline hydrogen phthalate as detecting agent yielded D-xylose (R*f* 0.28), L-arabinose (R*f* 0.21) and D-glucose (R*f* 0.18) by (Co-Pc and Co-TLC) (Lederer & Lederer 1957).

Permethylation (Kuhn et al. 1955)of compound **1** yielded methylated aglycone identified as 23 *α*-methoxy hederagenin confirmed that glycosidation was involved at C-3 and C-28 position and methylated sugars were identified as 2,3,4-tri-O-methyl-D-xylose (RG 0.94), 2,3-di-O-methyl-L-arabinose (RG 0.64) and 2,3,4,6-tetra-O-methyl-D-glucose (RG 1.0) which showed that C-1″ of D-glucose was attached to C-4′ of L-arabinose and C-1′ of L-arabinose was attached to C-3 position of aglycone and C-1‴ of D-xylose attached to carboxylic group at C-28 position of aglycone thus the interlinkage (1→4) was found between D-glucose and

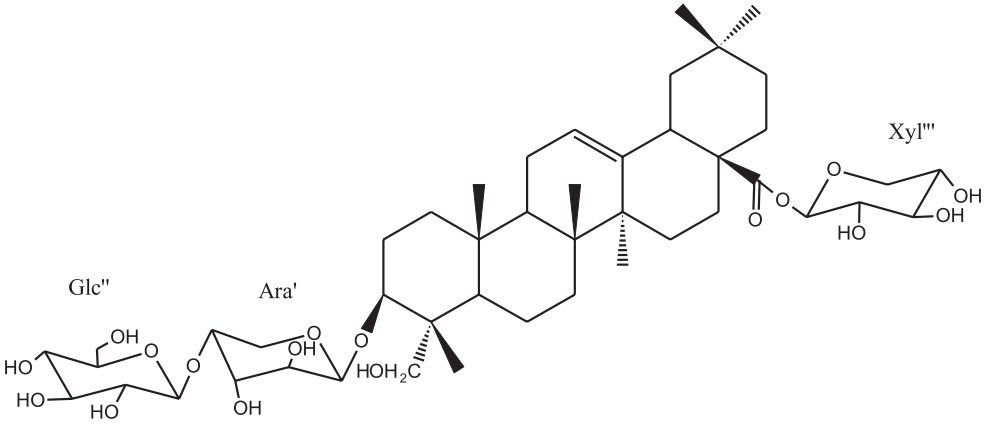

Figure 1. Compound **1**.

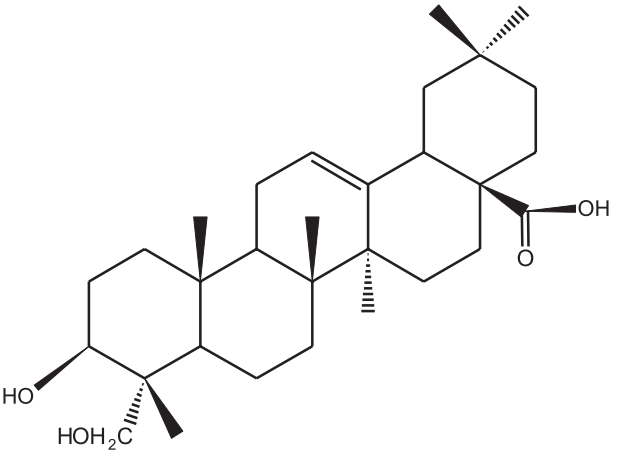

Figure 2. Compound **1a**.

L-arabinose and L-arabinose directly attached to C-3 position of aglycone. This linkage and the linkage between D-xylose attached to carboxylic group at C-28 position of aglycone were further confirmed by ^{13}C NMR spectral data. Periodate oxidation (Hirst & Jones 1949) of compound **1** confirmed that three sugars were present in pyranose form.

Enzymatic hydrolysis (Harborne 1965) of compound **1** with almond emulsin enzyme liberated D-glucose (R_f 0.18) first, followed by D-xylose (R_f 0.28) and proaglycone identified as 3-O-*α*-L-arabinopyranosyl hederagenin confirming the presence of *β*-linkage between D-glucose and Proaglycone as well as between D-xylose and Proaglycone. Proaglycone was further hydrolysed with enzyme takadiastase liberated L-arabinose (R_f 0.21) and aglycone confirming the presence of *α*-linkage between L-arabinose and aglycone.

Quantitative examination (Mishra & Rao 1960) of the sugars present in the hydrolysate revealed that the three sugars were present in equimolecular proportions indicating that the triterpenoid saponin consists of one molecule of each, aglycone (sapogenin), D-xylose, L-arabinose and D-glucose.

Antibacterial activity of compound **1** was screened against various Gram-positive and Gram-negative bacteria. The antimicrobial activity of compound **1** was carried out by agar disc diffusion method at different concentrations (25, 50, 75 and 100 µg/mL). Tetracyclin was used as a standard antibacterial drug. It was concluded that compound **1** showed maximum zone of inhibition (20.1 ± 0.32 mm) against *Bacillus subtilis* at 100 µg/ml concentration and minimum (13.8 ± 0.43 mm) against *Escherichia coli* at same concentration. (Table 1) and from Table 2, it was concluded that compound **1** had lowest MIC value of 1.56 µg/ml against *Bacillus subtilis* and highest MIC value of 6.25 µg/ml against *E. coli*.

On the basis of above, the structure of compound **1** was established as 3-O-*β*-D-glucopyranosyl-(1→4)]-*α*-L-arabinopyranosyl hederagenin 28-O-*β*-D- xylopyranosyl ester and exhibited antibacterial activity against various Gram-positive and Gram-negative bacteria.

3. Experimental

3.1. General experimental procedure

All the melting points were determined on a thermo electrical melting point apparatus and are uncorrected. The IR spectra were measured on Shimadzu 84,005 FTIR spectrophotometer using KBr matrix. UV spectra were recorded on Systronics-2201 UV/Vis Double Beam spectrophotometer in MeOH. The NMR spectral data were recorded at 300 MHz for ^{1}H NMR and 75 MHz for ^{13}C NMR on Bruker DRX 300 NMR spectrometer using TMS as internal standard and CD_3OD as solvent. The FABMS were recorded on a Joel SX (102) Mass spectrometer.

3.2. Plant material

The stems 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 (Bot/Her/A/3157) has been deposited in the Natural Products Laboratory, Department of Chemistry of this university.

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

	Concentration of compound **1** (µg/ mL)				
	25	50	75	100	Tetracycline (stand. drug) (10 µg/ mL)
Pathogenic bacteria	Zone of inhibition (mm)				
Escherichia coli	6.9 ± 0.05**	9.6 ± 0.12*	11.3 ± 0.10**	13.8 ± 0.43*	22.8 ± 0.15**
Bacillus subtilis	10.2 ± 0.09**	15.8 ± 0.18*	17.1 ± 0.07**	20.1 ± 0.32*	24.2 ± 0.12**
Staphylococcus aureus	7.3 ± 0.05**	10.1 ± 0.08**	12.9 ± 0.14*	16.5 ± 0.23*	27.3 ± 0.91*
Pseudomonas aeruginosa	8.2 ± 0.23*	10.8 ± 0.12*	13.01 ± 0.09**	15.1 ± 0.17*	23.1 ± 0.13**

Notes: Data are expressed as Mean ± S.E.
Experiments were conducted in triplicates.
ANOVA followed by Dunnett's test.
p value indicate calculated probability.
*$p < 0.05$, **$p < 0.01$ considered as significant.
**p* indicates that when *p* is less than 0.05 showed *statistically significant* values.
***p* indicates that when *p* is less than 0.01 showed *statistically highly significant* values.

Table 2. Minimum inhibitory concentration of compound **1**.

S. No.	Bacteria	MIC (μg/mL) of compound **1**	MIC (μg/mL) of tetracycline (positive control)
1.	*Escherichia coli*	6.25	0.78
2.	*Bacillus subtilis*	1.56	0.19
3.	*Staphylococcus aureus*	3.12	0.04
4.	*Pseudomonus aueruginosa*	3.12	0.39

3.3. Extraction and isolation

Air-dried and powdered stems (4.00 kg) of the plant were extracted with 95% ethanol (60–80°C) in Soxhlet apparatus for 74 h The total ethanol extract was concentrated under reduced pressure and successively partitioned with petroleum ether (40–60%), $CHCl_3$, $CH_3COOC_2H_5$, CH_3COCH_3 and CH_3OH. The methanol soluble fraction was concentrated under reduced pressure by rotary vacuum evaporator to yield brown viscous mass. It gave two spots on TLC examination using chloroform: methanol: water (6 : 4 : 2) as solvent and I_2 vapours as visualising agent indicating it to be mixture of two compounds **1** and **2**. These were separated by column chromatography over a silica gel column using $CHCl_3$: MeOH (3 : 6) as eluent and purified by TLC and studied separately.

3.4. Study of compound **1**

It was crystallised from methanol to give light brown crystalline compound (1.75 gm), m.p. 272–274°C, m.f. $C_{46}H_{74}O_{17}$ $[M]^+$ *m/z* 898(FABMS). Found (%): C 60.62, H 8.13, O 30.62 calcd. for m.f. $C_{46}H_{74}O_{17}$: C 61.46, H 8.24, O 30.28%, IR (KBr): 3475, 2935, 1669, 1715, 1038, 1353, 1220, 960 cm^{-1}; ^1H NMR (300 MHz, CD_3OD): δ 0.80 (3H, s, Me-24), 1.24 (3H, s, Me-25), δ 1.09 (3H, s, Me-26), 1.31 (3H, s, Me-27), 0.95 (3H, s, Me-29), 1.12 (3H, s, Me-30),1.52 (1H, m, H-1_a), 1.02 (1H, m, H-1_b), 1.83 (1H, m, H-2_a), 1.66 (1H, m, H-2_b), 3.49(1H, dd, *J* 11.2,3.8 Hz, H-3), 1.35 (1H, m, H-5), 1.48 (1H, m, H-6_a), 1.36 (1H, m, H-6_b), 1.59 (1H, m, H-7_a), 1.71 (1H, m, H-7_b), 1.62 (1H, m, H-9), 1.94 (2H, m, H-11), 5.29 (1H, *t*, 3.5 Hz, H-12), 1.79 (1H, m, H-15_a), 1.18 (1H, m, H-15_b), 1.85 (1H, m, H-16_a), 1.57 (1H, m, H-16_b), 2.78 (1H, m, H-18), 1.64 (1H, m, H-19_a), 1.14 (1H, m, H-19_b), 1.39 (1H, m, H-21_a), 1.21 (1H, m, H-21_b), 1.69 (1H, m, H-22_a), 1.53 (1H, m, H-22_b),), 3.52 (1H, d, *J* 11.5 Hz, H_a-23), 3.23 (1H, d, *J* 11.5 Hz, H_b-23)2.83 (1H, d, *J* 11.2 Hz, H-28), δ 4.82 (1H, d, *J* 6.3 Hz, H-1′), 3.68 (1H, dd, *J* 8.2,3.6 Hz, H-2′), 3.64 (1H, dd, *J* 8.3,3.0 Hz, H-3′), 3.75 (1H, m, H-4′), 3.83 (1H, dd, *J* 11.8,2.0 Hz, H-5_a′), 3.27 (1H, dd, *J* 11.2,3.0 Hz, H-5_b′); δ 4.42 (1H, d, *J* 7.2 Hz, H-1″), 3.35 (1H, dd, *J* 7.3, 9.0 Hz, H-2″), 3.54 (1H, t, *J* 9.0, Hz, H-3″), 3.46 (1H, t, *J* 9.0, Hz, H-4″), 3.40 (2H, dd, *J* 3.5,4.5 Hz, H-5″); 3.77 (1H, dd, *J* 3.5,12.0 Hz, H-6_a″), 3.67 (1H, dd, *J* 11.4,12.2 Hz, H-6_b″); δ 4.52 (1H, d, *J* 7.2 Hz, H-1‴), 3.29 (1H, dd, *J* 7.2,9.2 Hz, H-2‴), 3.48(1H, t, *J* 9.0 Hz, H-3‴), 3.69 (1H, m, H-4‴), 4.02 (1H, dd, *J* 5.0,11.2 Hz, H-5_a‴); 3.34 (1H, t, *J* 11.5 Hz, H-5_b‴); ^{13}C NMR (75 MHz, CD_3OD) δ 38.8 (C-1), 27.1 (C-2), 80.3 (C-3), 42.9 (C-4), 49.2 (C-5), 18.9 (C-6), 34.2(C-7), 41.2 (C-8), 50.2 (C-9), 36.8 (C-10), 25.5 (C-11), 123.2 (C-12), 147.4 (C-13), 43.5(C-14), 29.2 (C-15), 25.3 (C-16), 48.2 (C-17), 40.8 (C-18), 45.8 (C-19), 30.8 (C-20), 34.8 (C-21), 32.6 (C-22), 65.2 (C-23), 13.5 (C-24), 19.2(C-25), 17.2 (C-26), 24.4 (C-27), 177.2 (C-28), 31.5 (C-29), 24.8 (C-30), δ 105.2 (C-1′), 75.5 (C-2′), 73.2 (C-3′), 68.4 (C-4′), 70.2(C-5′); δ 103.8 (C-1″), 76.2 (C-2″), 78.5(C-3″), 72.4 (C-4″), 74.2 (C-5″),65.6 (C-6″), δ 104.3 C-1‴), 76.1 (C-2‴), 79.3(C-3‴), 71.4 (C-4‴), 67.5(C-5‴).

3.5. Study of compound 2

It was crystallised from methanol to give colourless crystalline compound (0.710 gm), m.p. 251–253°C, m.f. $C_{64}H_{104}O_{31}[M]^+$ *m/z* 1368 (FABMS). Found (%) : C 55.92%, H 7.23%, O 35.93%, calcd. for $C_{64}H_{104}O_{31}$: C 56.14%, H 7.61%, O 36.25%, IR (KBr): 3150, 2946, 2881, 1730, 1689, 1453, 1370, 1225, 1012, 739 cm^{-1}. It was identified as *3-O-β-D-glucopyranosyl-(1→4)-β-D-xylopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl hederagenin 28-O-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl ester* by comparison of its spectral data with reported literature values (Gulcemal et al. 2014).

3.6. Acid hydrolysis of compound 1

Compound **1** (500 mg) was dissolved in ethanol (30 mL) and refluxed with 10% H_2SO_4 (20 mL) on water bath for 7 h. The reaction mixture was concentrated and allowed to cool and filtered. The residue was extracted with diethyl ether. 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 (5 : 10) as solvent to give compound **1a** as aglycone, which was identified as hederagenin. The aqueous hydrolysate was neutralised with $BaCO_3$ and the $BaSO_4$ was filtered off. The filtrate was concentrated and subjected to paper chromatography examination using *n*-Butanol : Acetic Acid : Water [*n*BAW, 4 : 1 : 5] as solvent and aniline hydrogen phthalate as spraying reagent which showed the presence of D-xylose (R*f* 0.28), L-arabinose (R*f* 0.21) and D-glucose (R*f* 0.18) by (Co-Pc and Co-TLC).

3.7. Study of compound 1a

It had m.p. 231–233°C, molecular formula $C_{30}H_{48}O_4$ $[M]^+$ *m/z* 472 (FABMS). Found: C, 76.22; H, 10.21%; O 13.49 calcd. for m.f. $C_{30}H_{48}O_4$, C 76.27%; H, 10.16%, O 13.55. IR (KBr): 3478, 2940,1725,1670, 1120 cm^{-1}, ^{1}H NMR (300 MHz, CD_3OD): 0.83 (3H, s, Me-24), 1.23 (3H, s, Me-25), δ 1.02 (3H, s, Me-26), 1.32 (3H, s, Me-27), 0.97 (3H, s, Me-29), 1.12 (3H, s, Me-30),1.49 (1H, m, H-1_a), 1.13 (1H, m, H-1_b), 1.78 (1H, m, H-2_a), 1.64 (1H, m, H-2_b), 3.51(1H, dd, *J* 11.2,3.8 Hz, H-3), 1.39 (1H, m, H-5), 1.46 (1H, m, H-6_a), 1.33 (1H, m, H-6_b), 1.54 (1H, m, H-7_a), 1.73 (1H, m, H-7_b), 1.67 (1H, m, H-9), 1.91 (2H, m, H-11), 5.31 (1H, *t*, 3.5 Hz, H-12), 1.85 (1H, m, H-15_a), 1.12 (1H, m, H-15_b), 1.82 (1H, m, H-16_a), 1.59 (1H, m, H-16_b), 2.76 (1H, m, H-18), 1.69 (1H, m, H-19_a), 1.18 (1H, m, H-19_b), 1.42 (1H, m, H-21_a), 1.24 (1H, m, H-21_b), 1.69 (1H, m, H-22_a), 1.58 (1H, m, H-22_b),3.51 (1H, d, *J* 11.5 Hz, H_a-23), 3.28 (1H, d, *J* 11.5 Hz, H_b-23), 2.79 (1H, d, *J* 11.2 Hz, H-28), ^{13}C NMR (75 MHz, CD_3OD) 38.2 (C-1), 28.2 (C-2), 81.1 (C-3), 43.2 (C-4), 48.9 (C-5), 18.5 (C-6), 33.7(C-7), 39.8 (C-8), 49.5 (C-9), 35.1 (C-10), 24.8 (C-11), 122.5 (C-12), 146.3 (C-13), 44.1(C-14), 30.1 (C-15), 26.4 (C-16), 46.8 (C-17), 38.2 (C-18), 43.9 (C-19), 29.5 (C-20), 35.1 (C-21), 30.5 (C-22), 64.8 (C-23), 13.1 (C-24), 18.3(C-25), 17.5 (C-26), 23.7 (C-27), 176.5 (C-28), 30.4 (C-29), 25.5 (C-30).

3.8. Permethylation of compound 1

Compound **1** (35 mg) was dissolved in DMF (25 mL) and treated with MeI (5 mL) and Ag_2O (15 mg) in 150 mL round bottomed flask fitted with condenser and refluxed for two days. The reaction mixture was filtered and washed with DMF. The filtrate was concentrated under

reduced pressure and treated with $CHCl_3$ (25 mL) and washed with water. After removal of solvent a syrupy mass was obtained which was hydrolysed with 7% H_2SO_4 (5 mL) to give aglycone, identified as 23*α*-methoxy hederagenin. The aqueous hydrolysate obtained after the removal of aglycone was neutralised with $BaCO_3$ and the $BaSO_4$ was filtered off. The filtrate was concentrated under reduced pressure and subjected to paper chromatography examination on Whatman filter paper No.1 using *n*-Butanol : Ethanol : Water (5 : 1 : 4) as solvent system and aniline hydrogen phthalate as spraying agent. The methylated sugars were identified as 2,3,4-tri-O-methyl-D-xylose (RG 0.94), 2,3-di-O-methyl-L-arabinose (RG 0.64) and 2,3,4,6-tetra-O-methyl-D-glucose (RG 1.0).

3.9. Enzymatic hydrolysis of compound **1**

Compound **1** (30 mg) was dissolved in MeOH (20 mL) and hydrolysed with equal volume of enzyme almond emulsin. The content was allowed to stay at room temperature for two 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-glucose and D-xylose suggesting the presence of *β* linkage between D-glucose (R_f 0.18) and Proaglycone as well as between D-xylose (R_f 0.28) and Proaglycone. Proaglycone on further hydrolysis with enzyme takadiastase yielded L-arabinose (R_f 0.21) and aglycone suggesting the presence of α linkage between L-arabinose and aglycone.

3.10. Antibacterial activity of compound **1**

The antibacterial activity of compound **1** was performed by agar disc diffusion method (Doughari 2006). Compound **1** was dissolved in water and further diluted to obtain desired concentrations (25, 50, 75 and 100 µg/mL). Tetracyclin was used as a standard antibacterial drug. The bacterial strains were activated by inoculating a loopful of the strains in the nutrient broth (30 mL) and incubated for 6 h to maintain standard turbidity (10^6 cells/mL). After that 1 mL of inoculum of bacterial strains was swabbed in Muller Hinton agar plate. Disc (6 mm) was dipped in different concentrations (25, 50, 75 and 100 µg/mL) of compound **1** and allowed to dry. Then the disc was impregnated on the seeded agar plates. The plates were allowed to stand for 1 h for pre-diffusion of the compound **1** and incubated at 37°C for 24 h. The antimicrobial activity was taken on the basis of diameter of zone of inhibition (mm) around each paper disc. All the experiments were done in triplicates. The results obtained from experimental findings are recorded in the Table 1.

3.11. Minimum inhibitory concentrations of compound **1**

The minimum inhibitory concentrations of compound **1** were determined by two fold broth micro-dilution method. Bacterial cultures were activated by transferring a loopful of strains from stock culture to tubes and inoculated in Nutrient-broth (NB) medium then incubated for 24 h at 37°C. The bacterial cultures were diluted with fresh NB media to achieve optical density (600 nm) of 10^7 CFU/ml. Compound **1** was incorporated into NB medium to get a concentration of 100 µg/ml which was further serially diluted to 50, 25, 12.5, 6.25, 3.12, 1.56 and 0.78 µg/ml, respectively. The tubes were then inoculated with 20 µl microbial suspension

and incubated at 37°C for 24 h. Minimum concentrations of the assayed samples (Compound **1**), which inhibits the visible growth of the tested microorganisms were considered as MIC of the compound **1**. The results obtained from experimental findings are recorded in the Table 2 (Clinical & Laboratory Standards Institute 2012).

4. Conclusion

Thus, on the basis of above evidences the structure of compound **1** was established as 3-O-*β*-D-glucopyranosyl-(1→4)-*α*-L-arabinopyranosyl hederagenin 28-O-*β*-D- xylopyranosyl ester from the methanolic extract of the stems of the plant. Compound **1** showed significant antibacterial activity and thus it may be used as a potent antibacterial agent against diseases caused by these bacteria.

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