

NEW ANTIBACTERIAL TRITERPENOID SAPONIN FROM STEMS OF CASSIA ABSUS LINN.

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ABSTRACT

A new triterpenoid saponin 3-O-[α -L-rhamnopyranosyl-(1 \rightarrow 3)-O- β -D-xylopyranosyl-(1 \rightarrow 4)-O- β -D-glucopyranosyl]-bauerenol (1), together with two known compounds 3- β -hydroxyolean-12-ene-28-oic acid and β -sitosterol were isolated from ethanolic extract of the stems of *Cassia absus* Linn. Their structures were elucidated by various colour reactions, spectral analysis and chemical degradations. Compound 1 showed antibacterial activity.

KEYWORDS: *Cassia absus* Linn., Fabaceae, Triterpenoid saponin, Antibacterial activity.

INTRODUCTION

Cassia absus Linn.^[1-5] belongs to family Fabaceae and subfamily caesalpinioideae, which is commonly known as “**Chaksi**” or “**Chaksu**” in Hindi and “**Aranyakullithaka**” in Sanskrit. It is an erect annual plant with height in the range of 1-2 ft. It is found in Tropical Asia, continents of Australia, Africa, Central America and throughout India. Its leaves are hot, bitter and acrid; astringent to the bowels. It is traditionally used in the treatment of vata and kapha, tumours, cough, disease of nose, hiccup, asthma, hypertension, renal stones, hemorrhoids, leucoderma and hepatic diseases. Recent research studies have shown antioxidant, antidiabetic, antihypertensive, anti-inflammatory, antiglycation, antiacetylcholine effect, diuretic and stimulant properties. Its seeds are used in the treatment of ringworm, ophthalmia and skin affections. Earlier workers^[6-11] have reported various chemical constituents from this plant. In the present paper we report the isolation and structural elucidation of a new triterpenoid saponin 3-O-[α -L-rhamnopyranosyl-(1 \rightarrow 3)-O- β -D-xylopyranosyl-(1 \rightarrow 4)-O- β -D-glucopyranosyl]-bauerenol (1) along with two known

compounds 3- β -hydroxyolean-12-ene-28-oic acid (**2**) and β -sitosterol (**3**) from ethanolic extract of the stems of this plant.

RESULTS AND DISCUSSION

Chemical examination of ethanolic extract of stems of *Cassia absus* Linn., yielded a new compound **1**. It has molecular formula $C_{47}H_{78}O_{14}$, m. p. 232-234 $^{\circ}$ C, $[M]^+$ 866 (FABMS). Positive results with Liebermann-Burchard,^[12] haemolytic test,^[13] foam test^[14] and molisch's reagents evidenced its triterpenoidal saponin glycosidic nature. It also gave positive molisch test. The IR spectrum of compound **1** revealed an absorption band at 3372 cm^{-1} (OH), 1623(double bond), 1376 (gem dimethyl) and 1031(suggestive of a glycosidic compound). In 1H -NMR spectrum, a double doublet at δ 5.41 (1H, dd, J 2.7, 6.8Hz, H-7) showed the presence of trisubstituted olefinic proton which was further supported by ^{13}C -NMR values at δ 116.1 (C-7) and 145.3 (C-8). In 1H -NMR a double doublet at δ 3.16 (1H, dd, J 4.1, 11.2 Hz) was assigned to H-3. Signals at δ 2.16 (1H, m), δ 1.28 (1H, m) and δ 1.27 (1H, m) were assigned to H-5 α , H-9 α and H-18 β respectively. Eight singlets at δ 0.84, 0.95, 0.76, 0.98, 0.93, 1.03, 1.07 and 0.91 showed the presence of eight methyl groups at C-23, C-24, C-25, C-26, C-27, C-28, C-29 and C-30. The anomeric proton signals at δ 5.02 (1H, d, J 7.2Hz, H-1'), 5.05 (1H, d, J 7.6Hz, H-1'') and 4.93 (1H, d, J 7.8Hz, H-1''') were assigned for H-1', H-1'' and H-1''' of D-glucose, D-xylose and L-rhamnose respectively. In ^{13}C -NMR spectrum of compound **1** showed the presence of 47 carbon atoms. Seventeen carbon signals seen for the sugar moieties confirmed the presence of three monosaccharides one pentose and two hexose units. The remaining 30 signals were due to the triterpenoid aglycone. The downfield ^{13}C -NMR chemical shift at δ 79.2 suggested that compound **1** was a triterpenoid saponin with glycosidic linkage at C-3 through an ether bond.

In the mass spectrum of the compound **1**, characteristic ion peaks at $[M]^+$ m/z 866, 720, 588 and 426 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 and D-glucose was attached at C-3 position of aglycone.

Acid hydrolysis of compound **1** with 10% ethanolic H_2SO_4 gave aglycone **1-A**, m.p. 258-260 $^{\circ}$ C, m.f. $C_{30}H_{50}O$, $[M]^+$ 426 (EIMS). It responded all the colour reactions of triterpenoids.^[15] It was identified as bauerenol by comparison of its spectral data with reported literature values.^[16-18]

The aqueous hydrolysate after the removal of aglycone was neutralized with BaCO_3 and the 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 0.26) and D-glucose (R_f 0.19) (Co-PC and Co-TLC).^[19] Periodate oxidation of compound **1**, confirmed that all the sugars were present in the pyranose form.^[20]

Permethylation^[21] followed by acid hydrolysis of compound **1** yielded aglycone identified as bauerenol showed that glycosylation was involved at C-3 and methylated sugars were identified as 2, 3, 4-tri-O-methyl-L-rhamnose (R_G 1.01), 2, 4-di-O-methyl- D-xylose (R_G 0.66) and 2, 3, 6-tri-O-methyl-D-glucose (R_G 0.83), indicating that C-1''-OH of L-rhamnose was linked to C-3''-OH of D-xylose, C-1''-OH of D-xylose was linked to C-4'-OH of D-glucose and C-1'-OH of D-glucose was linked with C-3 position of the aglycone. Therefore it was concluded that interlinkages (1 \rightarrow 3) between L-rhamnose and D-xylose and interlinkages (1 \rightarrow 4) between D-xylose and D-glucose. The linkages were further confirmed by spectral data of ^{13}C -NMR.

Enzymatic hydrolysis^[22] of compound **1** with takadiastase liberated L-rhamnose (R_f 0.37) and proaglycone identified as 3-O- $[\beta$ -D-xylopyranosyl-(1 \rightarrow 4)-O- β -D-glucopyranosyl]-bauerenol confirming the presence of α -linkage between L-rhamnose and proaglycone. Proaglycone on further hydrolysis with enzyme almond emulsion liberated D-xylose (R_f 0.26) followed by D-glucose (R_f 0.19) suggesting the presence of β -linkage between D-xylose and D-glucose as well as between D-glucose and aglycone.

On the basis of above evidences, the structure of compound **1** was characterized as 3-O- $[\alpha$ -L-rhamnopyranosyl-(1 \rightarrow 3)-O- β -D-xylopyranosyl-(1 \rightarrow 4)-O- β -D-glucopyranosyl]-bauerenol.

Compound **1** was screened for antibacterial activity against various gram (+ve) gram (-ve) bacteria. The results reported in **Table I** show that compound **1** was found to be active against gram (+ve) bacteria *Bacillus subtilis*, *Micrococcus luteus* and gram (-ve) bacteria *E.coli* and no activity was found against gram (+ve) bacteria *Staphylococcus aureus*. So it was concluded that compound **1** may potentially be used active agent in diseases caused by these bacteria.

Compound **2**, has m.p. 302-304°C, m.f. $C_{30}H_{48}O_3$, $[M]^+$ 456 (EIMS). It was characterized as 3- β -hydroxyolean-12-ene-28-oic acid by comparison of its spectral data with reported literature values.^[23-25]

Compound **3**, has m.p. 132-134°C, m.f. $C_{29}H_{50}O$, $[M]^+$ 414 (EIMS). It was identified as β -sitosterol by comparison of its spectral data with reported literature values.^[26-27]

EXPERIMENTAL SECTION

Plant material

The stems 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 cm^{-1}). 1H -NMR and ^{13}C -NMR spectra were recorded using solvent $CDCl_3$ and TMS as internal standard on Bruker DRX-300 spectrometer.

Extraction and isolation

Air dried powdered plant stems (3.5kg) were extracted with 95% ethanol (45-60°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 (3.75gm), which was subjected to TLC examination using nBAW (4:1:5) as eluent and I_2 vapours as visualizing agent. It gave three spots indicating it to be a mixture of three compounds **1**, **2** and **3**. These compounds were separated by TLC and purified by column chromatography over silica gel using $CHCl_3$: MeOH (3:6) as eluent and studied separately.

Study of compound 1

It was crystallised from acetone to yield 1.25 gm. It has m.p. 182-184°C, m.f. $C_{47}H_{78}O_{14}$, $[M]^+$ 866 (FABMS); found(%): C 65.08, H 8.92, O 25.80 calcd.(%) for m.f. $C_{47}H_{78}O_{14}$: C 65.13, H 9.01, O 25.87; IR: ν_{max}^{KBr} (cm^{-1}), 3372(-OH), 2922, 2860, 1623(trisub. double bond), 1450, 1372, 1031, 930, 812; 1H -NMR (500 MHz, $CDCl_3$): δ (ppm): 1.06-1.59 (2H, m, H-1),

1.62 (2H, m, H-2), 3.16 (1H, dd, J 4.1, 11.2 Hz, H-3), 2.16 (1H, m, H-5), 1.94-2.12 (2H, m, H-6), 5.41 (1H, dd, J 2.7, 6.8 Hz, H-7), 1.28 (1H, m, H-9), 1.46-1.48 (2H, m, H-11), 1.12-1.57 (2H, m, H-12), 1.39-1.47 (2H, m, H-15), 1.45-1.50 (2H, m, H-16), 1.27 (1H, m, H-18), 1.12 (1H, m, H-19), 1.52 (1H, m, H-20), 1.18-1.48 (2H, m, H-21), 1.16-1.46 (2H, m, H-22), 0.84 (3H, s, Me-23), 0.95 (3H, s, Me-24), 0.76 (3H, s, Me-25), 0.98 (3H, s, Me-26), 0.93 (3H, s, Me-27), 1.03 (3H, s, Me-28), 1.07 (3H, s, Me-29), 0.91 (3H, s, Me-30), 5.02 (1H, d, J 7.2 Hz, H-1'), 4.32 (1H, d, J 8.0 Hz, H-2'), 4.42 (1H, d, J 8.0 Hz, H-3'), 4.63 (1H, m, H-4'), 4.21 (1H, d, J 9.0 Hz, H-5'), 4.45 (2H, m, H-6'), 5.05 (1H, d, J 7.6 Hz, H-1''), 3.50-4.41 (5H, m, H-2'', H-3'', H-4'', H-5''), 4.93 (1H, d, J 7.8 Hz, H-1'''), 4.82 (1H, d, J 2.4 Hz, H-2'''), 4.66 (1H, dd, J 3.1, 9.6 Hz, H-3'''), 4.32 (1H, m, H-4'''), 4.77 (1H, m, H-5'''), 1.06 (3H, J 6.2 Hz, Me-6'''). ^{13}C -NMR (125 MHz, CDCl_3), δ (ppm): 36.9 (C-1), 27.7 (C-2), 79.2 (C-3), 38.6 (C-4), 50.2 (C-5), 24.4 (C-6), 116.1 (C-7), 145.3 (C-8), 48.6 (C-9), 35.4 (C-10), 17.2 (C-11), 32.5 (C-12), 37.4 (C-13), 41.5 (C-14), 28.6 (C-15), 37.6 (C-16), 32.3 (C-17), 54.5 (C-18), 35.6 (C-19), 32.2 (C-20), 29.7 (C-21), 31.8 (C-22), 27.7 (C-23), 14.9 (C-24), 13.3 (C-25), 23.4 (C-26), 22.5 (C-27), 40.2 (C-28), 25.7 (C-29), 22.6 (C-30), 105.2 (C-1'), 83.8 (C-2'), 77.6 (C-3'), 73.2 (C-4'), 74.5 (C-5'), 61.4 (C-6'), 102.58 (C-1''), 79.53 (C-2''), 78.29 (C-3''), 70.89 (C-4''), 66.94 (C-5''), 101.3 (C-1'''), 71.5 (C-2'''), 70.3 (C-3'''), 73.7 (C-4'''), 69.5 (C-5'''), 18.6 (C-6''') and $[\text{M}]^+$ 866 (FABMS).

Acid hydrolysis of compound 1

Compound **1** (500 mg) was dissolved in ethanol (25 ml) and refluxed with 20 ml of H_2SO_4 on water bath for 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 (5:10) to give compound **1-A**, identified as bauerenol 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 0.37), D-xylose (R_f 0.26) and D-glucose (R_f 0.19) (Co-PC and Co-TLC).

Study of compound 1-A

It has m.f. $\text{C}_{30}\text{H}_{50}\text{O}$, m.p. 204-206°C, $[\text{M}]^+$ 426 (EIMS); found(%): C 84.43, H 11.68, O 3.72, calcd (%) for m.f. $\text{C}_{30}\text{H}_{50}\text{O}$, C 84.51, H 11.74, O 3.76; IR: $\nu_{\text{max}}^{\text{KBr}}$ (cm^{-1}), 3376 (-OH), 2926,

2864, 1626 (trisub. double bond), 1454, 1376 (gem dimethyl), 1036, 935, 814; $^1\text{H-NMR}$ (500 MHz, CDCl_3) : δ (ppm): 1.07-1.60 (2H, m, H-1), 1.63 (2H, m, H-2), 3.18 (1H, dd, J 4.2, 11.4 Hz, H-3), 2.17 (1H, m, H-5), 1.95-2.14 (2H, m, H-6), 5.40 (1H, dd, J 2.7, 6.8 Hz, H-7), 1.27 (1H, m, H-9), 1.45-1.50 (2H, m, H-11), 1.12-1.57 (2H, m, H-12), 1.39-1.47 (2H, m, H-15), 1.45-1.50 (2H, m, H-16), 1.27 (1H, m, H-18), 1.12 (1H, m, H-19), 1.52 (1H, m, H-20), 1.18-1.48 (2H, m, H-21), 1.16-1.46 (2H, m, H-22), 0.84 (3H, s, Me-23), 0.95 (3H, s, Me-24), 0.76 (3H, s, Me-25), 0.98 (3H, s, Me-26), 0.93 (3H, s, Me-27), 1.02 (3H, s, Me-28), 1.07 (3H, s, Me-29), 0.92 (3H, s, Me-30). $^{13}\text{C-NMR}$ (125 MHz, CDCl_3), δ (ppm): 37.0 (C-1), 27.9 (C-2), 79.2 (C-3), 38.8 (C-4), 50.1 (C-5), 24.3 (C-6), 116.3 (C-7), 145.2 (C-8), 48.8 (C-9), 35.2 (C-10), 17.1 (C-11), 32.6 (C-12), 37.5 (C-13), 41.7 (C-14), 28.8 (C-15), 37.9 (C-16), 32.2 (C-17), 54.6 (C-18), 35.5 (C-19), 32.3 (C-20), 29.5 (C-21), 31.8 (C-22), 27.7 (C-23), 14.9 (C-24), 13.3 (C-25), 23.4 (C-26), 22.5 (C-27), 40.2 (C-28), 25.7 (C-29), 22.6 (C-30).

Permethylation of compound 1

Compound **1** (30mg) was refluxed with MeI (5ml) and Ag_2O (20ml) in DMF (20 mg) for three days. The reaction mixture was filtered and washed with DMF. The filtrate was concentrated under reduced pressure and treated with CHCl_3 (20ml) and washed with water. After removal of solvent a syrupy mass was obtained which was hydrolyzed with 8% ethanolic H_2SO_4 (10ml) for 6-7 hrs, to give aglycone, identified as bauerenol. 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 (5:1:4) solvent and aniline hydrogen phthalate as spraying agent. The methylated sugars were identified as 2, 3, 4-tri-O-methyl-L-rhamnose (R_G 1.01), 2, 4-di-O-methyl- D-xylose (R_G 0.66) and 2, 3, 6-tri-O-methyl-D-glucose (R_G 0.83).

Enzymatic hydrolysis of compound 1

Compound **1** (25 mg) was dissolved in MeOH (10 ml) and hydrolysed with equal volume of takadiastase enzyme. The reaction mixture was allowed to stay at room temperature for 42 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 L-rhamnose (R_f 0.37). The proaglycone was dissolved in MeOH (20 ml) and further hydrolysed with equal volume of almond emulsion enzyme at room temperature as usual

procedure yielded aglycone and sugars were identified as D-xylose (R_f 0.26) and D-galactose (R_f 0.19).

Study of compound 2

It has m.f. $C_{30}H_{48}O_3$, m.p. 302-304°C, $[M]^+$ 456 (EIMS); found (%), C 78.82, H 10.46, O 10.48, calcd (%) for m.f. $C_{30}H_{48}O_3$, C 78.95, H 10.53, O 10.53; UV: λ_{max} nm: (MeOH) 236, 280; IR: ν_{max}^{KBr} (cm^{-1}), 3426 (O-H), 2928, 2850, 1710 (C=O), 1684, 1450, 1248, 1080, 1032, 998, 824, 688. 1H -NMR (300 MHz, $CDCl_3$): δ (ppm) 5.26 (1H, t, J 3.6 Hz, H-12), 3.12 (1H, t, J 6.9 Hz, H-3), 1.35 (2H, t, J 6.8 Hz, H-16), 2.76 (1H, dd, J 3.6, 14.2 Hz, H-18), 1.36 (2H, t, J 6.8 Hz, H-21), 0.76 (3H, s, Me-26), 0.88 (3H, s, Me-24), 0.92 (3H, s, Me-29), 0.98 (3H, s, Me-25), 1.06 (3H, s, Me-30), 1.15 (3H, s, Me-23), 1.21 (3H, s, Me-27). ^{13}C -NMR (75 MHz, $CDCl_3$): δ (ppm) 38.44 (C-1), 27.15 (C-2), 79.10 (C-3), 39.28 (C-4), 55.26 (C-5), 18.33 (C-6), 32.71 (C-7), 37.12 (C-8), 47.65 (C-9), 38.70 (C-10), 23.10 (C-11), 122.74 (C-12), 143.58 (C-13), 41.64 (C-14), 27.63 (C-15), 23.07 (C-16), 46.48 (C-17), 41.22 (C-18), 45.83 (C-19), 30.65 (C-20), 33.72 (C-21), 32.34 (C-22), 28.13 (C-23), 15.50 (C-24), 15.38 (C-25), 17.05 (C-26), 25.90 (C-27), 180.02 (C-28), 32.63 (C-29), 23.36 (C-30).

Study of compound 3

It has m.f. $C_{29}H_{50}O$, m.p. 132-134°C, $[M]^+$ 414 (EIMS); found (%), C 83.92, H 11.98, O 3.81, calcd (%) for m.f. $C_{29}H_{50}O$, C 84.06, H 12.08, O 3.86; IR: ν_{max}^{KBr} (cm^{-1}), 3422 (O-H), 2928 (aliphatic C-H stretching), 1582 (C=C), 1468 (C=O), 1032 (cycloalkane), 882. 1H -NMR (400 MHz, $CDCl_3$): δ (ppm) 3.68 (1H, m, H-3), 5.22 (1H, m, H-6), 5.16 (1H, m, H-23), 4.62 (1H, m, H-22), 2.34 (1H, m, H-20), 0.65 (3H, s, Me-18), 0.73 (3H, s, Me-29), 0.83 (3H, s, Me-27), 0.88 (3H, s, Me-26), 0.91 (3H, s, Me-21), 0.93 (3H, s, Me-19); ^{13}C -NMR (100 MHz, $CDCl_3$): δ (ppm) 37.14 (C-1), 28.44 (C-2), 79.07 (C-3), 38.7 (C-4), 45.6 (C-5), 118.86 (C-6), 34.26 (C-7), 32.61 (C-8), 50.41 (C-9), 37.17 (C-10), 21.3 (C-11), 38.4 (C-12), 39.6 (C-13), 55.8 (C-14), 28.5 (C-15), 29.75 (C-16), 55.10 (C-17), 19.35 (C-19), 35.6 (C-20), 17.75 (C-21), 139.5 (C-22), 29.9 (C-23), 50.6 (C-24), 29.83 (C-25), 26.6 (C-26), 21.9 (C-27), 27.2 (C-28), 15.8 (C-18, C-29).

Antibacterial activity of compound 1

The antibacterial activity of compound **1** was determined by Filter Paper Disc Diffusion method.^[28-29] The various Gram (+ve) and Gram (-ve) bacterial species were first incubated at

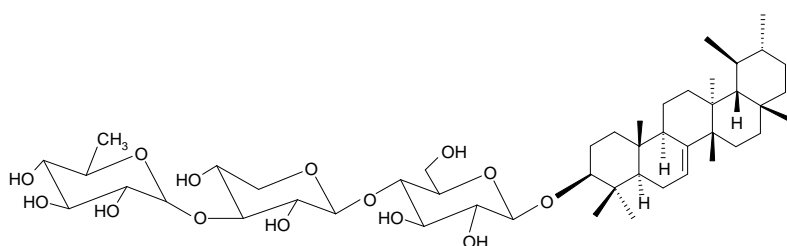
46°C for 44 hr. The sterile filter paper discs (8 mm) were soaked with standard antibacterial agent and various test samples and were dried at 46°C. The discs were then placed on soft nutrient agar (2%) petri plates previously seeded with suspension of each bacterial species. The diameters of zone of inhibition were measured at $38\pm1^\circ\text{C}$ after 24 hr. The results are recorded in **Table I**.

Table I: Antibacterial activity of compound 1.

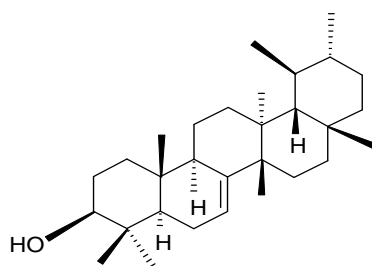
Sr. No.	Bacterial species	Diameters of zone of inhibition (mm) [*]				Std. ^{**}
		Concentration of compound 1 (%)				
		100	80	60	40	
1.	<i>Bacilius subtilis</i>	15.4	12.7	6.8	4.3	20.8
3.	<i>Escherichia coli</i>	13.2	9.8	5.9	-	20.5
2.	<i>Micrococcus luteus</i>	9.2	8.4	6.4	1.7	15.8
4.	<i>Staphylococcus aureus</i>	3.8	1.6	-	-	22.4

^{*}The zone of inhibition (mm) taken as average of four determination direction.

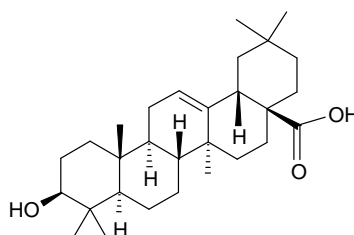
^{**}Ampicillin (10 mg/mL) used as standard antibacterial agent.



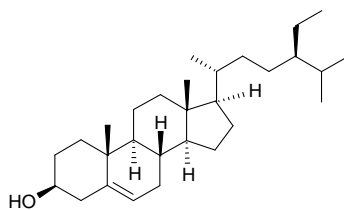
Compound 1



Compound 1A



Compound 2

**Compound 3**

CONCLUSIONS

Thus on the basis of above evidences the structure of compound **1** was established as 3-O-[α -L-rhamnopyranosyl-(1 \rightarrow 3)-O- β -D-xylopyranosyl-(1 \rightarrow 4)-O- β -D-glucopyranosyl]-bauerenol, along with two known compounds 3- β -hydroxyolean-12-ene-28-oic acid and β -sitosterol from ethanolic extract of the stems of *Cassia absus* Linn. Compound **1** showed significant antibacterial activity against various gram positive and gram negative bacteria. Hence, compound **1** can be used as a potent antibacterial agent.

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