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## ORIGINAL ARTICLE

# Two new phytosterols from the stem bark of *Ficus bengalensis* L.



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Bengalensisteroic acid  
acetate

**Abstract** Phytochemical investigation of the methanolic extract of the stem bark of *Ficus bengalensis* L. (Moraceae) resulted in the isolation of two new phytoconstituents characterized as lanost-5,24-dien-3 $\beta$ -yl- $\beta$ -D-glucopyranosid-2'-O-yl-docos-11"-enoate (lanostadienylglucosyl cetoleate) (**1**) and 3 $\beta$ -acetoxy-stigmast-22-en-26-oic acid (bengalensisteroic acid ester) (**2**) together with the known compounds heneicosanyl *cis*-octadec-9-enoate (heneicosanyl oleate) (**3**), urs-12-en-3 $\beta$ -yl acetate ( $\alpha$ -amyrin acetate) (**4**), and lup-20(29)-en-3 $\beta$ -ol (lupeol) (**5**). The structures of these phytoconstituents have been established on the basis of spectral data analysis, chemical reactions and comparison of literature.

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## 1. Introduction

*Ficus bengalensis* L. (Moraceae), commonly known as the banyan tree, occurs throughout the forest tracts of India, both in the sub-Himalayan region and in the deciduous forest of southern India (Kirtikar and Basu, 1993). It is a tree native to Bangladesh, India and Sri Lanka and now cultivated in Pakistan (Shivrajan, 2002). It has large dimensions, the leafy crown sometimes attains a circumference of 300–700 m. It is evergreen except in dry localities where it is leafless for a short time. It is drought resistant; it withstands mild frost (Kirtikar and Basu, 1993). Its bark is used in traditional medicine as antidiabetic (Rajagopal and Sasikala, 2008; Cherian and Augusti, 1993) and exhibited antidiarrhoeal (Mukherjee et al., 1998),

antioxidant (Augusti et al., 2005), antiasthmatic (Taur et al., 2007), hypocholesterolemic (Cherian and Augusti, 1993; Shukla et al., 1995; Rajagopal and Sasikala, 2008) and wound healing activities (Biswas and Mukherjee, 2003). Earlier flavonoids, aliphatic ketones, methyl ethers of leucoanthocyanins (Cherian and Augusti, 1995),  $\beta$ -sitosterol- $\alpha$ -D-glucoside and meso-inositol have been reported from the bark (Subramanian and Misra, 1978). The present paper describes the isolation and characterization of two new phytosterols **1** and **2** along with the three known phytoconstituents **3–5** from the bark of *F. bengalensis*.

## 2. Materials and methods

## 2.1. General

The melting points were determined on a Perfit apparatus and are uncorrected. The IR spectra were recorded on KBr pellet using a jasco FT/IR-5000 instrument (FTS 135, Hongkong). The  $^1\text{H}$  NMR (400 MHz) and  $^{13}\text{C}$  NMR (100 MHz) spectra were scanned on DRX-400 Avance 400 MHz spectrometer (Bruker-Biospin, Rheinstetten, Germany) using  $\text{CDCl}_3$  as

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solvent and TMS as internal standard. FAB-MS were measured using JEOL-JMS-DX 303 spectrometer (Peabody, MA, USA). Column (450 × 4 × 0.2 cm) chromatography was performed on silica gel (60–120 mesh, Qualigens, Mumbai, India) and thin layer chromatography on silica gel G-coated TLC plates (Merck) with solvents (chloroform, chloroform:methanol 97:3, petroleum ether, petroleum ether:chloroform 1:1, toluene:ethyl acetate:formic acid 1:1:0.3). Spots were visualized by exposing to iodine vapours, UV radiation, and spraying ceric sulphate solution.

## 2.2. Plant material

The bark of *F. bengalensis* was collected from the campus of Jamia Hamdard, New Delhi in August 2007. The plant was identified by M. P. Sharma, Taxonomist and Professor, Department of Botany, Faculty of Science, Jamia Hamdard, New Delhi. A voucher specimen (PRL/JH/07 No. 28) of drug is deposited in the herbarium of the Phytochemistry Research Laboratory, Department of Pharmacognosy and Phytochemistry, Faculty of Pharmacy, Jamia Hamdard, New Delhi.

## 2.3. Extraction and isolation

The *F. bengalensis* bark was sorted out by hand to remove impurities, dried in air and then in an oven at 45 °C. The material was then coarsely powdered. Exhaustive extraction of 1.7 kg of dried powdered drug was carried out in a Soxhlet apparatus using methanol as the extracting solvent. The methanolic extract was concentrated under reduced pressure to yield a dark brown viscous mass (148 g, 8.7%). The preliminary phytochemical screening of the extract showed the presence of triterpenes, carbohydrates, cardiac glycosides, saponins and amino acids. The extract was dissolved in a minimum amount of methanol and adsorbed on silica gel (60–120 mm, 245 g) for the preparation of slurry. The air dried slurry was chromatographed over the silica gel column packed in petroleum ether (60–80 °C). The column was eluted with petroleum ether (60–80 °C), petroleum ether–chloroform (9:1, 1:1, 1:3, v/v), chloroform, chloroform–methanol (99:1, 98:2, 95:5, 9:1, 3:1, 1:1, 1:3, v/v) and methanol in their various combinations in the order of increasing polarity to isolate the phytoconstituents.

## 2.4. Lanostadienylglucosyl cetoleate (1)

Elution of the column with chloroform afforded colourless semisolid mass of **1**, recrystallized from acetone, 134 mg (0.178% yield);  $R_f$  value: 0.58 (chloroform); m.p.: 38–39 °C; UV ( $\lambda_{\max}$  nm, MeOH): 207 (log  $\epsilon$  5.7); IR ( $\gamma_{\max}$  (KBr): 3433, 2922, 2851, 1731, 1645, 1462, 1380, 1265, 1073, 740  $\text{cm}^{-1}$ );  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ):  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  5.36 (1H, m, H-6), 5.12 (1H, m, H-24), 5.03 (1H, m, H-11''), 4.97 (1H, m, H-12''), 4.92 (1H, d,  $J = 7.1$  Hz, H-1'), 4.70 (1H, m, H-5'), 4.49 (1H, m, H-2'), 4.30 (1H, m, H-3'), 3.87 (1H, m, H-4'), 3.81 (1H, dd,  $J = 5.5, 9.3$  Hz, H-3 $\alpha$ ), 3.03 (1H, d,  $J = 6.9$  Hz, H<sub>2</sub>-6'a), 3.01 (1H, d,  $J = 6.9$  Hz, H<sub>2</sub>-6'b), 2.32 (1H, d,  $J = 6.9$  Hz, H<sub>2</sub>-2''a), 2.30 (1H, d,  $J = 6.9$  Hz, H<sub>2</sub>-2''b), 1.70 (3H, brs, Me-26), 1.62 (3H, brs, Me-27), 0.98 (3H, brs, Me-19), 0.91 (3H, d,  $J = 7.2$  Hz, Me-21), 0.87 (3H, brs, Me-28), 0.85 (3H, t,  $J = 6.3$  Hz, Me-22''), 0.82 (3H, brs, Me-29), 0.78 (3H, brs, Me-30), 0.70 (3H, brs, Me-18);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  39.72

(C-1), 32.81 (C-2), 76.49 (C-3), 42.22 (C-4), 139.07 (C-5), 121.53 (C-6), 33.76 (C-7), 33.71 (C-8), 48.33 (C-9), 38.69 (C-10), 22.58 (C-11), 36.04 (C-12), 47.86 (C-13), 49.85 (C-14), 25.37 (C-15), 28.05 (C-16), 52.20 (C-17), 13.61 (C-18), 19.25 (C-19), 35.50 (C-20), 18.25 (C-21), 34.06 (C-22), 27.11 (C-23), 114.01 (C-24), 135.97 (C-25), 26.42 (C-26), 26.01 (C-27), 18.47 (C-28), 24.77 (C-29), 21.02 (C-30), 101.36 (C-1'), 78.72 (C-2'), 71.66 (C-3'), 67.82 (C-4'), 79.61 (C-5'), 62.01 (C-6'), 171.55 (C-1''), 56.71 (C-2''), 32.81 (C-3''), 31.83 (C-4''), 29.59 (C-5''-C-9''), 45.22 (C-10''), 124.65 (C-11''), 130.73 (C-12''), 41.02 (C-13''), 31.79 (C-14''), 29.59 (C-15'' to C-19''), 29.53 (C-20''), 22.64 (C-21''), 14.01 (C-22''); +ve ion FAB MS  $m/z$  (rel. int.): 909  $[\text{M} + \text{H}]^+$  ( $\text{C}_{58}\text{H}_{101}\text{O}_7$ ) (1.5), 797 (11.3), 459 (10.3), 425 (29.8), 410 (70.1), 408 (26.3), 395 (41.2), 380 (13.7), 338 (32.7), 299 (19.3), 297 (22.1), 274 (17.8), 273 (17.5), 260 (15.8), 258 (19.8), 203 (60.7), 202 (42.7), 189 (41.8), 175 (53.2), 165 (26.3), 151 (19.8), 148 (53.3), 134 (52.9).

## 2.5. Hydrolysis of 1

Compound **1** (45 mg) was dissolved in 95% ethanol (5 mL), 1 N (mol  $\text{L}^{-1}$ ) NaOH solution (2 mL) was added and the reaction mixture was heated on a steam bath for 1 h. The solvent was evaporated under reduced pressure and the residue dissolved in chloroform to separate lanosta-5,24-dien-3 $\beta$ -ol,  $\gamma_{\max}$  3450  $\text{cm}^{-1}$ ;  $[\text{M}]^+$   $m/z$  426 ( $\text{C}_{30}\text{H}_{50}\text{O}$ ). The residue after the separation of triterpenoid was dissolved in  $\text{H}_2\text{O}$  (3 mL), acidified with dil. HCl to Congo red and extracted with  $\text{CHCl}_3$  (3 × 5 mL) to separate cetoleic acid, m.p. 32–33 °C, (co-TLC comparable) (Gunstone and Herslof, 2000). The aqueous solution was concentrated and chromatographed over TLC along with a standard solution of D-glucose using *n*-BuOH– $\text{H}_2\text{O}$ –AcOH (4:1:5) as a developing solvent.  $R_f$  0.12 was comparable.

## 2.6. Bengalensisteroic acid acetate (2)

Further elution of the same column with chloroform–methanol (97:3) eluent gave colourless crystals of **2** when recrystallized from acetone. 0.158 g (0.210% yield);  $R_f$ : 0.42 (chloroform:methanol, 97:3); m.p.: 50–52 °C; UV ( $\lambda_{\max}$  nm MeOH): 207 (log  $\epsilon$  5.6); IR ( $\gamma_{\max}$  (KBr): 3420, 2923, 2853, 1734, 1690, 1452, 1460, 1460, 1375, 1245, 1028  $\text{cm}^{-1}$ );  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  5.16 (1H, m, H-22), 5.12 (1H, m, H-23), 4.34 (1H, brm,  $w_{1/2} = 18.5$  Hz, H-3 $\alpha$ ), 1.86 (3H, brs, COCH<sub>3</sub>), 1.10 (3H, brs, Me-19), 0.87 (3H, d,  $J = 6.3$  Hz, Me-21), 0.80 (3H, d,  $J = 6.3$  Hz, Me-27), 0.72 (3H, t,  $J = 6.1$  Hz, Me-29), 0.54 (3H, brs, Me-18);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ): 37.39 (C-1), 32.93 (C-2), 80.23 (C-3), 44.90 (C-4), 47.60 (C-5), 18.13 (C-6), 31.55 (C-7), 31.13 (C-8), 49.70 (C-9), 36.67 (C-10), 22.45 (C-11), 40.72 (C-12), 40.05 (C-13), 55.54 (C-14), 24.45 (C-15), 28.96 (C-16), 54.95 (C-17), 11.35 (C-18), 19.45 (C-19), 35.21 (C-20), 18.65 (C-21), 129.45 (C-22), 127.63 (C-23), 46.76 (C-24), 29.33 (C-25), 176.78 (C-26), 25.75 (C-27), 23.81 (C-28), 14.32 (C-29), 170.34, 21.01 (COCH<sub>3</sub>); +ve ion FAB MS  $m/z$  (rel. int.): 486  $[\text{M}]^+$  ( $\text{C}_{31}\text{H}_{50}\text{O}_4$ ) (21.3), 413 (100), 317 (5.2), 274 (8.5), 257 (21.8), 215 (12.1), 208 (15.3), 200 (15.1), 169 (11.2), 168 (10.9), 154 (12.3), 114 (9.3).

## 2.7. Hydrolysis of (2)

Compound **2** (30 mg) was dissolved in 95% ethanol (5 mL), dil HCl (2 mL) was added and the mixture was heated on a steam

bath for 1 h. The solvent was evaporated and the residue was taken in methanol to get bengalensisteroic acid, m.p. 152–153 °C,  $m/z$  444  $[M]^+$  ( $C_{29}H_{48}O_3$ ) (21.6).

### 2.8. Heneicosanyl oleate (3)

Elution of the column with petroleum ether furnished colourless crystalline mass of **3**, recrystallized from acetone 0.145 g (0.193% yield);  $R_f$  value: 0.94 (petroleum ether); m.p.: 125–126 °C; +ve ion FAB MS  $m/z$  (rel. int.): 576  $[M]^+$  ( $C_{39}H_{76}O_2$ ) (16.2) (Hardell and Nilvebrant, 1999).

### 2.9. $\alpha$ -Amyrin acetate (4)

Elution of the column with petroleum ether:chloroform (1:1) yielded colourless crystals of **4**, recrystallized from acetone, 0.457 g (0.609% yield);  $R_f$  value: 0.69 [petroleum ether:chloroform (1:1)]; m.p.: 225–226 °C; +ve ion FAB MS  $m/z$  (rel. int.): 468  $[M]^+$  ( $C_{32}H_{52}O_2$ ) (88.3) (Ali et al., 2000).

### 2.10. Lupeol (5)

Elution of the column with petroleum ether:chloroform (1:1) yielded colourless crystals of amorphous powder of **5**, recrystallized from acetone, 0.724 g (0.965% yield);  $R_f$  value: 0.54 (toluene:ethyl acetate:formic acid; 1:1:0.3); m.p.: 213–215 °C; +ve ion FAB-MS  $m/z$  (rel. int.): 426  $[M]^+$  ( $C_{30}H_{50}O$ ) (Jamal et al., 2008).

## 3. Results and discussion

The compounds **3**, **4** and **5** were the known compounds identified as heneicosanyl oleate (Hardell and Nilvebrant, 1999),  $\alpha$ -amyrin acetate (Ali et al., 2000) and lupeol (Jamal et al., 2008), respectively.

The compound **1** designated as lanostadienylglucosyl cetoleate, was obtained as a colourless semisolid mass from chloroform eluants. It responded positively to Liebermann-Burchard test for sterols. Its IR spectrum exhibited characteristic absorption bands for hydroxyl groups ( $3433\text{ cm}^{-1}$ ), an ester group ( $1731\text{ cm}^{-1}$ ), unsaturation ( $1645\text{ cm}^{-1}$ ), and a long aliphatic chain ( $740\text{ cm}^{-1}$ ). On the basis of FAB mass and  $^{13}\text{C}$  NMR spectra, its molecular mass was established at  $m/z$  909  $[M+H]^+$  consisting of a molecular formula of triterpenic glycoside linked with  $C_{22}$  ester,  $C_{58}H_{101}O_7$ . The ion peaks arising at  $m/z$  797  $[M-C_8H_{15}$ , side chain] $^+$ , 459  $[793-CH_3(CH_2)_9CH=CH(CH_2)_9COO$ , ester group] $^+$ , 297  $[459-C_6H_{10}O_5$ , glucose unit] $^+$  and 338  $[CH_3(CH_2)_9CH=CH-(CH_2)_9COOH$ , cetoleic acid] $^+$  suggested that  $C_{22}$  ester unit was linked to a triterpenic glycoside. The ion peaks generating at  $m/z$  425  $[M-C_6H_{10}O_5CO(CH_2)_9CH=CH-(CH_2)_9CH_3]$ , 410  $[425-Me]^+$ , 395  $[410-Me]^+$ , 380  $[395-Me]^+$ , 273  $[314-\text{ring D fission}]^+$ , 299  $[314-Me]^+$  and 258  $[299-\text{ring D fission}]^+$  supported the presence of two double bonds in the triterpenic moiety, one each in tetracyclic carbon framework and another one in the side chain. The ion peaks forming at  $m/z$  151  $[C_{6,7}-C_{9,10}$  fission] $^+$ , 165  $[C_{6,7}-C_{9,10}$  fission] $^+$ , 175  $[C_{8,14}-C_{9,11}$  fission] $^+$ , 189  $[C_{8,14}-C_{11,12}$  fission] $^+$  and 203  $[C_{8,14}-C_{12,13}$  fission] $^+$  indicated the existence of the vinylic linkage at C-5, saturated nature of ring C and a carbinol carbon in ring A which was placed at C-3 on the basis of biological consideration. The ion fragments yielding at  $m/z$  408  $[425-OH]^+$ , 297  $[408-C_8H_{15}$ , side chain], 134, 148  $[C_{6,7}-$

$C_{9,10}$  fission] $^+$ , 274, 260  $[C_{7,8}-C_{9,10}$  fission] $^+$ , 174  $[C_{8,14}-C_{9,11}$  fission], 188  $[C_{8,14}-C_{12,13}$  fission] $^+$  and 202  $[C_{8,14}-C_{12,13}$  fission] $^+$  also indicated the location of the vinylic linkage at C-5 and saturated nature of the ring C (Phillips et al., 2005; Freire et al., 2005). The  $^1\text{H}$  NMR spectrum of **1** displayed four one-proton multiplets at  $\delta$  5.36, 5.12, 5.03, and 4.97 assigned to vinylic H-6, H-24, H-11'' and H-12'' protons, respectively. A one-proton double doublet at  $\delta$  3.81 ( $J = 5.5, 9.3$  Hz) was ascribed to the  $\alpha$ -oriented H-3 oxygenated methine proton. Three one-proton doublets at  $\delta$  4.92 ( $J = 7.1$  Hz) and at 3.03 ( $J = 6.9$  Hz) and 3.01 ( $J = 6.9$  Hz) were attributed to the anomeric H-1' and hydroxyl methylene H<sub>2</sub>-6' protons, respectively. The remaining sugar protons appeared as multiplets between  $\delta$  3.87–4.70. Two one-proton doublets at  $\delta$  2.32 ( $J = 6.9$  Hz) and 2.30 ( $J = 6.9$  Hz) were accounted to the methylene H<sub>2</sub>-2'' protons adjacent to the ester carbon. Two three-proton broad signals at  $\delta$  1.70 and 1.62 were associated with the C-26 and C-27 methyl protons located on the vinylic carbon C-25. A three-proton doublet at  $\delta$  0.91 ( $J = 7.2$  Hz), five three-proton broad signals at  $\delta$  0.70, 0.98, 0.87, 0.82 and 0.78 and a three-proton triplet at  $\delta$  0.85 ( $J = 6.3$  Hz) were accommodated correspondingly to the secondary C-21 methyl, tertiary C-18, C-19, C-28, C-29 and C-30 methyl and primary C-22'' methyl protons. The remaining methylene and methine protons resonated between  $\delta$  2.29–1.03. The  $^{13}\text{C}$  NMR values of **1** exhibited signals for vinylic carbons at  $\delta$  139.07 (C-5), 121.53 (C-6), 114.01 (C-24), 135.97 (C-25), 124.65 (C-11'') and 130.73 (C-12''), ester carbon at  $\delta$  171.55 (C-1''), anomeric carbons at  $\delta$  101.36 (C-1'), sugar carbons between  $\delta$  62.01–79.61, methyl carbons at  $\delta$  13.61 (C-18), 19.25 (C-19), 18.25 (C-21), 26.42 (C-26), 26.01 (C-27), 18.47 (C-28), 24.77 (C-29), 21.02 (C-30) and 14.01 (C-22'') and the remaining methylene and methine carbons in the range of  $\delta$  22.58–56.71. The  $^1\text{H}$  NMR signal H-2' at  $\delta$  4.49 and  $^{13}\text{C}$  NMR C-2' at  $\delta$  78.72 in the deshielded regions indicated the presence of the ester linkage at C-2' of the sugar moiety. The  $^1\text{H}$  and  $^{13}\text{C}$  NMR values of **1** were compared with the related lanostene-type triterpenoids (Khan et al., 2010; Ali, 2001). The  $^1\text{H}-^1\text{H}$  COSY spectrum of **1** showed correlations of H-3 with H<sub>2</sub>-2, H<sub>3</sub>-28 and H-1', H-6 with H<sub>2</sub>-7; H-24 with H<sub>2</sub>-23, H<sub>3</sub>-26 and H<sub>3</sub>-27; and H-11'' with H<sub>2</sub>-10'', H-12'' and H<sub>2</sub>-13''. The HMBC of **1** exhibited interactions of H<sub>2</sub>-2, H<sub>3</sub>-28 and H-1' with C-3; H-6, H<sub>2</sub>-7 and H<sub>3</sub>-29 with C-5; H-24, H<sub>3</sub>-26 and H<sub>3</sub>-27 with C-25; and H-2' and H<sub>2</sub>-2'' with C-1'' (Fig. 3). Alkaline hydrolysis of **1** yielded lanost-5, 2H-dien-3 $\beta$ -ol, D-glucose and cetoleic acid. On the basis of spectral data analysis and chemical reactions, the structure of **1** has been established as lanosta-5,24-dien-3 $\beta$ -yl- $\beta$ -

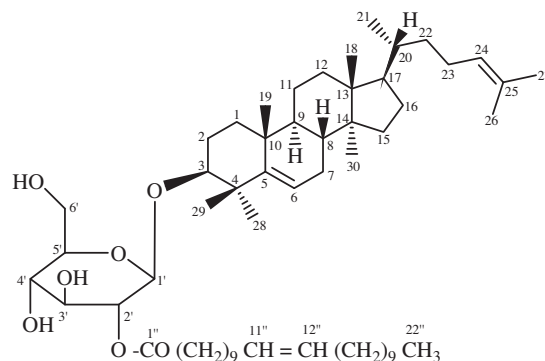


Figure 1 Lanostadienylglucosyl cetoleate **1**.

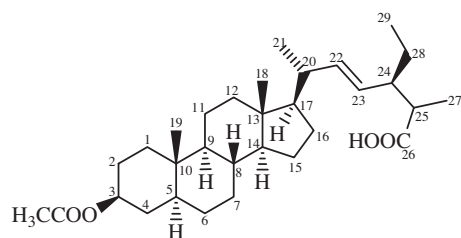


Figure 2 Bengalensisteroic acid acetate **2**.

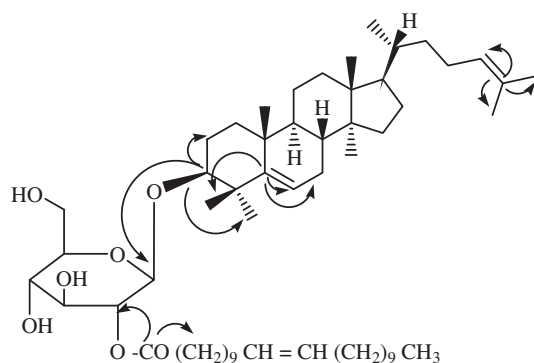


Figure 3 HMBC correlations of compound **1**.

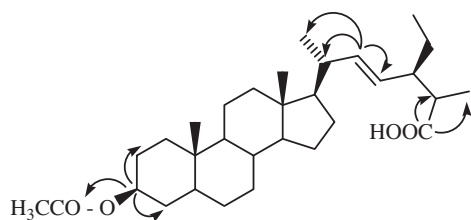


Figure 4 HMBC correlations of compound **2**.

D-glucopyranosid-2'-O-yl-docos-11''-enoate (Fig. 1). This is a new triterpenic glycoside isolated from a natural source for the first time.

Compound **2**, designated as bengalensisteroic acid acetate, was obtained as a colourless crystalline mass from chloroform–methanol (97:3) eluents. It produced effervescence with sodium bicarbonate solution indicating the presence of a carboxylic function in the molecule. It showed a positive Liebermann–Burchard test for steroids. Its IR spectrum exhibited characteristic absorption bands for a carboxylic group ( $3420$ ,  $1690$   $\text{cm}^{-1}$ ), an ester group ( $1734$   $\text{cm}^{-1}$ ) and unsaturation ( $1652$   $\text{cm}^{-1}$ ). On the basis of FAB mass and  $^{13}\text{C}$  NMR spectral data, the molecular weight of **2** was established at  $m/z$  487  $[\text{M} + \text{H}]^+$  consisting of the molecular formula of steroidal acetate,  $\text{C}_{31}\text{H}_{51}\text{O}_4$ . The ion fragments produced at  $m/z$  426  $[\text{M} - \text{AcOH}]^+$ , 411  $[426 - \text{Me}]^+$  and 396  $[411 - \text{Me}]^+$  indicated the presence of an acetoxy moiety in the molecule. The ion peaks generated at  $m/z$  317  $[\text{M} - 169, \text{side chain}]^+$ , 257  $[317 - \text{AcOH}]^+$ , 242  $[257 - \text{Me}]^+$  and 215  $[257 - \text{ring D}]^+$  suggested the steroidal compound containing an unsaturated side chain with carboxylic function. The ion fragment arising at  $m/z$  114  $[\text{C}_{4,5} - \text{C}_{1,10} \text{ fission}]^+$ , 203  $[\text{M} - 114 - \text{C}_{10}\text{H}_{17}\text{O}_2, \text{side chain}]^+$ , 130  $[\text{C}_{5,6} - \text{C}_{9,10} \text{ fission}]^+$ , 187  $[\text{M} - 130 - \text{side chain}]^+$ , 144  $[\text{C}_{6,7} - \text{C}_{9,10} \text{ fission}]^+$ ,

173  $[\text{M} - 144 - \text{side chain}]^+$  and 159  $[\text{C}_{7,8} - \text{C}_{9,10} \text{ fission-side chain}]^+$  supported the saturated nature of the rings A and B and the location of the oxygenated methine in ring A which was placed at C-3 on biogenetic analogy. The ion peaks forming at  $m/z$  207, 279  $[\text{C}_{8,14} - \text{C}_{12,13} \text{ fission}]^+$ , 110  $[279 - \text{side chain}]^+$ , 124  $[\text{C}_{8,14} - \text{C}_{12,13} \text{ fission-side chain}]^+$  and 138  $[\text{C}_{8,14} - \text{C}_{9,11} \text{ fission-side chain}]^+$  supported the saturated nature of ring C. The ion peaks appearing at  $m/z$  414  $[\text{M} - \text{CH}(\text{CH}_3)\text{COOH}]^+$ , 169  $[\text{C}_{10}\text{H}_{17}\text{O}_2, \text{side chain}]^+$  and 274  $[\text{M} - \text{side chain} - \text{CH}_3\text{CO}]^+$  also indicated the existence of the unsaturated  $\text{C}_{10}$ -side chain containing one carboxylic function at C-26 (Phillips et al., 2005; Freire et al., 2005). The  $^1\text{H}$  NMR spectrum of **2** displayed two one-proton multiplets at  $\delta$  5.12 and 5.14 assigned to vinylic H-22 and H-23 protons, respectively. A one-proton broad multiplet at  $\delta$  4.34 with the coupling interaction of 18.5 Hz was ascribed to the oxygenated methine H-3 proton and its appearance in the deshielded region indicated the presence of acetyl group at C-3. Two three-proton broad signals at  $\delta$  0.54 and 1.10 were ascribed to C-18 and C-19 tertiary methyl protons, respectively. Two three-proton doublets at  $\delta$  0.87 ( $J = 6.3$  Hz), 0.80 ( $J = 6.3$  Hz) and a three-proton triplet at  $\delta$  0.72 ( $J = 6.1$  Hz) were attributed correspondingly to secondary C-21 and C-27 and primary C-29 methyl protons. The acetyl protons appeared as a three proton broad signal at  $\delta$  1.86. The remaining methylene and methine protons resonated in the range of  $\delta$  1.08–2.60. The presence of methyl signal between  $\delta$  0.54–1.10 suggested their location on saturated carbons. The  $^{13}\text{C}$  NMR spectrum of **2** showed signals for the vinylic carbons at  $\delta$  129.42 (C-22) and 127.63 (C-23), carbinol carbon at  $\delta$  80.23 (C-3), methyl carbons at  $\delta$  11.35 (C-18), 19.45 (C-19), 18.65 (C-21), 25.75 (C-27) and 14.32 (C-29) and acetyl carbons at  $\delta$  170.34 and 21.01. The  $^1\text{H}$  and  $^{13}\text{C}$  NMR values of **2** were compared with the other related steroids (Alam et al., 2010; Bagri et al., 2009). The DEPT spectrum of **2** showed the presence of six methyl, ten methylene, eleven methine and four quaternary carbons. The  $^1\text{H} - ^1\text{H}$  COSY spectrum of **2** showed correlations of H-3 with H<sub>2</sub>-2 and H<sub>2</sub>-4; H-22 with H-20 and H-23; and H-24 with Me-26 and H<sub>3</sub>-27. The HMBC of **2** exhibited correlations of H<sub>2</sub>-2, H<sub>2</sub>-4 and Ac with C-3; H-20, Me-21 and H-23 with C-22; and H-25 and H<sub>3</sub>-27 with C-26 (Fig. 4). Acid hydrolysis of **2** yielded bengalensisteroic acid. On the basis of these evidences the structure of **2** has been elucidated as 3 $\beta$ -acetoxy-stigmast-22-en-26-oic acid (Fig. 2). This is a new phytosterol isolated from a natural source.

#### 4. Conclusion

The present work characterized the lanostadienyl glucosyl cetoluate and bengalensisteroic acid ester as the new phytoconstituents from the methanolic extract of the stem bark of *F. bengalensis*. The existing knowledge regarding its phytoconstituents may be increased by the present phytochemical investigation which is useful as this drug is used in the traditional Indian System of Medicine and may be used as chromatographic marker of the drug and for medicinal importance of the drug.

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