

New Naphthyl Substituted Phytosterol and Lanostane Type-triterpenic Esters from the Stem Bark of *Ficus religiosa* L.

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ABSTRACT

Background: *Ficus religiosa* L. (Moraceae) is considered as a holy tree in most of the part of south-eastern Asia. Traditionally, its bark is used in the treatment of burns, diarrhoea, dysentery, gastrohelcosis and gonorrhoea, glandular swellings of the neck, scabies, piles, urogenital disorders, anxiety, vomiting, skin diseases and prescribed to improve the skin complexion. **Materials and Methods:** The methanol extract of stem bark of *F. religiosa* was obtained by continuous hot extraction process. Isolation of phytoconstituents was done by silica gel column chromatography. Analytical thin layer chromatography was used to check the homogeneity of eluted fractions. The structures of isolated compounds were established on the basis of 1D and 2D NMR, FT-IR, UV and MS data and chemical means. **Results:** Phytochemical investigation of the methanol extract of *F. religiosa* stem bark led to the isolation of a new naphthyl substituted phytosterol characterized as naphthyl-1',3'-diol-1'-3 β -sitosteryl-3'-linoleinate (1) and a new lanostane type-triterpenic ester elucidated as lanostan-19-oic acid-3 β -olyl-oleate (2). **Conclusion:** The present work has enhanced the phytochemical profile of *F. religiosa*. Compound 1 and 2 have been isolated for the first time from this plant and might be used as chromatographic markers for the quality control analysis of its marketed herbal formulations.

Key words: *Ficus religiosa*, Stem bark, Phytosterol ester, Lanostanol ester, Isolation, characterization, β -sitosteryl naphthadiolyl linoleinate, Lanostanoic acid oleate.

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INTRODUCTION

Ficus religiosa L. (Moraceae) tree, known as peepal, bodhi and ashwattha tree, is a native to India and south-eastern Asian countries. It is distributed in Egypt, Chad, Mexico and South America. It is a deciduous, laticiferous, fast growing tree, up to 25 m high; smooth bark grey in colour; spreading branches; simple, alternate, spiral ovate-lanceolate, puberulous leaves; unisexual, small, red flowers; inflorescence a syconia, sessile, axillary, in pairs, obovoid or globose; fruits small figs, purple on ripening.¹⁻³ The leaves are purgative, leaf ash mixed with coconut oil is applied to subside boils; latex is tonic and put on to cure bleeding and swelling gums; fruits are aphrodisiac, purgative and

given to relieve menstrual disorders, seeds are alterative and refrigerant. Traditionally it is used to treat asthma, bacterial infections, bronchitis, chicken pox, constipation, cough, diabetes, diarrhoea, elephantiasis, epilepsy, gastric problems, gonorrhoea, inflammation, leprosy, liver diseases, menstrual irregularities, migraine, sexual disorders, stomatitis, tuberculosis, ulcers and vomiting.^{4,5} The bark possesses aphrodisiac, anti-inflammatory, astringent, antiseptic and refrigerant properties, used to treat anxiety, boils, blisters, burns, diarrhoea, diabetes, dysentery, gastrohelcosis, gonorrhoea, inflammation, gout, piles, skin diseases, urogenital disorders, vomiting, wounds

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and to improve skin complexion. The root bark is beneficial to control diabetes and its complications.^{1,6-8} Ayurvedic formulations, such as *Nalpamaraditailam*, *Nyagrodhadi churna* and *Saribadyasavam* contain *F. religiosa* bark as an important ingredient.^{9,10}

Unsaturated and saturated fatty acids, cyclic fatty acid ester, fatty alcohols,¹¹ tetrahydroxyoctanoic and tetrahydroxydecanoic acids, steryl naphthyl esters,¹² phytosteroids, naphthyl esters, lanostanoic acid linolenate,¹³ lupen-3-one, lanosterol, coumarins, vitamin K₁,¹⁴⁻¹⁷ anthocyanidins, saponin, lupeol, α -amyrin acetate, tannins and wax had been reported from the *F. religiosa* stem bark.^{18,19} Fruits contain fibre, hemicellulose, cellulose, lignin, pectin, asparagine, tyrosine, myricetin, quercetin, kaempferol and several volatile components. However, leaves furnish minerals, amino acids, campesterol, stigmasterol, sitosterol, α -amyrin, β -amyrin and lupeol.^{5,20} This article reports the isolation and structure elucidation of a new naphthyl substituted phytosterol and a new lanostane type-triterpenic ester from the stem bark of *F. religiosa* for the first time.

MATERIALS AND METHODS

General procedures

Melting points of isolated phytoconstituents were determined by a thermoelectrical heated Perfit melting point apparatus. The IR spectra were recorded on an FT-IR (Bio-Rad) spectrometer in KBr pellet. Ultraviolet spectra were acquired with a Lambda Bio 20 spectrometer in methanol. ¹H (500 MHz), ¹³C (125 MHz), COSY and HMBC NMR spectra of phytoconstituents were obtained on Bruker spectropin spectrometer using TMS as internal standard. ESI MS studies were executed on a Waters Q-TOF Premier Mass spectrometer (Manchester, UK).

Chromatographic conditions

Isolation of phytoconstituents was performed on glass column chromatography using silica gel (mesh size: 60-120, Merck, India). Silica gel G (mesh size: 200-300) was utilized for preparative thin layer chromatography. Precoated TLC plates (Silica gel 60 F₂₅₄) were utilized for analysis of eluents and the spots were visualized under UV lamp (254 and 366 nm).

Plant material

Fresh stem bark of *F. religiosa* was collected from Delhi region and authenticated by Dr. H.B. Singh (taxonomist), National Institute of Science Communication and Information Resources (NISCAIR), New Delhi. A bark voucher specimen was placed with a number NISCAIR/

RHMD/Consult/-2010-11/1665/263 in the herbarium for future reference.

Extraction and isolation

The stem bark of *F. religiosa* (1 kg) was air dried and pulverized. Extraction of coarsely powdered bark was done exhaustively with methanol (2.5 L) using a Soxhlet apparatus at 80°C for 12 h. The extract was filtered and evaporated in vacuum (337 mbar) at 40°C to produce a brown semi-solid mass (193.3 g). The semi-solid mass (100 g) was dissolved in double distilled water (500 ml) by continuous stirring and petroleum ether (500 ml) was added. The aqueous fraction (95.14 g) was collected, dried and loaded over a silica gel (60-120 mesh) glass column.¹² Elution of the column with CHCl₃ alone and CHCl₃-MeOH (97:3 v/v) mixture yielded compounds 1 and 2 (Figure 1). Further isolated compounds were purified by preparative thin layer chromatography and recrystallization.

RESULTS

The structure of new isolated phytoconstituents 1 and 2 were elucidated on the basis of NMR, IR, UV and ESI MS spectral data analysis.

β -Sitosteryl naphthadiolyl linoleinate (1)

Elution of the column with chloroform yielded yellow sticky mass of 1, purified by preparative TLC (chloroform-methanol, 99:1), 412 mg (0.412 % yield): R_f 0.85 (chloroform-methanol, 99:1); UV λ_{\max} (MeOH): 247 nm; m.p. 295-297°C; IR ν_{\max} (KBr): 2928, 2841, 1721, 1642, 1525, 1441, 1362, 1240, 1167, 1081, 973, 759 cm⁻¹; ¹H NMR (CDCl₃): δ 7.67 (1H, d, J = 8.0 Hz, H-9'), 7.52 (1H, d, J = 3.0 Hz, H-2'), 7.31 (1H, d, J = 3.0 Hz, H-4'), 7.29 (1H, m, H-7'), 7.03 (1H, m, H-8'), 6.45 (1H, dd, J = 3.0, 8.5 Hz, H-6'), 5.79 (1H, m, H-10''), 5.38 (1H, m, H-12''), 5.30 (1H, m, H-15''), 5.22 (1H, m, H-13''), 5.10 (1H, m, H-9''), 4.95 (1H, m, H-16''), 4.07 (1H, brm, $w_{1/2}$ = 16.5 Hz, H-3 α), 2.72 (2H, m, CH₂), 2.31 (2H, t, J = 7.5 Hz, H₂-2''), 1.06 (3H, brs, Me-19), 0.95 (3H, d, J = 6.5 Hz, Me-21), 0.87 (3H, d, J = 6.1 Hz, Me-26), 0.83 (3H, t, J = 6.0 Hz, H₂-18''), 0.66 (3H, brs, Me-18), 2.27-1.18 (49H, m, 21 \times CH₂, 7 \times CH); ¹³C NMR (CDCl₃): δ 37.27 (C-1), 31.94 (C-2), 71.76 (C-3), 42.26 (C-4), 140.76 (C-5), 121.67 (C-6), 33.90 (C-7), 31.66 (C-8), 50.13 (C-9), 37.12 (C-10), 21.24 (C-11), 39.43 (C-12), 43.50 (C-13), 56.77 (C-14), 23.07 (C-15), 27.22 (C-16), 56.05 (C-17), 11.88 (C-18), 19.31 (C-19), 37.89 (C-20), 19.07 (C-21), 33.94 (C-22), 25.64 (C-23), 45.85 (C-24), 29.53 (C-25), 17.72 (C-26), 18.02 (C-27), 26.09 (C-28), 12.01 (C-29), 166.82 (C-1'), 138.31 (C-2'), 152.25 (C-3'), 144.33 (C-4'), 130.89 (C-5'), 134.55 (C-6'), 115.48 (C-7'), 117.58

(C-8'), 125.12 (C-9'), 150.87 (C-10'), 173.28 (C-1''), 34.21 (C-2''), 29.77 (C-3''), 24.76 (C-4''), 29.62 (C-5''), 29.64 (C-6''), 29.68 (C-7''), 29.71 (C-8''), 128.05 (C-9''), 118.84 (C-10''), 25.71 (C-11''), 128.85 (C-12''), 129.68 (C-13''), 25.74 (C-14''), 114.06 (C-15''), 109.83 (C-16''), 20.62 (C-17''), 14.11 (C-18''); ESI-MS m/z (rel. int.): 816 [M]⁺ (C₅₇H₈₄O₃) (2.8), 413 (30.1), 397 (11.2), 277 (33.5), 261 (3.7).

Lanostanoic acid oleate (2)

Elution of the column with chloroform-methanol (97:3) furnished yellow crystals of 2, recrystallized from acetone, 378 mg (0.378 % yield): R_f 0.34 (chloroform-methanol, 99:1); UV λ_{\max} (MeOH): 209 nm; m.p. 225-226°C; IR ν_{\max} (KBr): 3432, 2938, 2738, 1721, 1698, 1642, 1417, 1389, 1242, 1169, 1024, 763 cm⁻¹; ¹H NMR (CDCl₃): δ 5.32 (1H, m, H-9'), 5.25 (1H, m, H-10'), 4.18 (1H, dd, $J = 5.0, 8.5$ Hz, H-3 α), 0.95 (3H, d, $J = 6.5$ Hz, Me-21), 0.91 (3H, brs, Me-28), 0.85 (3H, d, $J = 6.6$ Hz, Me-26), 0.83 (3H, d, $J = 6.5$ Hz, Me-27), 0.81 (3H, t, $J = 6.1$ Hz, Me-18'), 0.78 (3H, brs, Me-30), 0.75 (3H, brs, Me-29), 0.72 (3H, brs, Me-18), 2.32-1.11 (56H, m, 25 \times CH₂, 6 \times CH); ¹³C NMR (CDCl₃): δ 39.29 (C-1), 29.10 (C-2), 80.41 (C-3), 40.83 (C-4), 48.96 (C-5), 18.07 (C-6), 27.50 (C-7), 31.92 (C-8), 51.36 (C-9), 38.46 (C-10), 22.81 (C-11), 37.41 (C-12), 47.48 (C-13), 55.61 (C-14), 23.66 (C-15), 27.84 (C-16), 55.90 (C-17), 15.84 (C-18), 178.76 (C-19), 38.65 (C-20), 16.31 (C-21), 40.37 (C-22), 27.48 (C-23), 41.75 (C-24), 30.79 (C-25), 20.53 (C-26), 20.81 (C-27), 26.28 (C-28), 16.63 (C-29), 19.18 (C-30), 171.15 (C-1'), 35.16 (C-2'), 29.65 (C-3'), 29.57 (C-4'), 29.48 (C-5'), 29.18 (C-6'), 29.33 (C-7'), 29.71 (C-8'), 128.88 (C-9'), 128.62 (C-10'), 29.71 (C-11'), 29.65 (C-12'), 29.33 (C-13'), 29.71 (C-14'), 27.41 (C-15'), 27.48 (C-16'), 22.71 (C-17'), 14.16 (C-18'); ESI-MS m/z (rel. int.): 724 [M]⁺ (C₄₈H₈₄O₆) (2.3), 459 (4.3), 443 (2.8), 281 (12.2), 265 (10.3).

DISCUSSION

Compound 1, named β -sitosterol naphthadiolyl linoleinate, was obtained as a yellow sticky mass from chloroform eluants. Its IR spectrum showed absorption bands for ester groups (1721 cm⁻¹), unsaturation (1642 cm⁻¹), aromatic ring (1525, 1081 cm⁻¹) and long aliphatic chain (759 cm⁻¹). On the basis of mass and ¹³C NMR spectra the molecular ion peak of 1 was determined at m/z 816 consistent with a molecular formula of a β -sitosteryl naphthyl ester, C₅₇H₈₄O₃. The ion fragments arising at m/z 413 [C_{1'}-O fission, C₂₉H₄₉O]⁺ and 397 [O-C₃ fission, C₂₉H₄₉]⁺ indicated that β -sitosteryl unit was attached to the naphthyl moiety. The ion peaks generating at m/z 261 [O-C_{1'} fission, CO

(CH₂)₇ (CH=CH CH₂)₃ CH₃]⁺ and 277 [C₃-O fission, OCO(CH₂)₇ (CH=CH CH₂)₃ CH₃]⁺ suggested the attachment of linoleinyl group to another carbon of the naphthyl moiety. The ¹H NMR spectrum of 1 exhibited four one-proton doublets at δ 7.67 ($J = 8.0$ Hz), 6.45 ($J = 8.5$ Hz), 7.52 ($J = 3.0$ Hz) and 7.31 ($J = 3.0$ Hz) assigned to aromatic ortho-coupled H-9' and H-6' and to meta-coupled H-2' and H-4' protons, respectively. The vinylic protons appeared as one-proton multiplets from δ 5.79 to 4.95. A one-proton broad multiplet at δ 4.07 was attributed to oxygenated methine H-3 α proton and its half width of 16.5 Hz suggested alpha orientation of the proton. A two-proton triplet at δ 2.31 ($J = 7.5$ Hz) was due to methylene H₂-2'' protons adjacent to the ester group. Three three-proton doublets in the upward region at δ 0.95 ($J = 6.5$ Hz, Me-21), 0.87 ($J = 6.1$ Hz) and 0.85 ($J = 6.3$ Hz) were attributed to C-21, C-26 and C-27 methyl protons. Two three-proton broad singlets at δ 1.01 and 0.66, a three-proton doublet at δ 0.95 ($J = 6.5$ Hz) and a three-proton triplet at δ 0.83 ($J = 6.0$ Hz) were associated correspondingly with the tertiary C-19 and C-18, secondary C-21 and primary C-18'' methyl protons, all of them located on the saturated carbons. The other methylene and methine protons resonated in the range of δ 2.27-1.18.

The ¹³C NMR spectrum of 1 showed signals for ester carbon at δ 173.28 (C-1''), aromatic carbons between δ 166.82-115.48, vinylic carbons between δ 140.76-109.43 and methyl carbons from δ 22.59 to 11.88. The ¹H and ¹³C NMR spectral data of the steroidal unit of 1 were compared with the reported data of similar steroids.²¹⁻²³ The ¹H-¹H COSY spectrum of 1 exhibited correlations of H-3 with H₂-2; H-6 with H₂-7 and Me-19; H-24 with H₂-23, Me-26 and Me-27; H-4' with H-2' and H-6'; and H-12'' with H-10'', H₂-11'' and H-13''. The HMBC spectrum of 1 displayed interactions of H-3, Me-19 and H-6 with C-5; H₂-23, H-24, Me-26 and Me-27 with C-25; H-2', H-9' and H-3 with C-1'; H-2' and H₂-2'' with C-1'';

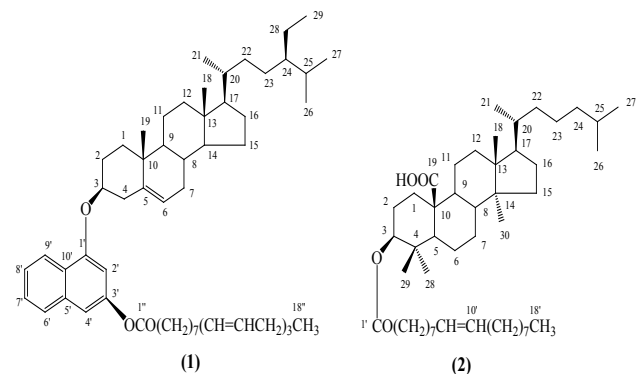


Figure 1: Structure of isolated compounds 1 and 2 from the stem bark of *F. religiosa* L.

H-4', H-6' and H-7' with C-5'; and H-10'', H₂-11'' and H-13'' with C-12''. On the basis of above discussion, the structure of 1 has been characterized as naphthyl-1',3'-diol-1'-3 β -sitosteryl-3'-linoleinate (Figure 1). This is a new naphthyl substituted phytosterol isolated for the first time from this plant.

Compound 2, designated as lanostanoic acid oleate, was obtained as a yellow crystalline mass from chloroform-methanol (97:3) eluants. It responded positively to Liebermann-Burchardt test for triterpenoid and yielded effervescences with sodium bicarbonate solution suggesting the presence of a carboxylic group. Its IR spectrum displayed absorption bands for an ester group (1721 cm⁻¹), carboxylic function (3432, 1698 cm⁻¹), unsaturation (1642 cm⁻¹) and long aliphatic chain (763 cm⁻¹). On the basis of mass and ¹³C NMR spectra the molecular ion peak of 2 was determined at *m/z* 724 consistent with the molecular formula of a lanostanoic acid ester, C₄₈H₈₄O₆. The ion fragments arising at *m/z* 459 [C₁-O fission, C₃₀H₅₁O₃]⁺, 443 [O-C₃ fission, C₃₀H₅₁O₂]⁺, 281 [CH₃-(CH₂)₇-CH=CH-(CH₂)₇-COO, C₁₈H₃₃O₂]⁺ and 265 [CH₃-(CH₂)₇-CH=CH-(CH₂)₇-CO, C₁₈H₃₃O]⁺ indicated the attachment of oleil group to lanostan-19-oic acid unit. The ¹H NMR spectrum of 2 exhibited two one-proton multiplets at δ 5.32 and 5.25 assigned to vinylic H-9' and H-10' protons, respectively. A one-proton double doublet at δ 4.18 with coupling interactions of 5.0 and 8.5 Hz was attributed to oxygenated methine H-3 α proton. Three three-proton doublets at δ 0.95 (*J* = 6.5 Hz), 0.85 (*J* = 6.6 Hz) and 0.83 (*J* = 6.5 Hz) were ascribed to C-21, C-26 and C-27 methyl protons, respectively. Four three-proton broad singlets at δ 0.91, 0.78, 0.75 and 0.72 were associated correspondingly with the tertiary C-28, C-30, C-29 and C-18 methyl protons. A three-proton triplet at δ 0.81 (*J* = 6.1 Hz) was due to primary C-18' methyl protons. The other methylene and methine protons resonated in the range of δ 2.32-1.11.

The ¹³C NMR spectrum of 2 showed signals for carboxylic carbon δ 178.76 (C-19), ester carbon at δ 171.15 (C-1'), vinylic carbons at δ 128.88 (C-9') and 128.62 (C-10') and methyl carbons from δ 26.28 to 14.16. The ¹H and ¹³C NMR spectral data of the triterpenic unit of 2 were compared with the reported data of lanostene-type triterpenoids.²⁴⁻²⁶ The ¹H-¹H COSY spectrum of 2 showed correlations of H-3 with H₂-2 and Me-28; H-5 with Me-29, H₂-6 and H₂-7; H-25 with H₂-24, Me-26 and Me-27; and H-12' with H-10', H₂-11' and H₂-13'. The HMBC spectrum of 2 showed interactions of Me-28, Me-29, H-3 and H-6 with C-5; H₂-23, H₂-24, Me-26 and Me-27 with C-25; H₂-1 and H-9 with C-19; and H-10', H₂-11' and H₂-13' with C-12'.

On the basis of above discussion the structure of 2 has been elucidated as lanostan-19-oic acid-3 β -oleyl-oleate (Figure 1). This is a new lanostane type-triterpenic ester isolated for the first time from this plant.

CONCLUSION

Chromatographic separation of methanolic extract of *F. religiosa* stem bark yielded a new naphthyl substituted phytosterol and a lanostane type-triterpenic ester for the first time. These new isolated compounds have enhanced the phytochemical profile of *F. religiosa* and may play important role as chromatographic chemical markers in quality analysis of its traditional formulations.

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CONFLICT OF INTEREST

The authors confirm that the article content has no conflict of interest

ABBREVIATIONS

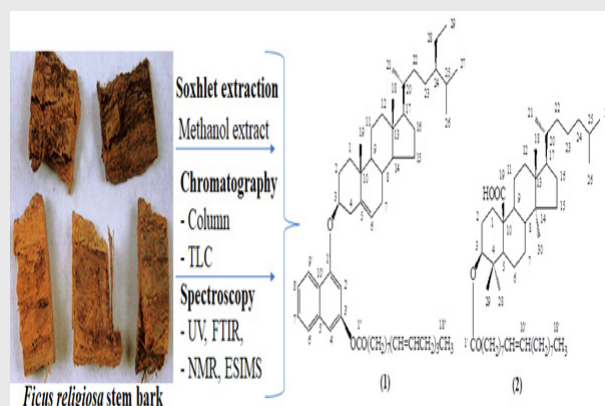
IR: Infrared Spectroscopy; **KBr:** Potassium Bromide; **FT-IR:** Fourier-Transform Infrared Spectroscopy; **NMR:** Nuclear Magnetic Resonance; **COSY:** Correlation Spectroscopy; **HMBC:** Heteronuclear Multiple Bond Correlation; **TMS:** Tetramethylsilane; **ESI MS:** Electrospray Ionization Mass Spectrometry; **UV:** Ultraviolet-Visible Spectroscopy; **TLC:** Thin-Layer Chromatography; **CHCl₃:** Chloroform; **CDCl₃:** Deuterated Chloroform; **MeOH:** Methanol; **d:** Doublet; **dd:** Double doublet; **m:** Multiplet; **brm:** Broad Multiplet; **Me:** Methyl; **Brs:** Broad Singlet; **rel. int.:** Relative Intensity.

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PICTORIAL ABSTRACT



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SUMMARY

Traditionally, *F. religiosa* bark is used in the treatment of burns, diarrhoea, dysentery, gastrohelcosis, gonorrhoea, glandular swellings of the neck, scabies, piles, urinogenital disorders, anxiety, vomiting, skin diseases and prescribed to improve the skin complexion. Phytochemical investigation of the methanolic extract of *F. religiosa* stem bark led to the isolation of a new naphthyl substituted phytosterol characterized as naphthyl-1',3'-diol-1'-3 β -sitosteryl-3'-linoleinate (1) and a new lanostane type-triterpenic ester elucidated as lanostan-19-oic acid-3 β -olyl-oleate (2). The structure of these isolated compounds was established on the basis of spectroscopic analysis and chemical reactions. This work has enhanced the phytochemical profile of *F. religiosa*. These two compounds have reported for the first time from this plant and may consider as chromatographic markers for the quality control analysis of its marketed herbal formulations.

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