



PHYTOCHEMICAL INVESTIGATION OF THE LEAVES OF *FICUS PANDURATA* SANDER

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Article Received on 08/08/2016

Article Revised on 29/08/2016

Article Accepted on 20/09/2016

ABSTRACT

Ficus pandurata Sander, syn. *F. lyrata* Warb. (Moraceae) is a medium size tree native to tropical Africa, Mediterranean region, India, Saudi Arabia, China and Japan. Its fruit, root and leaves are used to relieve gastrointestinal, respiratory, inflammatory and cardiovascular disorders, asthma, cough, sexual diseases, ear-ache, toothache, migraine, eye troubles, scabies, gonorrhea, paralysis, bone fracture and as an anthelmintic, antiseptic, antitumor and astringent. Phytochemical investigation of a methanolic extract of the leaves of *F. pandurata* resulted in the isolation of β -sitosterol (1), ursolic acid (2), siresinolic acid (3) and β -sitosterol 3- β -D-glucopyranoside (4). The structures of all the isolated phytoconstituents have been established on the basis of spectral data analysis and chemical reactions.

KEYWORDS: *Ficus pandurata* Sander, leaves, chemical constituents, isolation, characterization.

INTRODUCTION

Ficus pandurata Sander; syn. *Ficus lyrata* Warb. (Moraceae), known as fiddle leaf fig or banjo fig, is an evergreen tree, upright, medium size tree with an attractive shape and large green fiddle-shaped leaves, up to 15 m in height. It is a native to tropical western and central Africa from Cameroon west to Sierra Leone^[1]; grown as an ornamental and shade tree in the United States, Florida, El Salvador, Nicaragua, Columbia, Chile, Cameroon, Hawai'ian Islands^[2-4] in the Mediterranean region from Turkey to Spain and Portugal; and to a small extent, in India, Saudi Arabia, China and Japan^[5,6]. Its trunk is solitary with rough, scaling bark; no aerial and surface roots, leaves simple, alternate, fiddle-shaped in outline, with prominent venation, undulate margins, dark green^[2].

The bark of the species is used for its gums and resins^[7,8]. Its fruit, root and leaves are utilized to treat colic, indigestion, loss of appetite, diarrhea, sore throats, coughs, bronchial problems, inflammation and cardiovascular disorders. Fig is useful as a cardiovascular, respiratory, antispasmodic and anti-inflammatory remedy^[6]. In Pakistan, the whole plant is taken to alleviate gastrointestinal problems, diabetes, asthma, cough, sexual disorders, diarrhea, earache, toothache, migraine, eye troubles, scabies, gonorrhea, bleeding, paralysis, bone fracture and as an anthelmintic, antiseptic, antitumor and astringent^[9]. *F. pandurata* possessed bioactive compounds such as arabinose,

β -amyirin, β -carotenes, glycosides, β -sitosterol and xanthotoxin^[10,11]. The fruits and leaves contained flavonoids, phenolic acids and fatty acids^[12]. The leaves yielded 5, 6-dihydroxy-2-methylchromone, flavonoids, β -sitosterol-D-glucoside and ursolic acid^[13,14]. A saponified *n*-hexane extract of the leaves afforded *n*-heptacosane, β -sitosterol and palmitic, carboceric, and linoleic acids^[15]. The plant part extracts exhibited antimicrobial^[6,14,16,17], analgesic, antinociceptive^[18] and antioxidant^[15,19] activities. This paper describes isolation and characterization of steroidal and triterpenic constituents from the leaves of *F. pandurata*.

EXPERIMENTAL

General

Melting points were determined on a Perfit melting apparatus (Ambala, Haryana, India) and are uncorrected. UV spectra were measured with a Lambda Bio 20 spectrophotometer (Perkin Elmer Rotkreuz, Switzerland) in methanol. Infra red spectra were recorded on a Bio-Rad FTIR 5000 (FTS 135, Kawloon, Hong Kong) spectrophotometer using KBr pellets. ¹H and ¹³C NMR spectra were scanned on Advance DRX Bruker spectrometer 400 and 100 MHz, respectively, instruments (Karlsruhe, Germany) using TMS as an internal standard. Mass spectra were obtained by effecting FAB ionization at 70 eV on a JEOL-JMS DX 303 spectrometer (Japan) equipped with direct inlet probe system. Column chromatography was performed on a silica gel (60-120 mesh, Qualigen, Mumbai, India) column. TLC was run

on silica gel G (Qualigen) coated plates. Spots were visualized by exposing to iodine vapors, UV radiation and spraying with ceric sulfate solution.

Plant Material

The leaves of *F. pandurata* were procured from Delhi and identified by Prof. M.P. Sharma, Department of Botany, Jamia Hamdard, New Delhi.

Extraction and Isolation

The air-dried aerial parts (500 g) were coarsely powdered and extracted with methanol exhaustively in a Soxhlet apparatus. The combined extracts were filtered and concentrated under reduced pressure to get a dark brown viscous mass (51.2 g, 10.2%). The dried extract was dissolved in minimum quantity of methanol and adsorbed on silica gel (60-120 mesh) for preparation of a slurry. It was dried in air and chromatographed over a silica gel column (1.6 m x 16 mm x 2 mm) packed in petroleum ether. The column was eluted successively with different solvents in increasing order of polarity in various combinations of petroleum ether, chloroform and methanol in order of increasing polarity. The fractions were collected separately and matched by TLC to check homogeneity. Similar fractions having the same R_f values were combined and crystallized. The isolated compounds were recrystallized to get pure compounds. The following compounds were isolated from the methanolic extract of the leaves of *F. pandurata*:

β -Sitosterol (1)

Elution of the column with petroleum ether – chloroform (3:1) yielded colourless amorphous powder of **1**, yield 127 mg, m.p. 137-138 °C; UV $\lambda_{\max}$ (MeOH): 211 nm (log ϵ 2.9); IR $\nu_{\max}$ (KBr): 3401, 1654 cm^{-1} ; ^1H NMR (CDCl_3): δ 5.34 (1H, m, H-6), 3.54 (1H, brs, w $\frac{1}{2}$ = 18.5 Hz, H-3), 1.01 (3H, brs, Me-19), 0.94 (3H, d, J = 6.2 Hz, Me-21), 0.87 (3H, d, J = 6.5 Hz, Me-27), 0.84 (3H, J = 6.3 Hz, Me-26), 0.82 (3H, t, J = 6.1 Hz, Me-29), 0.67 (3H, brs, Me-18), 2.28 – 1.05 (29H, 11 x CH_2 , 7 x CH); ^{13}C NMR (CDCl_3): δ 37.28 (C-1), 31.93 (C-2), 71.81 (C-3), 42.34 (C-4), 140.78 (C-5), 121.68 (C-6), 29.33 (C-7), 34.23 (C-8), 50.21 (C-9), 36.14 (C-10), 22.66 (C-11), 38.89 (C-12), 39.81 (C-13), 56.80 (C-14), 27.21 (C-15), 28.22 (C-16), 56.11 (C-17), 11.85 (C-18), 19.33 (C-19), 36.73 (C-20), 19.03 (C-21), 33.98 (C-22), 26.18 (C-23), 45.90 (C-24), 29.68 (C-25), 21.07 (C-26), 19.78 (C-27), 24.94 (C-28), 11.97 (C-29); +ve ion FAB MS m/z (rel.int.): 415 $[\text{M}+\text{H}]^+$ ($\text{C}_{29}\text{H}_{51}\text{O}$) (15.1), 398 (100), 383 (13.5).

Ursolic acid (2)

Elution of the column with petroleum ether – chloroform (1:1) eluent gave crystals of **2**, recrystallized from acetone, 79 mg; m.p. 286 – 288 °C; Liebermann–Burchard test positive; IR $\nu_{\max}$ (KBr): 3358, 3211, 2925, 2853, 1691, 1645, 1455, 1381, 1256, 1167, 1035, 981 cm^{-1} ; ^1H NMR (CDCl_3): δ 5.25 (1H, d, J = 10.9 Hz, H-12), 3.48 (1H, dd, J = 5.6, 9.1 Hz, H-3 β), 1.24 (3H, s, Me-23), 1.03 (3H, s, Me-25), 0.91 (3H, s, Me-24), 0.87

(3H, d, J = 6.5 Hz, Me-30), 0.81 (3H, d, J = 6.5 Hz, Me-29), 0.78 (3H, s, Me-27), 0.65 (3H, s, Me-26), 2.01-1.17 (23 H, 9 x CH_2 , 5 x CH); ^{13}C NMR (CDCl_3): δ 39.05 (C-1), 27.67 (C-2), 76.86 (C-3), 39.14 (C-4), 55.45 (C-5), 18.67 (C-6), 33.46 (C-7), 39.79 (C-8), 47.73 (C-9), 37.23 (C-10), 23.94 (C-11), 124.53 (C-12), 139.67 (C-13), 42.38 (C-14), 28.29 (C-15), 24.53 (C-16), 47.53 (C-17), 53.08 (C-18), 39.99 (C-19), 39.92 (C-20), 31.16 (C-21), 34.67 (C-22), 28.97 (C-23), 15.66 (C-24), 15.89 (C-25), 16.72 (C-26), 23.52 (C-27), 177.75 (C-28), 17.69 (C-29), 21.73 (C-30); +ve FAB MS m/z 457 $[\text{M}+\text{H}]^+$ ($\text{C}_{30}\text{H}_{49}\text{O}_3$) (16.6), 440 (21.7), 410 (19.1), 248 (32.1), 189 (31.3), 174 (15.9), 159 (17.8).

Siaresinolic acid (3)

Elution of the column with chloroform eluent afforded colourless crystals of **3**, recrystallized from acetone, 114 mg; m.p. 272 – 273 °C, IR $\nu_{\max}$ (KBr): 3425, 3238, 2929, 2861, 1693, 1637, 1455, 1384, 1031, 981 cm^{-1} ; ^1H NMR (CDCl_3): δ 5.17 (1H, d, J = 8.4 Hz, H-12), 4.32 (1H, d, J = 5.1 Hz, H-19 β), 3.39 (1H, dd, J = 5.6, 9.5 Hz, H-3 β), 2.97 (1H, s, J = 5.1 Hz, H-18 β), 1.25 (3H, s, Me-23), 1.08 (3H, s, Me-25), 0.93 (6H, s, Me-29), 0.91 (3H, s, Me-26), 0.88 (3H, s, Me-24), 0.73 (3H, s, Me-27), 0.65 (3H, s, Me-30), 2.27-1.27 (20 H, 9 x CH_2 , 2 x CH); ^{13}C NMR (CDCl_3): δ 38.32 (C-1), 22.27 (C-2), 80.64 (C-3), 37.29 (C-4), 55.34 (C-5), 18.71 (C-6), 32.66 (C-7), 39.90 (C-8), 47.63 (C-9), 36.96 (C-10), 23.53 (C-11), 121.53 (C-12), 145.38 (C-13), 41.83 (C-14), 26.29 (C-15), 26.23 (C-16), 32.53 (C-17), 47.37 (C-18), 76.69 (C-19), 31.22 (C-20), 34.86 (C-21), 37.13 (C-22), 16.78 (C-23), 28.47 (C-24), 15.51 (C-25), 16.25 (C-26), 25.92 (C-27), 178.08 (C-28), 33.39 (C-29), 19.47 (C-30); +ve FAB MS m/z 473 $[\text{M}+\text{H}]^+$ ($\text{C}_{30}\text{H}_{49}\text{O}_4$) (2.6), 427 (9.1), 265 (8.1), 248 (11.2), 220 (21.3), 207 (12.2), 203 (24.9), 189 (25.4), 174 (14.9), 119 (74.7), 107 (61.1).

β -Sitosterol 3- β -D-glucopyranoside (4)

Elution of the column with chloroform- methanol (9:1) furnished colourless amorphous powder of **4**, recrystallized from methanol, yield: 200 mg; R_f 0.35 (chloroform: methanol, 9:1); m.p. 265- 267 °C, UV $\lambda_{\max}$ (MeOH): 241 nm (log ϵ 2.9); IR $\nu_{\max}$ (KBr): 3401, 3321, 2918, 2849, 1654, 1377, 1261, 1172, 1082 cm^{-1} ; ^1H NMR (CDCl_3): δ 5.34 (1H, m, H-6), 5.11 (1H, d, J = 7.2 Hz, H-1'), 4.37 (1H, m, H-5'), 4.31 (1H, m, H-4'), 4.16 (1H, m, H-3'), 3.91 (1H, m, H-2'), 3.54 (1H, brs, w $\frac{1}{2}$ = 18.5 Hz, H-3), 3.37 (2H, brs, H-2'-6'), 0.99 (3H, brs, Me-19), 0.92 (3H, d, J = 6.2 Hz, Me-21), 0.87 (3H, brs, Me-27), 0.84 (3H, brs, Me-26), 0.82 (3H, brs, Me-29), 0.67 (3H, brs, Me-18); ^{13}C NMR (CDCl_3): δ 37.28 (C-1), 31.93 (C-2), 70.62 (C-3), 42.33 (C-4), 140.31 (C-5), 122.10 (C-6), 29.33 (C-7), 34.23 (C-8), 50.21 (C-9), 36.14 (C-10), 22.66 (C-11), 38.89 (C-12), 39.78 (C-13), 56.18 (C-14), 27.21 (C-15), 28.22 (C-16), 56.12 (C-17), 11.85 (C-18), 19.33 (C-19), 36.73 (C-20), 19.03 (C-21), 33.98 (C-22), 26.18 (C-23), 45.68 (C-24), 29.68 (C-25), 21.07 (C-26), 19.78 (C-27), 24.94 (C-28), 14.07 (C-29), 101.20 (C-1'), 73.92 (C-2'),

68.61 (C- 3'), 73.62 (C- 4'), 78.58 (C- 5'), 62.11 (C- 6'); +ve ion FABMS m/z (rel.int.): 576 $[M]^+$ ($C_{35}H_{60}O_6$) (11.3), 413 $[M - C_6H_{11}O_5]^+$ (10.1), 398 (100).

RESULTS AND DISCUSSION

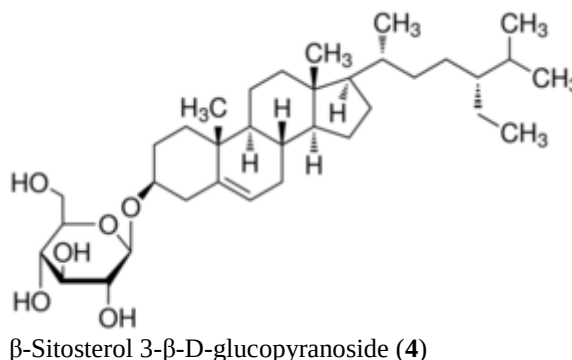
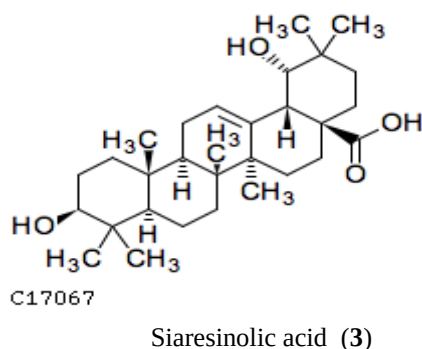
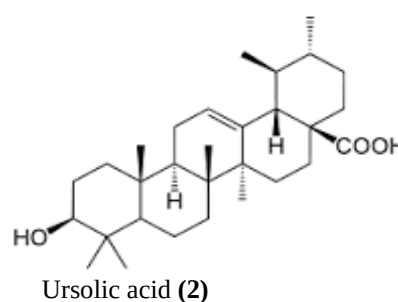
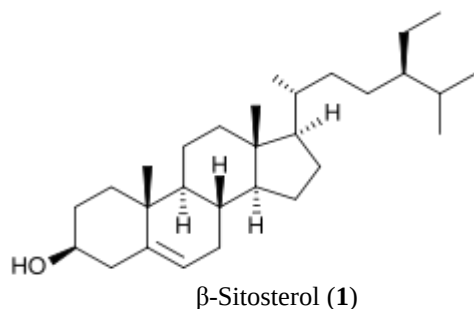
Compound **1** showed IR absorption bands for hydroxyl group (3401 cm^{-1}) and unsaturation (1654 cm^{-1}). Its mass spectrum had a molecular ion peak at m/z 414 ($C_{29}H_{50}O$). The ^1H NMR spectrum of **1** displayed signals for carbinol proton at δ 3.54 as a broad singlet with half width of 18.5 Hz, vinylic proton signal at δ 5.34 and methyl proton signals between δ 1.01 – 0.67. Its ^{13}C NMR spectrum exhibited signals for vinylic (δ 140.78, C-5; 121.68, C-6) and carbinol (δ 71.81) carbons. On the basis of spectral data analysis and comparison with the reported values^[20-22] the structure of **1** was determined as β -sitosterol.

Compound **2**, $[M+H]^+$ at m/z 457 ($C_{30}H_{49}O_3$) was characterized as ursolic acid on the basis of spectral data analysis and comparison of the spectral values with the reported data^[23,24].

Compound **3** responded for Liebermann-Burchard test positively and yielded effervescences with sodium bicarbonate solution. Its IR spectrum showed absorption bands for hydroxyl groups (3425 cm^{-1}), carboxylic function ($3238, 1693\text{ cm}^{-1}$) and unsaturation (1637 cm^{-1}). On the basis of its mass and ^{13}C NMR spectra, its molecular ion peak was established at m/z 473 $[M+H]^+$ consistent with a molecular formula of a pentacyclic triterpenoid $C_{30}H_{49}O_4$. The important ion peaks arising at m/z 207 and 265 arising due to retro-Diels Alder

fragmentation, 189 $[207 - H_2O]^+$, 174 $[189 - Me]^+$, 427 $[M - COOH]^+$ and 220 $[265 - COOH]^+$ indicated the existence of the vinylic linkage in ring C at C-12, one of the hydroxyl group placed at C-3 on the basis of biogenetic consideration and carboxylic and hydroxyl functions in rings D/E. The ^1H NMR spectrum of **3** displayed a one-proton deshielded doublet at δ 5.17 ($J = 8.4\text{ Hz}$) assigned to vinylic H-12, a one-proton doublet at δ 4.32 ($J = 5.1\text{ Hz}$) ascribed to β -oriented H-19, a one-proton double doublet at δ 3.39 ($J = 5.6, 9.5\text{ Hz}$) accounted to carbinol H-3 β proton and seven three-proton singlets between δ 1.25 – 0.65 associated with the tertiary methyl protons, all of them were attached to the saturated carbons. The remaining methine and methylene protons appeared from δ 2.27 to 1.27. The ^{13}C NMR spectrum of **3** exhibited the presence of 30 carbon signals. The important signals appeared for vinylic carbons at δ 121.53 (C-12) and 145.38 (C-13) and carboxylic carbon at δ 178.08 (C-28), carbinol carbons at δ 80.64 (C-3) and 76.69 (C-19) and methyl carbons between δ 33.39 – 15.51. On the basis of the foregoing account the structure of **3** has been established as olean-12-en-3 β , 19 α -diol-28-oic acid (siaresinolic acid). This is a rare triterpenic acid and recently isolated from *Sabicea grisea* var. *grisea*^[25].

The compound **4**, $[M]^+$ at m/z 576, responded to glycoside tests positively and showed IR absorption bands for hydroxyl groups ($3401, 3321\text{ cm}^{-1}$) and unsaturation (1654 cm^{-1}). On the basis of ^1H and ^{13}C NMR spectra analysis^[20-22] its structure was elucidated as β -sitosterol 3- β -D-glucopyranoside.



CONCLUSION

Phytochemical investigation of the leaves of *F. pandura* furnished steroids and pentacyclic triterpenoids which

may be used as chromatographic markers for identification of the drug.

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