Journal of Scientific and Innovative Research 2014; 3(4): 409-413
Available online at: www.jsirjournal.com

Research Article

ISSN 2320-4818
JSIR 2014; 3(4): 409-413

Received: 05-09-2014
Accepted: 21-09-2014

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Phytochemical investigation of the stem bark of *Ficus hispida* L.

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Abstract

Ficus hispida L. (Moraceae) is a hairy moderate sized tree used as an astringent, antidysenteric, bitter and refrigerant and to treat psoriasis, anaemia, piles, jaundice and haemorrhage. Its stem bark was extracted with methanol and the concentrated extract was subjected to silica gel column. The column was run with solvents of increasing polarity to isolate two new phytoconstituents characterized as β-sitosteryl capriate and lanost-5-enyl caprylate along with known compounds α-amyrin acetate , lupeol acetate, lupeol and α-amyrin. Their structures were elucidated on the basis of spectral data analysis. All these compounds were isolated for the first time from this plant.

Keywords: *Ficus hispida*, Moraceae, stem bark, triterpenoids, steroids.

Introduction

Ficus hispida L. (Moraceae), commonly known as gobla, devil fig and hairy fig, is a coarsely hairy moderate sized tree, up to 10 m tall, with spreading branches and many aerial roots. It is widely distributed throughout India, Sri Lanka, Myanmar, southern region of the Republic of China, New Guinea, Australia and Andaman Islands in damp localities, open lands and river banks up to 1200 m in altitude.[1] The plant parts are astringent, antidysenteric, bitter and refrigerant and used to treat psoriasis, anaemia, piles, jaundice and haemorrhage.[2-4] The fruit acts as a refrigerant and tonic. The juice obtained from the fig is taken with jaggery as a mild purgative. A mixture of honey and its juice is a good antihemorrhage.[5] Its bark contained lupeol acetate, β-amyrin acetate, β-sitosterol, acetates of *n*-triacontanol and gluanol[6] and 10-ketotetracosanyl arachidate[7]. Phenanthroindolizidine alkaloids, 6-O-methyltylophorinidine, 2-demethoxytylophorine and biphenylhexahydroindolizine hispidine are reported from the stem and leaves.[8, 9] A volatile oil containing 9,12-octadecadienoic acid, hexadecanoic acid ethyl ester, linalool, 1-hydroxylinalool and benzyl alcohol is present in the plant.[10] Hispidin[11], ficustriol, steroids, triterpenoids and bergaptine[12] have been reported from the plant. In the present work, we report the isolation and characterization of triterpenoids and steroids from the stem bark of *F. hispida* collected from Delhi.

Materials and Methods

General

Melting points were determined on a Perfit melting apparatus (Ambala, Haryana, India)

and are uncurrected. UV spectra were measured with a Lambda Bio 20 spectrophotometer (Perkin-Elmer-Rotkreuz, Switzerland) in methanol. Infra red spectra were recorded on Bio-Rad FTIR 5000 (FTS 135, Kawloon, Hong Hong) spectrophotometer using KBr pellets; γmax values are given in cm^{-1}. ^{1}H and ^{13}C NMR spectra were screened on advance DRX 400, Bruker spectrospin 400 and 100 MHz instrument in 5 mm spinning tubes at 27 ºC, respectively (Karlesruthe, Germany) using TMS as an internal standard. Mass spectra were scanned 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 silica gel (60-120 mesh; Qualigen, Mumbai, India). TLC was run on silica gel G (Qualigen). Spots were visualised by exposing to iodine vapours, UV radiation and spraying with ceric sulphate solution.

Plant material

The stem bark of *F. hispida* was obtained from local market of Delhi, Khari Baoli, India. The plant material was authenticated by Dr. H.B. Singh, Head, RHMD, National Institute of Science Communication and Information Resources (CSIR), New Delhi. A voucher specimen (No. NISCAIR/RHMD/Consult/-2010-11/1465/63) is deposited in the herbarium of Department of Raw Materials Herbarium and Museum, NISCAIR, New Delhi.

Extraction and isolation

Air-dried and coarsely powdered stem bark (3 kg) was exhaustively extracted in a Soxhlet apparatus using methanol as an extracting solvent. The methanolic extract was subjected to dryness under reduced pressure to obtain a dark brown viscous mass (yield 153 g, 5.3 %). The resulted dried mass was dissolved in small amount of methanol and adsorbed on silica gel (60-120 mesh, 200 g) to prepare a slurry. The air-dried slurry was chromatographed over silica gel column loaded in petroleum ether. The column was eluted with petroleum ether, petroleum ether-chloroform (3:1, 1:1, 1:3) mixtures and chloroform to isolate the following compounds:

α-Amyrin acetate (1)

Elution of the column with petroleum ether gave colorless crystals of **1**, recrystallized from acetone–methanol (1:1), 0.79% yield. R_f: 0.39 (petroleum ether); m.p. 239-240° C; IR ν_{max} (KBr): 1733, 1638 cm^{-1}; +ve TOF MS *m/z* (rel. int.): 468 $[M]^+$ ($C_{32}H_{52}O_2$) (14.1).

Lupeol acetate (2)

Elution of the column with petroleum ether – chloroform (3:1) furnished colorless crystals of **2,** recrystallized from acetone, yield 0.61%, R_f : 0.52 (petroleum ether-chloroform; 1:1); m.p. 216 - 218^0 C; IR ν_{max} (KBr): 1732, 1639 cm^{-1}; +ve TOF MS *m/z* (rel. int.): 468 $[M]^+$ ($C_{32}H_{52}O_2$) (1.5).

β–Sitosteryl capriate (3)

Elution of the column with petroleum ether–chloroform (1:1) furnished colorless crystals of **3,** recrystallized from acetone–methanol (1:1), yield 1.61%. R_f: 0.50 (petroleum ether-chloroform; 1:1); m.p. 62 – 64 ºC; λmax (MeOH): 208 nm (log ε 4.7); IR ν_{max} (KBr): 1725, 1616, 722 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 5.37 (1H, m, H-6), 4.51 (1H, brm, w ½ = 18.1 Hz, H-3 α), 2.33 (2H, t, J=7.2 Hz, H_2-2'), 1.28 (12H, brs, 6 x CH_2), 1.01 (3H, brs, Me–19), 0.93 (3H, d, J = 6.5 Hz, Me–21), 0.89 (3H, d, J = 6.1 Hz, Me – 26), 0.87 (3H, d, J = 6.0 Hz, Me–27), 0.85 (3H, t, J = 6.9 Hz, Me–10'), 0.83 (3H, d, 6.5 Hz, Me–29), 0.63 (3H, brs, Me–18); ^{13}C NMR ($CDCl_3$): δc 37.39 (C-1), 31.66 (C-2), 71.86 (C-3), 42.21 (C-4), 141.78 (C-5), 121.05 (C-6), 40.05 (C-7), 32.07 (C-8), 50.35 (C-9), 36.49 (C-10), 21.11 (C-11), 29.73 (C-12), 42.13 (C-13), 56.81 (C-14), 24.23 (C-15), 28.19 (C-16), 56.09 (C-17), 11.03 (C-18), 18.87 (C-19), 33.31 (C-20), 18.54 (C-21), 33.86 (C-22), 26.17 (C-23), 45.90 (C- 24), 29.05 (C-25), 20.75 (C-26), 20.83 (C-27), 18.47 (C-28), 10.38 (C-29), 173.03 (C-1′), 54.18 (C-2′), 33.15 (C-3′), 31.42 (C-4′), 29.31 (C-5′), 29.08 (C-6′), 27.69 (C-7′), 25.23 (C-8′), 22.71 (C-9′), 15.13 (C-10′); +ve TOF MS *m/z* (rel. int.): 568 $[M]^+$ ($C_{39}H_{68}O_2$) (10.2).

Lupeol (4)

Further elution of the column with petroleum ether–chloroform (1:1) gave colorless crystals of **4,** recrystallized from acetone–methanol (1:1), yield 0.11%. R_f: 0.49 (petroleum ether-chloroform; 1:1); m.p.: 213 - 215˚C; +ve TOF MS *m/z* (rel. int.): 424 $[M]^+$ ($C_{30}H_{48}O$) (2.8).

α–Amyrin (5)

Further elution of the column with petroleum ether–chloroform (1:1) yielded colourless crystals of **5**, recrystallized from acetone–methanol (1:1); 0.07% yield; R_f: 0.61 (petroleum ether-chloroform; 1:1); m.p.: 181 - 183˚C; IR ν_{max} (KBr): 3422, 1638 cm^{-1}; +ve TOF MS *m/z* (rel. int.): 426 $[M]^+$ ($C_{30}H_{50}O$) (13.2).

Lanost-5-enyl caprylate (6)

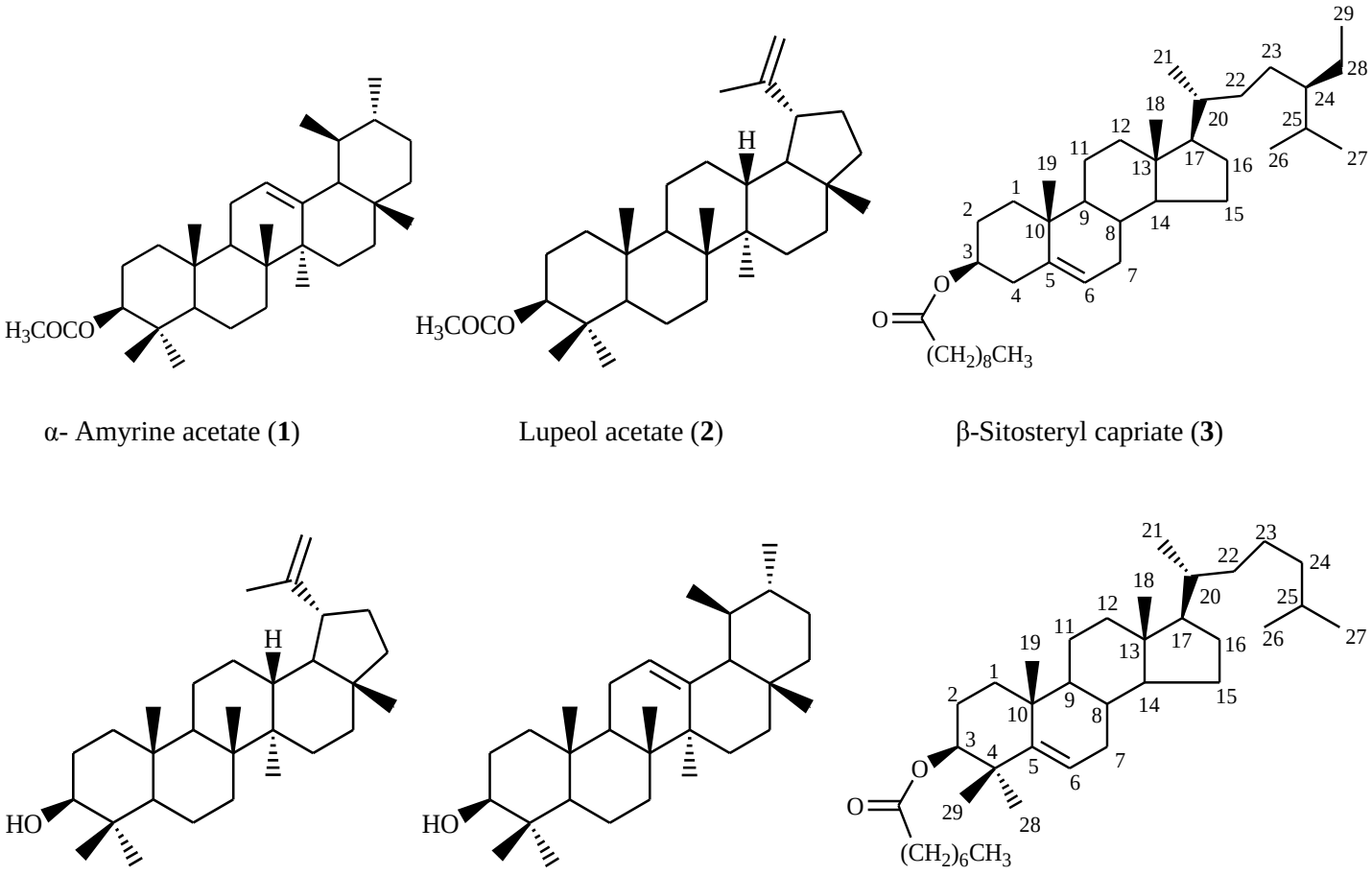
Elution of the column with petroleum ether– chloroform (1:3), furnished colourless crystals of **6**, recrystallized from acetone–methanol (1:1), yield 0.88%; R_f value: 0.62 (petroleum ether-chloroform; 1:3) m.p. 133 - 136˚C; λmax (MeOH): 205, 249 nm (log ε 3.9, 5.3); IR ν_{max} (KBr): 1723, 1646, 723 cm^{-1}; ^1H NMR ($CDCl_3$): δ 5.33 (1H, m, H–6), 4.55 (1H, dd, J = 5.5, 9.6 Hz, H-3α), 2.36 (2H, t, J = 7.2 Hz, H_2 – 2′), 1.28 (3H, brs, Me–30), 1.14 (3H, brs, Me–29), 1.05 (3H, brs, Me–19), 0.97 (3H, d, J = 6.5 Hz, Me–21), 0.94 (3H, d, J = 6.3 Hz, Me–26), 0.91 (3H, d, J = 6.0 Hz, Me–27), 0.89 (3H, brs, Me–28), 0.86 (3H, t, J=6.3 Hz, Me–8′), 0.74 (3H, brs, Me–18); ^{13}C NMR ($CDCl_3$): δc 34.22 (C-1), 27.23 (C-2), 73.05 (C-3), 39.78 (C-4), 140.37 (C-5), 121.04 (C-6), 28.73 (C-7), 41.64 (C-8), 48.13 (C-9), 37.18 (C-10), 21.15 (C-11), 23.87 (C-12), 46.13 (C-13), 52.16 (C-14), 36.03 (C-15), 41.22 (C-16), 50.35 (C-17), 11.28 (C-18), 19.85 (C-19), 30.92 (C-20), 20.16 (C-21), 36.30 (C-22), 29.11 (C-23), 31.88 (C-24), 29.04 (C-25), 26.41 (C-26), 26.49 (C-27), 22.84 (C-28), 30.06 (C-29), 16.72 (C-30), 171.81 (C-1′), 56.09 (C-2′) , 34.18 (C-3′), 29.36 (C-4′), 29.36 (C-5′) , 29.36 (C-6′) , 22.36 (C-7′), 14.22 (C-8′); +ve TOF MS *m/z* (rel. int.): 554 $[M]^+$ ($C_{38}H_{66}O_2$) (18.3).

Results and Discussion

Compounds **1, 2, 4,** and **5** were the known phytoconstituents characterized as α-amyrin acetate, lupeol acetate, lupeol and α-amyrin, respectively.

Compound **3,** named β–sitosteryl capriate, was obtained from petroleum ether-chloroform (1:1) eluent. Its IR spectrum showed characteristic absorption bands for ester group (1725 cm^{-1}), unsaturation (1616 cm^{-1}) and long aliphatic chain (722 cm^{-1}). On the basis of mass and ^{13}C-NMR spectra, the molecular ion peak of **3** was determined at *m/z* 568 constituent with the molecular formula of a steroidal ester, $C_{39}H_{68}O_2$. The ^1H NMR spectrum of **3** exhibited a one–proton multiplet at δ 5.37 assigned to vinylic H–6 proton. A one–proton broad multiplet at δ 4.51 with half-width of 18.1 Hz was due to oxygenated α–oriented methine H–3 proton. A two–proton triplet at δ 2.33 (J = 7.2 z) was ascribed to methylene H_2 – 2′ protons adjacent to the ester group. Two three–proton broad singlets at δ 1.01 and 0.63 and three doublets at δ 0.93 (J = 6.5 Hz), 0.89 (J = 6.1 Hz) and 0.87 (J = 6.0 Hz) integrating for three protons each were accounted to tertiary C–19 and C–18 and secondary C–21, C–26 and C–27 methyl protons, respectively. A three–proton doublet at δ 0.83 (J= 6.5 Hz) and as a triplet at δ 0.85 (J= 6.9 Hz) were due to primary C–29 and C–10 methyl protons, respectively. The ^{13}C NMR spectrum of **3** exhibited signals for ester carbon at δ 173.03 (C–1′), vinylic carbons at δ 141.78 (C–5) and 121.05 (C– 6), oxygenated methine carbon at δ 71.86 (C-3) and methyl carbons from δ 20.83 to 10.38. The ^1H and ^{13}C NMR spectral values of the sterol unit were compared with the spectral values of the reported compounds[13,14]. On the basis of the foregoing discussion, the structure of **3** has been elucidated as stigmast–5-en-3β olyl *n*-decanoate.

Compound **6**, designated as lanost -5- en-3β-olyl caprylate, was obtained from petroleum ether-chloroform (1:3) eluent. It gave positive Liebermann–Burchardt's test for triterpenoids. Its IR spectrum showed characteristic absorption bands for ester group (1723 cm^{-1}), unsaturation (1646 cm^{-1}) and long aliphatic chain (723 cm^{-1}). On the basis of mass and ^{13}C NMR spectral data, the molecular ion peak of **6** was established at *m/z* 554 corresponding to the molecular formula of a triterpenic ester, $C_{38}H_{66}O_2$. The ^1H NMR spectrum of **6** displayed a one–proton multiplet at δ 5.33 assigned to vinylic H–6. A one–proton double doublet at δ 4.55 with coupling interactions of 5.5 and 9.6 Hz was ascribed to oxygenated α-oriented methine H–3 proton. A two–proton triplet at δ 2.36 (J = 7.2 Hz) was attributed to methylene H_2 – 2′ protons adjacent to the ester function. The other methylene protons of the ester side chain resonated at δ 1.55 (2H) and 1.31 (8H). Five three–proton broad singlets at δ 1.28, 1.14, 1.05, 0.89, and 0.74, three doublets at δ 0.97 (J =6.5 Hz), 0.94 (J = 6.3 Hz) and 0.91 (J = 6.0 Hz) and a triplet at δ 0.86 (J = 6.3 Hz) integrating for three–protons each were associated correspondingly to tertiary C-30, C-29, C-19, C-28 and C-18, secondary C-21, C-26 and C-27 and primary C-8′ methyl protons, all attached to the saturated methyl carbons. The ^{13}C NMR spectrum of **6** showed carbon signals for ester carbon at δ 171.81 (C-1′), vinylic carbons at δ 140.37 (C-5) and 121.04 (C-6), oxygenated methine carbon at δ 73.05 (C–3) and methyl carbons from δ 30.06 to 11.28. The ^1H and ^{13}C NMR spectral values of the lanostene nucleus were compared with reported data[15,16]. On the foregoing account the structure of **6** has been formulated as lanost- 5- en- 3β-olyl *n*-octanoate.



α- Amyrine acetate (**1**) Lupeol acetate (**2**) β-Sitosteryl capriate (**3**)

Lupeol (**4**) α- Amyrin (**5**) Lanost-5-en-3β-olyl caprylate (**6**)

Figure 1: Structural formulae of phytoconstituents isolated from *Ficus hispida* stem bark

Conclusion

The stem bark of *Ficus hispida* contained steroids and triterpenoids. These phytoconstituents are reported for the first time from the stem bark which may be responsible for the medicinal properties of the plant.

Acknowledgement

The authors wish to thank the Incharge, AIRF, Jawaharlal Nehru University, New Delhi for recording spectral data of the compounds.

Conflict of Interest

Authors have no conflict of interest.

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