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Research Article

PHYTOCONSTITUENTS FROM THE STEM BARK OF *MANGIFERA INDICA* VARIETY "*SAFEDA*"

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ABSTRACT
Phytochemical investigation of the stem bark of *mangifera indica* variety "*safeda*" yielded four new constituents characterised as 5-oxo-*n*-heptyl-n-hexadecanoate, 6α-hydroxypiperitonoic acid, 6β- hydroxypiperitonoic acid and eicos-2-en-11-one-12β, 14β-diol-1,5-olide along with the known phytoconstituents dimethyl terephthalate, β-sitosterol and its β-D-glucoside. The structures of these phytoconstituents have been elucidated on the basis of spectral data analysis and chemical reactions.

KEYWORDS: *Mangifera indica* var. *safeda*, Anacardiaceae, pipertonoic acids, eicosane lactone.

INTRODUCTION

Mangifera indica L. (Anacardiaceae), commonly known as Am or mango, is a large evergreen tree with a heavy dome shaped crown and straight, stout bole. It occurs throughout India, other parts of temperate Asia, southern Europe and America[1]. It is the prominent fruit crop and over 1,000 mango types are grown in various parts of India, each having its own peculiar taste, flavour and consistency of pulp. The mango stem bark is astringent, anthelmintic and used to treat haemoptysis, haemorrhage, nasal catarrh, diarrhoea, ulcers, diphtheria, rheumatism and for lumbrici[2]. The stem bark stops vomiting[3]. Aliphatic constituents, coumarin, mangiferine[4-6], sequiterpinenoids[6,7], triterpinoids[8,9] and phenolics[10] have been reported from the stem barks of different cultivars of *M. indica*. This paper describes the isolation and characterization of phytoconstituents from the bark of *Mangifera indica* var. *safeda*.

MATERIALS AND METHODS

The melting points were determined on a Perfit apparatus and are uncorrected. The IR spectra were recorded in KBr pellet on Win IR FTS 135 instrument (Biorad, USA). ^{1}H NMR (300 MHz) and ^{13}C NMR (75 MHz) spectra were recorded by Bruker spectrospin NMR instrument in $CDCl_3$ using TMS as internal standard. EIMS were scanned at 70 eV on a Jeol D-300 instrument (Jeol, USA). Column chromatography was performed on silica gel (Merck, 60–120 mesh) and thin layer chromatography on silica gel G-coated TLC plates (Merck).

Plant material

Stem barks of *Mangifera indica* variety 'Safeda" was collected from Baghpat, U.P. and identified by Prof. M.P. Sharma, Taxonomist, Department of Botany, Faculty of Science, Jamia Hamdard (Hamdard University). A voucher specimen No. PRL/JH/08/45 was deposited in the herbarium of the Faculty of Pharmacy, Jamia Hamdard (Hamdard University), New Delhi, India.

Extraction and isolation of compounds

The dried and powdered stem bark (3.0 kg) was extracted with ethanol in a Soxhlet apparatus. The extracts were combined and the solvent evaporated under reduced pressure to obtain a dark brown viscous mass (195 g). The dried alcoholic extract was dissolved in minimum amount of methanol and adsorbed on silica gel to form slurry. The slurry was air-dried and chromatographed over silica gel column prepared in petroleum ether. The column was eluted with petroleum ether, chloroform and methanol in order of increasing polarity to isolate the following compounds:

Dimethyl terephthalate (1)

Elution of the column with petroleum ether furnished colourless amorphous powder of **1**, needles from ethyl acetate, m.p. 139-141° (lit m.p. 141-142°), 1.23 g (0.04% yield), R_f = 0.21 (petroleum ether), UV λ_{max} MeOH: 235 nm (log ε 6.5); IR γ_{max} (KBr): 2965, 1720, 1635, 1523, 955, 875, 815, 715 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 7.23 (2H, m, H-2, H-6), 6.60 (2H, m, H-3, H-5), 3.50 (6H, brs, 2×OCH_3); EIMS *m/z* (rel. int.); 194 $[M]^+$ ($C_{10}H_{10}O_4$), (64.2).

Oxoheptyl palmitate (2)

Further elution of the column with petroleum ether gave colourless amorphous powder of **2**, recrystallized from $CHCl_3$-MeOH (1:1), 2.50 g (0.08% yield), R_f=0.42 (petroleum ether–benzene, 1:1), m.p. 72-73° UV λ_{max} MeOH: 207 nm (log ε 6.5); IR γ_{max} (KBr): 2921, 2852, 1738, 1712, 1467, 1230, 1170, 723 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 4.10 (2H, m, H_2-1), 2.33 (2H, m, H_2-4), 2.26 (2H, m, H_2-6), 2.13 (2H, m, H_2-2′), 1.96 (2H, m, CH_2), 1.60 (4H, m, 2 × CH_2), 1.52 (4H, m, 2 × CH_2), 1.23 (20H, brs, 10 × CH_2), 0.86 (3H, t, *J*=6.3 Hz, Me-7), 0.82 (3H, t, *J*=6.5 Hz, Me-16′); ^{13}C NMR ($CDCl_3$): δ 205.21 (C-5), 170.69 (C-1′), 63.48 (C-1), 34.56 (CH_2), 32.73 (CH_2), 29.36 (CH_2), 29.14 (12 × CH_2), 28.91 (CH_2), 24.13 (CH_2), 22.50 (CH_2), 14.35 (Me-16′), 14.18 (Me-7); EIMS *m/z* (rel. int.): 368 $[M]^+$ ($C_{23}H_{44}O_3$) (23.8), 353 (7.3), 339 (36.9), 325 (6.3), 311 (16.8), 255 (48.6), 239 (11.8), 129 (54.4), 115 (23.8), 113 (11.5), 101 (17.5), 73 (95.2), 57 (100).

β-Sitosterol

Elution of the column with petroleum ether-chloroform (9:1) yielded a colourless product **3**, recrystallized from ethyl acetate, 3.3 g (0.11% yield), R_f=0.18 (petroleum ether-chloroform, 4:1), m.p. 137-138°, $[\alpha]_D^{25}$=+22.3°, (C 0.754, MeOH); IR γ_{max} (KBr): 3450, 1635,1440, cm^{-1}; EIMS *m/z* (rel. int.): 414 $[M]^+$ ($C_{29}H_{50}O$) (11.2).

6α-Hydroxypiperitonic acid (4)

Elution of the column with petroleum ether-chloroform (4:1) afforded colourless crystals of **4**, recrystallized from methanol, 4.2 g (1.4% yield), R_f= 0.33 (benzene : chloroform, 4:1); m.p 140-141°; UV λ_{max} MeOH: 222 nm (log ε 6.8); IR γ_{max} (KBr): 3457, 3307, 2977, 2835, 1705, 1690, 1635, 1469, 1410, 1348, 1315, 1255, 1197, 1039, 966, 866 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 6.85 (1H, brs, H-2), 4.33 (1H, dd, *J*=5.2, 5.8 Hz, H-6β), 2.85 (1H, ddd, *J*=6.5, 4.8, 6.2 Hz, H-4), 2.63 (1H, m, H-8), 2.01 (1H, m, H-5a), 1.95 (1H, m, H-5b), 1.50 (3H, brs, Me-7), 1.23 (3H, d, *J*=6.2 Hz, Me-10); ^{13}C NMR ($CDCl_3$): δ 202.37 (C-3), 180.26 (C-9), 141.58 (C-1), 122.76 (C-2), 79.15 (C-6), 46.27 (C-4), 38.61 (C-8), 29.48 (C-5), 23.16 (C-7), 18.45 (C-10); EIMS *m/z* (rel. int.): 198 $[M]^+$ ($C_{10}H_{14}O_4$) (49.5), 183 (9.8), 180, (22.8), 152 (100), 137 (13.5), 125 (21.8), 107 (12.3).

6β-Hydroxypiperitonoic acid (5)

Further elution of the column with petroleum ether-chloroform (4:1) furnished coloursless crystalline product **5**, purified by preparative TLC (chloroform-methanol, 4:1), 1.2 g (0.40% yield), R_f=0.58 (benzene:chloroform, 3:1), m.p. 134-135°, $[\alpha]^{25}$ = +12.5 (C 1.003, methanol); UV λ_{max} MeOH: 223, 279 nm (log ε 6.7, 6.0); IR γ_{max}

(KBr): 3460, 3290, 2980, 1710, 1692, 1640, 1535, 1469, 1384, 1315, 1251, 1196, 1036, 965 cm^{-1}; ^1H NMR ($CDCl_3$): 6.83 (1H, brs, H-2), 4.35 (1H, dd, *J*=5.3, 9.2 Hz, H-6α), 2.83 (1H, m, H-4), 2.60 (1H, m, H-8), 1.98 (2H, m, H2-5), 1.52 (3H, brs, Me-7), 1.21 (3H, d, *J*=6.1 Hz, Me-10); ^{13}C NMR ($CDCl_3$): 203.16 (C-3), 179.83 (C-9), 140.77 (C-1), 121.84 (C-2), 78.69 (C-6), 45.80 (C-4), 36.25 (C-8), 29.36 (C-5), 22.98 (C-7), 18.31 (C-10); EIMS *m/z* (rel. int.): 198 $[M]^+$ ($C_{10}H_{14}O_4$) (46.8), 183 (19.6), 180 (20.9), 163 (14.2), 152 (90.3), 137 (16.4), 125 (20.3).

Dihydroxy eicosanyl lactone (6)

Elution of the column with chloroform gave colurless crystal of **6**, recrystallized from methanol, 3.8 g (0.42% yield), R_f= 0.11($CHCl_3$), m.p. 60-61º, $[\alpha]_D^{25}$= - 18.5º, (C 0.972, MeOH); UV λ_{max} MeOH: 214, 237 nm (log ε 6.5, 6.9); IR γ_{max} (KBr): 3440, 2920, 2850, 1715, 1705, 1625, 1465, 1260, 1170, 1025, 820, 800, 720 cm^{-1}; ^1H NMR ($CDCl_3$): δ 6.40 (1H, dd, *J*=6.7, 5.5 Hz, H-3), 5.30 (1H, d, *J* = 5.5 Hz, H-2), 4.10 (2H, d, *J*=6.5 Hz, H_2-5), 3.90 (1H, dd, *J*=5.1, 8.3 Hz, H-12α), 3.48 (1H, brm, $w_{1/2}$= 16.5 Hz, H-14α), 2.30 (1H, brm, $w_{1/2}$=6.9Hz, H-4α), 2.13 (2H, m, H_2-10), 1.98 (2H, m, H_2=13), 1.62 (2H, m, CH_2), 1.29 (18H, brs, 9 × CH_2), 0.86 (3H, t, *J*=6.3 Hz, Me-21); ^{13}C NMR ($CDCl_3$): 203.15 (C-11), 169.87 (C-1), 63.26 (C-5), 68.51 (C-12), 66.37 (C-14), 45.20 (C-4), 36.15 (CH_2), 31.76 (CH_2), 29.42 (7×CH_2), 25.33 (CH_2), 22.16 (CH_2), 14.17 (Me-21); EIMS *m/z* (rel. int.): 368 $[M]^+$ ($C_{21}H_{36}O_5$) (11.1), 195 (15.6), 173 (14.2), 167 (16.3), 143 (14.3), 129 (21.8), 99 (19.7), 97 (37.2).

β- Sitosterol D-glucoside (7)

Elution of the column with chloroform-methanol (9:1) gave colourless crystals of **7**, recrystallized from chloroform-methanol (1:1), 4.05 g (0.13% yield), R_f =0.61(methanol-acetone, 1:1), m.p. 280-282º (lit. m.p. 283-286º); IR γ_{max} (KBr): 3450, 3400, 3300, 1600, 1430, 1355 cm^{-1}; EIMS *m/z* (rel. int.): 576 $[M]^+$ ($C_{35}H_{60}O_6$)(3.7), 413 (38.3).

RESULT AND DISCUSSION

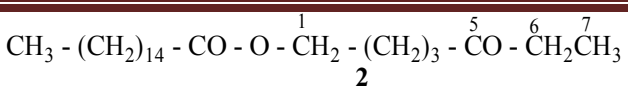
Compound **1**, **3** and **7** are the known compounds, chararcterised as dimethyl terephthalate, β-sitosterol and β-sitosterol-3β-D-glycoside respectively.

Compound **2**, named oxoheptyl palmitate was obtained as colourless amorphous powder from petroleum ether eluent. Its IR spectrum showed characteristic absorption bands for ester (1738 cm^{-1}) and keto (1712 cm^{-1}) groups and long aliphatic chain. On combination of its mass and ^{13}C NMR spectra, the molecular weight of **2** was determined at *m/z* 368 corresponding to the molecular formula of fatty acid ester $C_{23}H_{44}O_3$. The ion peaks arising at *m/z* 239 $[CH_3(CH_2)_{14}CO]^+$, 255 $[CH_3(CH_2)_{14}COO]^+$, 129 $[M-239]^+$ and 113 $[M-255]^+$ indicated that palmitic acid was esterified with oxoheptyl alcohol. The ion fragments appearing at *m/z* 57, 311 $[C_4\text{-}C_5$ fission$]^+$ and 339 $[M\text{-}C_2H_5]^+$ suggested the location of carbonyl function at C-5. The ^1H NMR spectrum of **2** exhibited a two-proton multiplet at δ 4.10 assigned to oxygenated methylene H_2-1, methylene protons from δ 2.33 to 1.23 and two three-proton triplets at δ 0.86 (*J*=6.3 Hz) and 0.82 (*J*=6.5 Hz) ascribed to terminal C-7 and C-16′ primary methyl protons. The ^{13}C NMR spectrum of **2** displayed signals for carbonyl carbon at δ 205.21 (C-5), ester carbon at δ 170.69 (C-1′), oxygenated methylene carbon at δ 63.48 (C-1), methylene carbon between δ 34.56-22.50 and methyl carbons at δ 14.35 (C-16′) and 14.18 (C-7). Alkaline hydrolysis of **2** yielded palmitic acid. On the basis of these evidences, the structure of **2** was established as 5-oxo-*n*-heptyl *n*-hexadecanoate. This is a new fatty acid ester.

Compound **4**, a piperitone-type monoterpene, was obtained as a colourless product from petroleum ether-chloroform (4:1) eluents. It gave effervescences with sodium bicarbonate solution. Its IR spectrum exhibited characteristic absorption bands for hydroxyl group (3457 cm^{-1}), carboxylic function (3307, 1690 cm^{-1}), carbonyl group (1705 cm^{-1}) and unsaturation (1635 cm^{-1}). On the basis of mass and ^{13}C NMR spectra, the molecular ion peak of **4** was determined at *m/z* 198 consistent to the molecular formula of oxomonoterpenic carboxylic acid, $C_{10}H_{14}O_4$. The ion peaks arising at *m/z* 183 $[M\text{-}Me]^+$, 152 $[M\text{-}HCOOH]^+$, 137 $[152\text{-}Me]^+$ and 180 $[M\text{-}H_2O]^+$ supported the presence of carboxylic and hydroxyl groups in the molecule. The ion fragments appearing at *m/z* 125 $[M\text{-}CH(CH_3)COOH]^+$ and 107 $[125\text{-}H_2O]^+$ suggested the existence of the carboxylic function in the isopropanoic unit and hydroxyl function in the cyclohexyl ring of the methane carbon framework. The ^1H NMR spectrum of **4** showed a one-proton broad signal at δ 6.85 assigned to vinylic H-2 proton and its appearance in the deshielding field suggested its location nearby to carbonyl function. A one-proton double doublet at δ 4.33 with coupling interactions of 5.2 and 5.8 Hz was ascribed to β-oriented carbinol H-6 proton. Two three-proton signals as a broad signal at δ 1.50 and as a doublet at δ 1.23 (*J*=6.2 Hz) were attributed to C-7 methyl protons located on the vinylic carbon and to C-10 secondary methyl protons, respectively. The other methylene and methine protons resonated from δ 2.85 to 1.95. The ^{13}C NMR spectrum of **4** displayed important signals for carbonyl carbon at δ 202.37 (C-3), carboxylic carbon at δ 180.26 (C-9), vinylic carbons at δ 141.58 (C-1) and 122.76 (C-2) and carbinol carbons at δ 79.15 (C-6). On the basis of these evidences, the structure of **4** has been elucidated as 6α-hydroxypiperitonoic acid (*p*-menth-1-en-3-on-6α-ol-9-oic acid). It is a new *p*-menthane-like monoterpenoic acid.

Compound **5**, an isomer of **4**, was also obtained from petroleum ether-chloroform (4:1) eluents. Mass fragmentation pattern and NMR spectral data analysis indicated the presence of similar functional group in the *p*-methane skeleton. The ^1H NMR spectrum of **4** showed proton signals identical to that of **5**. However, the H-6 carbinol signal at δ 4.35 was observed as double doublet with coupling interactions of 5.3 and 9.2 Hz suggesting its α-orientation. Hence, the structure of **5** was determined as 6β-hydroxypiperitonoic acid. It is also a new monoterpenoic acid.

Compound **6**, named dihydroxyeicosanyl lactone, was obtained as a colourless crystalline mass from chloroform eluents. Its IR spectrum showed characteristic absorption bands for hydroxyl groups (3440 cm^{-1}), δ-lactone ring (1735 cm^{-1}), keto function (1705 cm^{-1}), unsaturation (1625 cm^{-1}) and long aliphatic chain (720 cm^{-1}). On the basis of mass and ^{13}C NMR spectra, the molecular ion peak of **6** was established at *m/z* 368 corresponding to dihydroxy δ-lactone, $C_{21}H_{36}O_5$. The ion peaks generating at *m/z* 97 $[C_5H_5O_2$, $C_4\text{-}C_6$ fission$]^+$, 167 $[C_5H_5O_2\text{-}(CH_2)_5$, $C_{10}\text{-}C_{11}$ fission$]^+$ and 195 $[C_{11}\text{-}C_{12}$ fission$]^+$ indicated the presence of δ-lactone at the terminal position and carbonyl function at C-11. The ion fragments arising at *m/z* 173 $[M\text{-}195]^+$, 143 $[C_{12}\text{-}C_{13}$ fission$]^+$, 129 $[C_{13}\text{-}C_{14}$ fission$]^+$ supported the location of the hydroxyl group at C-12 and C-14. The ^1H NMR spectrum of **6** showed two one-proton signals as a double doublet at δ 6.40 (*J*=6.7, 5.5 Hz) and a doublet at δ 5.30 (*J*=5.5 Hz) assigned to vinylic H-3 and H-2, respectively. A two-proton doublet at δ 4.10 (*J*=6.5 Hz) was ascribed to oxygenated methylene H_2-5 protons. A one-proton double doublet at δ 3.90 (*J*=5.1, 8.3 Hz) and a one-proton broad multiplet at δ 3.48 width half-width of 16.5 Hz were attributed to α-oriented carbinol H-12 and H-14 protons, respectively. The methylene protons resonated as two-proton multiplets at δ 2.13, 1.98 and 1.62 and as abroad signal at δ 1.29 (18H). A three-proton triplet at δ 0.86 (*J*=6.3 Hz) was accounted to terminal C-21 primary methyl protons. The ^{13}C NMR spectrum of **6** exhibited important signals for carbonyl carbon at δ 203.15 (C-11), lactone carbon at δ 169.87 (C-1), carbinol carbons at δ 68.51 (C-12) and 66.37 (C-14), oxygenated methylene carbon at δ 63.26 (C-5) and methyl carbon at δ 14.17 (C-21). These data led to formulate the structure of **6** as eicos-2-en-11-one-12β, 14β-diol-1,5-olide. This is a new δ-lactone of natural origin. The other δ-lactone containing constituents have been isolated from *Oriza sativa*[12], *Pluchea lanceolata*[13] and *Desmotrichum finibrianum*[14].



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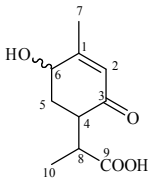
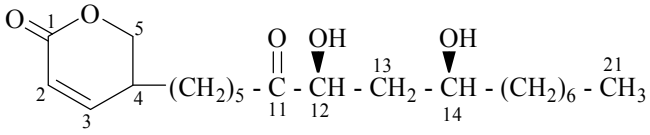


4. 6α-OH

5. 6β-OH



6

CONCLUSION

The phytochemical investigation of stem bark of *M.indica* var. *Safeda* gave two new methanone-type monoterpenoids and one each fatty ester and aliphatic δ-lactone compound as the new phytoconstituents. These compounds may be used as chromatographic markers for quality control of the stem bark of this species.

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REFERENCES

1. Anonymous, The Wealth of India, Raw Material, , National Institute of Science Communication and Information Resources, CSIR, New Delhi 2003; 6 (supp. 4): 265-285.
2. Sharma SK, Ali M. Evaluation of chemical constituents of *Mangifera indica* (Mango). Hamdard Medicus, 1992; 35(3), 110-133.
3. Mhaskar KS, Blatter E, Caius JF. Kirtikar and Basu's Illustrated medicinal Plants, Sri Satguru Publication, 2000; 3: 916-922.
4. Sharma SK, Ali M. Chemical constituents of stem bark of *Mangifera indica* cultivar Dusehri. Indian Drugs 1993; 30: 446-449.
5. Sharma SK, Ali M. Chemical constituents of stem bark of *Mangifera indica* cultivar Dusehri. Indian J Pharmaceut Sci 1994; 56: 53-56.
6. Sharma SK, Ali M. Chemical constituents of stem bark of *Mangifera indica* cultivar Deshi. J Indian Chem Soc 1995; 72: 339-342.
7. Sharma SK, Ali M. Sesquiterpenic constituents of stem bark of *Mangifera indica* cultivar Chausa. Indian J Nat Prod 1993a; 9: 3-5.
8. Anjaneyulu V, Babu JS, Krishna MM, Connolly JD. 3-Oxo-20*S*, 24*R*-epoxy-dammarane-25ξ, 26-diol from *Mangifera indica,* Phytochemistry 1993; 32(2): 469-471.
9. Anjaneyulu V, Babu JS, Connolly JD. 29-hydroxymangiferonic acid from *Mangifera indica*. Phytochemistry 1994; 35(5): 1301-1303.
10. Ghosal S, Chakrabarti DK. Differences in phenolic and steroidal constituents between healthy and infected florets of *Mangifera indica*. Phytochemistry 1988; 27(5): 1339-1343.
11. Ali M. Techniques in Terpinoid Identification. Birla Publications, Delhi, 2001; 384.
12. Chung IM, Ali M, Chun S-C, Lee O-K, Ahmad A. Sativalanosteroyl glycoside and Oryzatriacontolide constituents from hulls of *Oriza sativa*. Asian J Chem 2007; 19: 1535-1543.
13. Ali M, Siddiqui NA, Ramachandran R. Phytochemical investigation of aerial parts of *Pluchea lanceolata* C.B. Clarke, Indian J Chemistry 2001; 40 B: 698-706.
14. Roy SK, Ali M, Sharma MP, Ramachandran R. Phytochemical investigations of *Desmotrichum fimbrianum* Blume. Indian J Nat Prod 2002; 18 (1): 13-17.

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