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Isolation of Fatty Acids and Other Constituents from *Callicarpa macrophylla* Fruits

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Two diterpenoids, 16α-hydroxy-17-acetoxy-3-oxophyllocladane, 16α,17-dihydroxy-3-oxophyllocladane and four fatty acids, linoleic acid, stearic acid, myristic acid, octacosanoic acid along with four steroids, β-sitosterol, stigmasterol, oleanolic acid, β-sitosterol-3-O-β-D-glucoside were isolated and identified from the fruits of *Callicarpa macrophylla*. The structures of all compounds were elucidated by ^{1}H NMR and ^{13}C NMR aided by EIMS, FABMS and IR spectra and also compared with reported values.

Key Words: *Callicarpa macrophylla*, Verbanaceae, Known compounds.

INTRODUCTION

Callicarpa macrophylla Vahl. (Verbenaceae), a shrub growing abundantly in the upper Gangetic plain[1] and due to medicinal importance of *C. macrophylla* in the Indian system of medicine[2], chemical investigations of different parts like seeds, leaves and bark of the plant have been carried out by several groups[3–16]. However, the fruits have not been investigated chemically; for instance, its fruits are used by the Tharus of Kheri District, Uttar Pradesh, India for blisters and boils on tongue[17]. Recently we reported callicarpenol[18] from its fruits and further investigation of its fruits, and identified the more known compounds in minor quantities as 16α-hydroxy-17-acetoxy-3-oxophyllocladane (calliterpenone monoacetate) (**1**), 16α,17-dihydroxy-3-oxophyllocladane (calliterpenone) (**2**), β-sitosterol (**3**), linoleic acid (**4**), stigmasterol (**5**), stearic acid (**6**), myristic acid (**7**), octacosanoic acid (**8**), oleanolic acid (**9**), β-sitosterol-3-O-β-D-glucoside (**10**) and characterized by using 500 MHz NMR spectral methods, *viz.*, ^{1}H and ^{13}C NMR aided by EIMS, FABMS and IR. Earlier the compounds (**1, 2, 3, 9**) were reported from seeds and leaves and other compounds (**4, 5, 6–8, 10**) are reported for the first time from fruits.

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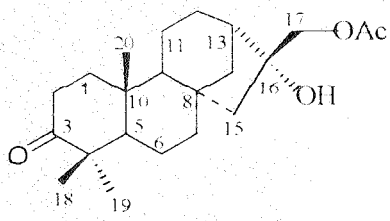

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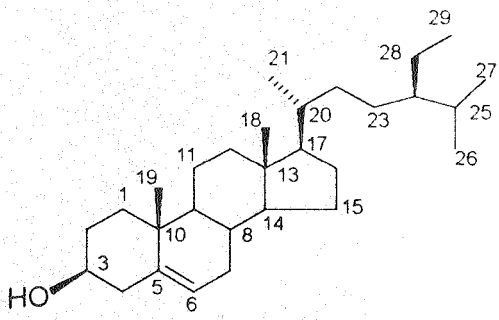

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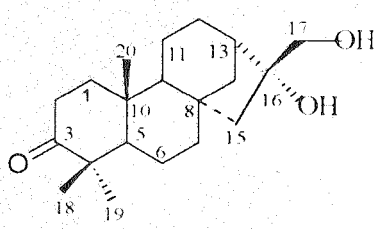

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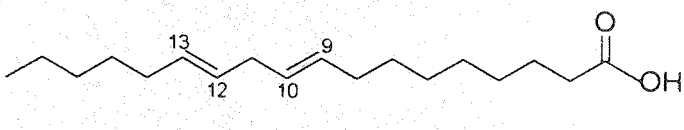

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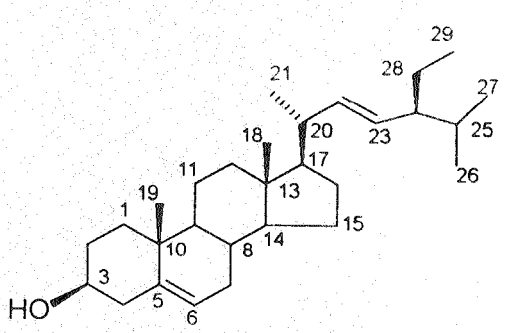

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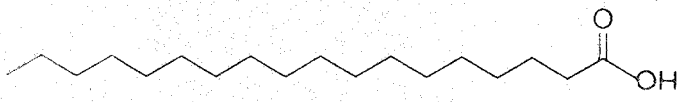
Chemical structures of 1–5



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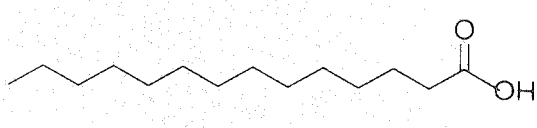

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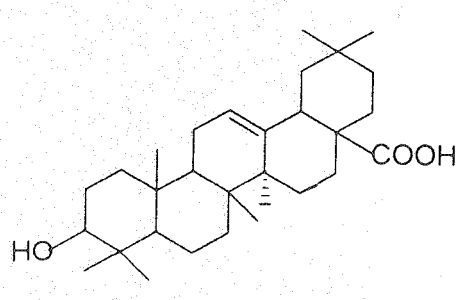
$CH_3(CH_2)_{24}CH_2CH_2COOH$

8



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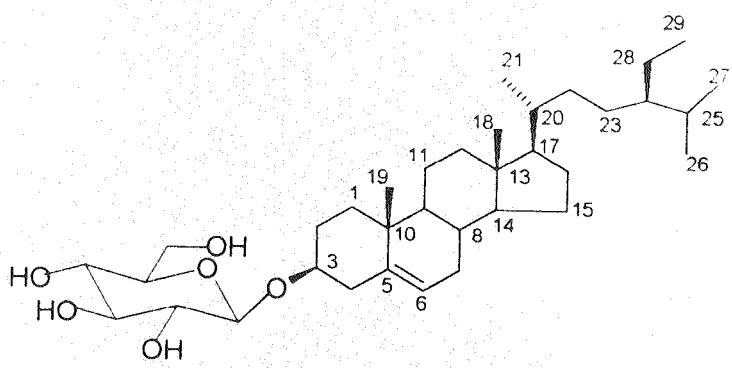

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Chemical structures of **6–10**

EXPERIMENTAL

Melting points were determined on Electrochemical Eng. melting point apparatus. IR spectra were recorded on a Thermo Mattson 60-AR spectrophotometer. UV spectra were recorded using a UV-Vis spectrometer TU-180_{PC}. ^1H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectra were obtained on a Bruker Avance (DRX-500) using $CDCl_3$, CD_3OD, C_5D_5N as solvents. EI-Mass spectra were recorded on a Jeol JMS-SX 102 A and FABMS on a Jeol JMS-AX 505 WA. Column chromatography was performed over silica gel 70–230 (Merck) or ODS silica gel [Lichroprep RP-18 (40–63 μm)] (Merck). TLC analyses were performed on

precoated silica gel glass plates $60F_{254}$ (Merck) and visualized under UV light and by spraying with vanillin (1 g)-sulfuric acid (5 mL)-ethanol (94 mL) solution followed by heating (100–110°C). The authentic samples (linoleic acid, stearic acid, myristic acid, β-sitosterol, stigmasterol, octacosanoic acid and oleanolic acid) were purchased from Sigma Company for Co-TLC.

Plant material: Fruit tissue (250 g) of *C. macrophylla* were collected from the Himalayas of Kumaun region, Nanital (India) in August 2001 when white in colour and dried at low temperature in oven and authenticated by Prof. Y.P.S. Pangtey, Plant Taxonomist, Department of Botany, Kumaun University, India. A voucher specimen (No. KUPH 70-71) was deposited at the herbarium of Kumaun University, Nainital, India.

Extraction and isolation: The dried powdered fruit tissues (250 g) of *C. macrophylla* after defatting with petroleum ether (40–60°C) were extracted with 95% EtOH (3 × 1 L) at room temperature. The combined extract was evaporated to dryness (30 g). The residue was separated on a silica gel column (70–230 mesh, 700 g, 5.0 × 75 cm) and eluted with a gradient of hexane (hex), hex/EtOAc (9 : 1, 8 : 2, 7 : 3, 1 : 1), EtOAc, EtOAc/MeOH (9.5 : 0.5, 9 : 1, 7 : 3) and MeOH (each fraction 500 mL): fractions 1–2 in hex, 3–5 in hex-EtOAc (9 : 1), 6–11 in hex-EtOAc (8 : 2), 12–15 in hex-EtOAc (7 : 3), 16–20 in hex-EtOAc (1 : 1), 21–22 in EtOAc, 23–28 in EtOAc-MeOH (9.5 : 0.5), 29–30 in EtOAc-MeOH (9 : 1), 31–34 in EtOAc-MeOH (7 : 3) and 35–40 in MeOH. The fraction 5 after further column chromatography obtained a calliterpenone monoacetate (**1**, 3 mg), while fraction 6 (2.4 g) was chromatographed on a silica gel (70–230 mesh, 100 g) by normal column chromatography (CC) using CH_2Cl_2-MeOH (99.8 : 0.2, 99.6 : 0.4, 99.4 : 0.6, 99.2 : 0.8, 99 : 1, each fraction 200 mL) as eluants to yield five fractions. Fractions 1–3 were combined on the basis of TLC to give pure compound β-sitosterol (**2**, 100 mg) and also confirmed with authentic sample from Sigma through Co-TLC. Fraction 9 after mixing and further purification by CC over silica gel with methylene dichloride and methanol afforded the compound calliterpenone (**3**, 4 mg). Fraction 11 after CC over silica gel methylene chloride and methanol yielded the compound linoleic acid (**4**, 15 mg). Fractions 12–13 after CC over silica gel methylene chloride and methanol yielded the pure compound stigmasterol (**5**, 10 mg) and also confirmed with authentic sample from Sigma through Co-TLC. Fractions 15–16 after CC over silica gel methylene chloride and methanol yielded two pure compounds stearic acid (**6**, 25 mg) and myristic acid (**7**, 12 mg). Fractions 16–17 were crystallized in a tube and the solid compound insoluble in hexane and after filtration through sintered funnel impure compound was obtained; the same was chromatographed on an ODS silica gel (Lichroprep RP-18 (Merck) by normal CC using sequential mixtures of H_2O/MeOH as eluants (elution order 80 : 20, 60 : 40, 40 : 60, 20 : 80), MeOH 100% and each fraction (150 mL) to yield five fractions From fraction 4 to yield octacosanoic acid (**8**, 6 mg). Fraction 21 (2.1 g) and fractions 23 (1.2 g) after CC over silica gel using $CHCl_3$/MeOH yielded one compound from each fraction, oleanolic acid (**9**, 5 mg), β-sitosterol-3-O-β-D-glucoside (**10**, 80 mg). The oleanolic acid and β-sitosterol-3-O-p-D-glucoside were identified through Co-TLC with the previously isolated sterol glycosides from several plants.

Calliterpenone monoacetate (1): White compound, R_f 0.32 (Hex : EtOAc, 8 : 2); m.p. 121–123°C; IR (KBr, cm^{-1}) ν_{max}: 3402, 2920, 1705, 1408, 988; ^1H

NMR ($CDCl_3$): δ 1.66 and 1.88 (H-1), 2.47 and 2.39 (H-2), 1.21 (H-5), 1.10 and 1.04 (H-6), 1.70 and 1.42 (H-7), 0.85 (H-9), 1.26 (H-11), 1.92 and 1.36 (H-12), 2.31 (H-13), 2.47 and 1.00 (H-14), 2.15 and 1.57 (H-15), 4.01 and 4.05 (H-17), 1.07 (H-18), 0.94 (H-19), 0.84 (H-20); ^{13}C NMR ($CDCl_3$): 8 38.0 (C-1), 33.9 (C-2), 216.2 (C-3), 46.4 (C-4), 55.1 (C-5), 21.3 (C-6), 40.9 (C-7), 43.9 (C-8), 55.8 (C-9), 37.0 (C-10), 19.8 (C-11), 26.5 (C-12), 44.3 (C-13), 48.5 (C-14), 44.9 (C-15), 84.1 (C-16), 65.5 (C-17), 26.7 (C-18), 21.3 (C-19 × 2 CH_3), 14.3 (C-20), 171.9 (OAc); EIMS m/z (rel. int., %) 362 $[M]^+$ (21.2) ($C_{22}H_{34}O_4$); FABMS (positive mode) m/z 363 $[M + H]^+$; ^1H, ^{13}C NMR and MS similar to reported literature values[5, 7, 9, 12–14].

β-Sitosterol (2): White crystals; R_f 0.32 ($CDCl_3$: MeOH; 9.5 : 0.5), m.p. 139–142°C, IR (KBr, cm^{-1}) ν_{max}: 3429, 2936, 1644, 1462, 1376, 1057, EIMS: m/z (rel. int., %) 414 $[M]^+$ ($C_{29}H_{50}O$), 396 $[M\text{-}H_2O]^+$ (70.4), 381 (37.9), 367 (10.2), 351 (8.7), 329 (38.5), 303 (31.1), 289 (11.4), 273 (26.2), 255 (48.5), 241 (7.6), 231 (21.7), 213 (36.2), 199 (13.7), 173 (16.1), 159 (27.6), 145 (33.0), 133 (23.2), 107 (25.2), 95 (26.7), 81 (27.0), 55 (22.9). FABMS (positive mode) m/z 415 $[M + H]^+$, ^1H and ^{13}C NMR similar to reported literature values[19, 21, 22].

Calliterpenone (3): White compound, R_f 0.21 (Hex : EtOAc, 8 : 2); m.p. 150–153°C; IR (KBr, cm^{-1}) ν_{max}: 3420, 2903, 1695, 1388, 898, ^1H NMR ($CDCl_3$): δ 1.34 and 1.95 (H-1), 2.44 (H-2), 1.38 (H-5), 1.42 and 1.49 (H-6), 1.47 and 1.63 (H-7), 1.08 (H-9), 1.57 (H-11), 1.50 and 1.60 (H-12), 2.04 (H-13), 1.61 and 1.87 (H-14), 1.39 and 1.52 (H-15), 3.67 and 3.77 (H-17), 1.06 (H-18), 1.01(H-19), 1.06 (H-20); ^{13}C NMR ($CDCl_3$): δ 39.2 (C-1), 33.9 (C-2), 218.2 (C-3), 47.1 (C-4), 54.1 (C-5), 21.6 (C-6), 40.8 (C-7), 44.3 (C-8), 55.3 (C-9), 38.5(C-10), 18.8 (C-11), 26.0 (C-12), 45.2 (C-13), 36.8 (C-14), 52.6 (C-15), 81.7 (C-16), 66.1 (C-17), 27.2 (C-18), 20.9 (C-19), 17.7 (C-20); EIMS m/z (rel. int., %) 320 $[M]^+$ ($C_{20}H_{32}O_3$); FABMS (positive mode) m/z 321 $[M + H]^+$; ^1H, ^{13}C NMR and MS similar to reported literature values[5, 7, 9, 12, 14].

Linoleic acid (4): Colorless oil; R_f 0.32 (Hex : EtOAc, 7 : 3); IR (KBr, cm^{-1}) ν_{max}: 3009, 2917, 2849, 2672, 1707, 1462, 1293, 758; ^1H NMR ($CDCl_3$) δ: 2.01–2.06 (m, H-2, H-8, H-14), 1.27 (m, H-3 to H-7, H-15, 16, 17), 5.36 (m, H-9, 10 and H-12, H-13), 2.36 (t, J = 6.0 Hz, H-11), 0.92 (t, H-18); ^{13}C NMR ($CDCl_3$) δ: 180.00 (C-1), 32.24 (C-2), 29.91 (C-3), 29.90 (C-4), 29.89 (C-5), 29.87 (C-6), 29.86 (C-7), 31.76 (C-8), 130.44 (C-9), 130.25 (C-10), 34.23 (C-11), 128.31 (C-12), 128.14 (C-13), 32.15 (C-14), 29.99 (C-15), 24.91 (C-16), 22.91 (C-17), 14.31 (C-18); EIMS m/z (rel. int., %) 280 $[M]^+$ ($C_{18}H_{32}O_2$) (28.0), 256 (100), 213 (42), 185 (18), 171 (15), 129 (30), 79 (33), 55 (40). FABMS (positive mode) m/z 281 $[M + H]^+$; ^1H and ^{13}C NMR similar to reported literature values[23].

Stigmasterol (5): Crystalline solid; R_f 0.34 ($CHCl_3$: MeOH 9.5 : 0.5); m.p. 165–167°C; IR (KBr, cm^{-1}) ν_{max}: 3430, 2956, 1653, 1459, 1373, 1035; EIMS m/z (rel. int., %) 412 $[M]^+$ ($C_{29}H_{48}O$) (0.8), 396 (100), 394 $[M\text{-}H_2O]^+$ (4.5), 378 (19), 363 (98), 337 (43), 271 (27), 253(57), 239 (8.9), 227 (9), 211 (22), 199 (17), 171 (16.7), 157 (30), 143 (26.3), 131 (11.6),119 (15), 107 (15.5), 95 (13.6), 81 (26.5), 69 (43.4). FABMS (positive mode) m/z 413 $[M + H]^+$; ^1H and ^{13}C NMR similar to reported literature values[21].

Stearic acid (6): Colorless solid; R_f 0.41 (Hex : EtOAc, 7 : 3); m.p. 69–70°C;

IR (KBr, cm^{-1}) ν_{max}: 3020, 2918, 2890, 1702, 1407, 1295, 1094, 758; ^1H NMR ($CDCl_3$) δ: 2.36 (t, J = 5.6 Hz, H-2), 1.65 (m, H-3), 1.38 (br s, H-4 to H-17), 0.88 (t, J = 5.2 Hz, H-18); ^{13}C NMR ($CDCl_3$) δ: 179.75 (C-1), 34.28 (C-2), 32.24 (C-3), 30.02 (C-4), 30.00 (C-5), 29.99 (C-6), 29.97 (C-7), 29.96 (C-8), 29.91 (C-9), 29.76 (C-10), 29.68 (C-11), 29.56 (C-12), 29.39 (C-13), 25.03 (C-14), 23.02 (C-15), 24.92 (C-16), 22.91 (C-17), 14.31 (C-18); EIMS m/z (rel. int., %) 284 $[M]^+$ ($C_{18}H_{36}O_2$) (5.2), 256 (100), 238 (12), 213 (39), 185 (22), 129 (48), 57 (76); FABMS (positive mode) m/z 285 $[M + H]^+$. ^1H and ^{13}C NMR similar to reported literature values[23].

Myristic acid (7): Colourless solid; R_f 0.38 (Hex : EtOAc; 7 : 3); m.p. 58-59°C; IR (KBr, cm^{-1}) ν_{max}: 3439 (weak signal), 3108, 2914, 2847, 1696, 1461, 1404, 1292, 1023; ^1H NMR ($CDCl_3$) δ: 2.35 (t, J = 7.4 Hz, H-2), 1.63 (m, H-3), 1.30 (br s, H-4 to H-13), 0.89 (t, J = 6.3 Hz, H-14); ^{13}C NMR ($CDCl_3$) δ: 179.95 (C-1), 34.22 (C-2), 29.91 (C-3), 29.90 (C-4), 29.89 (C-5), 29.86 (C-6), 29.81 (C-7), 29.65 (C-8), 29.58 (C-9), 29.46 (C-10), 24.29 (C-11), 24.91 (C-12), 22.91 (C-13), 14.32 (C-14); EIMS m/z (rel. int., %): 228 $[M]^+$ ($C_{14}H_{28}O_2$) (5.6); FABMS (positive mode) m/z 229 $[M+H]^4$; ^1H and ^{13}C NMR similar to reported literature values[24].

Octacosanoic acid (8): Colorless solid; R_f 0.39 ($CHCl_3$: MeOH; 9.5 : 0.5); IR (KBr, cm^{-1}) ν_{max}: 3350, 2918, 2849, 1705, 1464, 1455, 1425, 725; ^1H NMR (C_5D_5N, 500 MHz) δ: 2.56 (1H, d, J = 7.3 Hz, H_2-a), 2.53 (1H, d, J = 7.4 Hz, H_2-2b), 2.13 (2H, m, H_2-3), 1.84 (2H, m, H_2-4), 1.81 (2H, m, H_2- 5), 1.42 (2H, m, H_2-6), 1.36 (18H, br s, 9 × CH_2), 1.28 (24H, br s, 12 × CH_2), 0.88 (3H, t, J = 6.2 Hz, Me-28); ^{13}C NMR (C_5D_5N, 125 MHz): δ 176.4 (COOH-1), 36.7 (CH_2), 35.3 (CH_2), 32.5 (CH_2), 32.5 (CH_2), 30.4 (10 × CH_2), 30.3 (6 × CH_2), 30.2 (CH_2), 30.2 (CH_2), 30.1 (CH_2), 30.0 (CH_2), 30.0 (CH_2), 29.91 (CH_2), 28.0 (CH_2), 26.6 (CH_2), 26.1 (CH_2), 23.3 (CH_2), 14.7 (Me-28); EIMS m/z (rel. int., %)): 424 $[M]^+$ ($C_{28}H_{56}O_2$) (1.7), 396 (15.1), 381 (22.1), 367 (100), 353 (72.8), 339 (78.5), 325 (17.5), 311 (15.6), 297 (15.3), 283 (11.0), 269 (13.3), 255 (10.4), 241 (15.4), 227 (10.8), 213 (10.1), 199 (7.8), 185 (23.8), 171 (13.6), 157 (6.6), 143 (8.5), 129 (57.2), 115 (17.4), 113 (12.9), 97 (41.3), 85 (35.4), 83 (44.3), 73 (70.1), 71 (48.9), 57 (79.5); FABMS (positive mode) m/z 425 $[M + H]^+$; ($C_{28}H_{57}O_2$)[25].

Oleanolic acid (9): White crystals; R_f 0.34 $CHCl_3$: MeOH, 9 : 1); m.p. 300–302°C; IR (KBr, cm^{-1}) ν_{max}: 3400, 2920, 1625, 1376, 973; ^1H NMR (MeOD) δ: 5.24 (1H, t, H-12), 3.21 (1H, dd, J = 10.8, 4.8 Hz, H-3), 2.82 (2H, dd, J = 13.8,3.8, H-2), 0.92 (3H, s), 0.77 (3H, s), 0.75 (3H, s), 0.89 (3H, s), 0.91 (3H, s), 1.12 (3H, s), 0.97 (3H, s); ^{13}C NMR (MeOD) δ: 38.5 (C-1), 27.3 (C-2), 79.1 (C-3), 38.8 (C-4), 55.4 (C-5), 18.4 (C-6), 32.8 (C-7), 39.4 (C-8), 47.7 (C-9), 37.2 (C-10), 23.1 (C-11), 122.7.4 (C-12); 143.6 (C-13), 41.7 (C-14), 27.8 (C-15), 23.5 (C-16), 46.6 (C-17), 41.2 (C-18), 46.0 (C-19), 30.7 (C-20), 33.9 (C-21), 32.5 (C-22), 28.1 (C-23), 15.6 (C-24), 15.3 (C-25), 17.2 (C-26), 26.0 (C-27), 182.5 (C-28), 33.0 (C-29), 23.6 (C-30); EIMS m/z (rel. int., %) 456 $[M]^+$ ($C_{30}H_{48}O_3$); FABMS (positive mode) m/z 457 $[M + H]^+$. The NMR data of 9 were identical to the published data of oleanolic acid[19, 20].

β-Sitosterol-3-O-β-D-glucoside (10): White solid; m.p. R_f 0.47 ($CHCl_3$:

MeOH, 9 : 1); IR (KBr, cm^{-1}) ν_{max}: 3429, 2933, 1635, 1376, 1073 cm^{-1}; EIMS: m/z (rel. int., %) 414 $[M\text{-glucose}]^+$ (Calc. for $C_{29}H_{50}O$) (25.7%), 396 $[M\text{-}H_2O]^+$ (100), 382 (42.8), 367 (9.4), 354 (4.0), 329 (9.9), 303 (9.0), 288 (11.5), 275 (14.8), 255 (33.2), 279 (9.2), 213 (20.5), 199 (8.4), 173 (9.4), 159 (19.5), 147 (28.3), 133 (17.0), 107 (19.3), 95 (22.9), 81 (25.3), 55 (20.8) (19, 22); FABMS (positive mode) m/z 577 $[M + H]^+$ [22].

RESULTS AND DISCUSSION

In continuation of our studies of the fruits of *C. macrophylla* reported acyclic triterpene, callicarpenol[18] and after further investigation of its fruits ethanol extract fractions, the latter was identified some more known compounds in minor quantities as calliterpenone monoacetate (**1**), β-sitosterol (**2**), calliterpenone (**3**), linoleic acid (**4**), stigmasterol (**5**), stearic acid (**6**) myristic acid (**7**) octacosanoic acid (**8**), oleanolic acid (**9**), β-sitosterol-3-O-β-D-glucoside (**10**). Due to known compounds there is no need to describe all compounds and all possible spectroscopic data are given in the experimental section and compared Co-TLC with authentic standard of Sigma. Most of the known compounds complete spectroscopic data not available in literature. The plants are also extensively used in the indigenous system of medicine, specially Ayurvedic, for rheumatism due to its major compoment of diterpenoids (calliterpenone monoacetate and calliterpenone) in leaves and seeds. Earlier there is no report of chemical investigation of its fruits and therefore we decided to study the fruits of *C. macrophylla*. This is the first chemical investigation report of known compounds *C. macrophylla* fruits.

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