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Chemical Constituents from the Stem bark of *Vitex negundo* L.

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Abstract *Vitex negundo* L. (Verbenaceae) is found in south- eastern Asia, Afghanistan, India, China, Tanzania, Madagascar, Europe, West Indies and North America as a much branched aromatic shrub. The plant parts are used to treat asthma, body pains, diarrhoea, dysentery, dyspepsia, fever, liver, skin and spleen diseases, malaria, piles, rheumatism, sinusitis, spermatorrhoea, sprains, swellings, urticaria, vomiting, weakness and whooping cough. Phytochemical investigation of a methanolic extract of the stem bark led to isolate ten chemical constituents characterized as the fatty esters *n*-undecanyl oleate (**1**), *n*-undecanyl stearate (**2**) and *n*-tetracosanyl capriate (**6**), long chain alkanes *n*-pentacosane (**3**), *n*-henetriacontane (**4**) and *n*-pentatriacontane (**5**) and triterpenoids lanostan-3-olyl 1′-(3′- eicosanyl) anthraquinone (**7**), lanostan-3-olyl 1′-(4′-arachidyloxy)-xanthone (**8**), oleanolic acid 3-O-α-D-glucopyranosyl-2′-arachidate (**9**) and betulinic acid 3β-O-α-D-glucopyranosyl-2′-lignocerate (**10**). The structures of all the isolated phytoconstituents have been established on the basis of spectral data analysis and chemical reactions.

Keywords *Vitex negundo*, stem bark, chemical constituents, isolation, structure elucidation

Introduction

Vitex negundo L., syn. *V. cannabifolia* Siebold et Zucc., *V. incise* Lam. (Verbenaceae), known as Chinese chastetree, five-leaved chaste tree, horseshoe vitex or nirgundi, is a native to southern Asia, Afghanistan, India, China, Japan, Korea, Malaysia, Thailand, Tanzania, Madagascar, Europe, West Indies and North America [1]. It is a much branched aromatic shrub, 2-5 m in height, with quadrangular tomentose, densely whitish tomentose branchlets, bark thin, grey; leaves palmately compound, 3-5 foliolate; leaflets lanceolate, crenate, glabrous above, whitish tomentose beneath; flowers bluish-purple, small, in peduncled cymes; fruit a succulent drupe, black when ripe, 5-6 mm in diameter. The plant parts are used as an astringent, anthelmintic, aphrodisiac, carminative, diuretic, expectorant, febrifuge, tonic, vermifuge and to treat abscess, asthma, backache, carbuncles, cellulitis, diarrhoea, dysentery, dyspepsia, fever, gout, headache, jaundice, liver, skin and spleen diseases, malaria, piles, rheumatism, sinusitis, spermatorrhoea, sprains, swellings, toothache, urticaria, vomiting, weakness, whooping cough [2–6].

The leaf essential oil was composed of viridiflorol (26.5%), β-caryophy/lene (13.2%), 4-terpineol (4.4%), linalool (2.04%), globulol, elemol, farnesene and aromadendrene [7-9]. The leaves possessed casticin, flavonoids, chrysophenol D, p-hydroxybenzoic acid, D-fructose, nishindine, hydrocotylene, 4H-chrome-4-ones, negundoside, agnuside, vitegnoside, triterpenoids, vitamin C, stilbenes, gardenins, aucubin, squalene, 1β-D-glucosyl-(3′,4′dihydroxy-benzoyloxymethyl)-5-ketocyclopenta[c]pyran-4-carboxylic acid and 6′-p-hydroxy benzoyl-mussaenosidic acid. The stem bark yielded flavonoids, vanillic acid, oleanolic acids, vitexin cafeate, p-

hydroxybenzoic acid and β-sitosterol. The seeds contained phenylnaphthalene-type lignans, triterpenoids, alkanes, 5-oxyisophthalic acid, vitedoamine A, artemetin, 3,4-dihydroxybenzoic acid, 3,4-dihydro-2-naphthaldehyde derivative, vitedoins A and B, vitedoamine A, p-hydroxybenzoic acid and β-sitosterol. Its roots contained oleanane triterpenoids, vitexin, isovitexin, vitexoside, agnuside, negundins A and B; diasyringaresinol, lyoniresinol, vitrofolals E and F, β-sitosterol, 6,7-dihydronaphtho (2,3-b)furan, lignans, 3,4-dihydro-2-naphthaledehyde derivative and furanoeremophilane [10–23]. The present study was aimed to isolate and characterize phytoconstituents from the stem bark of *V. negundo*.

Materials and Methods

General Procedures

Melting points were determined on a Perfit melting point apparatus (Ambala, Haryana, India). UV spectra were measured on Shimadzu UV-1601 spectrophotometer in methanol. IR spectra were recorded on KBr discs, using a Jashco FTIR-410 spectrophotometer. ^{1}H and ^{13}C NMR spectra were obtained using Bruker Advance DRX 400 and 100 spectrospin instruments (Karlsruhe, Germany), respectively, using TMS as an internal standard. FAB mass spectra were recorded on a Jeol D-300 spectrometer. Column chromatography was performed on silica gel 60-120 mesh (Merck, Mumbai, India) and silica gel G coated TLC plates (Merck, Mumbai, India) were used for thin-layer chromatography. Spots were visualized by exposing to iodine vapors and UV radiation and spraying with ceric sulfate solution.

Plant Material

The stem bark of *V. negundo* was collected from Khari Baobli market, Delhi and authenticated by Prof. M.P. Sharma, Department of Botany, Jamia Hamdard, New Delhi. A voucher specimen was preserved for further verification.

Extraction and Isolation

The air-dried bark powder (1.0 kg) was extracted with methanol exhaustively in a Soxhlet apparatus. The methanolic extract was concentrated under reduced pressure to obtain a reddish brown viscous mass (114 g, 11.4 % yield). A portion of the extract was analyzed chemically to determine the presence of different chemical constituents. It was dissolved in small amount of methanol and adsorbed on silica gel (60-120 mesh) for column chromatography for preparation of a slurry. The slurry was dried in air and subjected to chromatography over a silica gel column packed in petroleum ether. The column was eluted successively with petroleum ether, mixture of petroleum ether and chloroform (9:1, 3:1, 1:1, 1:3), chloroform and the mixture of chloroform and methanol (99:1, 97:3, 95:5, 92:8, 9:1, 3:1, 1:1, 1:3). Various 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 purified to get the following phytoconstituents:

n-Undecanyl oleate **(1)**

Elution of the column with petroleum ether furnished a pale yellow semisolid of **1**, yield 137 mg, IR γ_{max} (KBr): 2925, 2853, 1734, 1645, 1458, 1360, 1245, 1114, 725 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 5.34 (1H, m, H-9), 5.28 (1H, m, H-10), 4.16 (2H, t, J = 6.6 Hz, H_2-1′), 2.53 (2H, t, J = 7.8 Hz, H_2-2), 2.36 (2H, m, H_2-8), 1.93 (2H, m, H_2-11), 1.88 (2H, m, CH_2-2′), 1.46 (2H, m, CH_2), 1.26 (36H, brs, 18 × CH_2), 0.96 (3H, t, J = 6.1 Hz, Me-18), 0.89 (3H, t, J = 6.6 Hz, Me-11′); ^{13}C NMR ($CDCl_3$): δ 173.24 (C-1), 129.11 (C-9), 119.53 (C-10), 66.02 (C-1′), 33.61 (C-2), 31.37 (CH_2), 29.14 (19 x CH_2), 28.95 (CH_2), 27.73 (CH_2), 25.19 (CH_2), 22.68 (CH_2), 13.17 (Me - 18), 12.31 (Me - 11′); TOF MS *m/z* (rel.int.): 436 $[M]^+$ ($C_{29}H_{56}O_2$) (22.6), 281 (10.1).

n-Undecanyl stearate **(2)**

Further elution of the column with petroleum ether furnished a colourless mass of **2**, yield 159 mg, m. p. 87 - 88 °C; IR γ_{max} (KBr) : 2919, 2848, 1735, 1641, 1466, 1377, 1171, 1110, 722 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 4.06 (2H, t, J = 6.8 Hz, H_2-1′), 2.30 (2H, t, J = 8.0 Hz, H_2-2), 1.62 (2H, m, CH_2-2′), 1.44 (2H, m, CH_2), 1.25 (44H, brs, 22 ×

CH_2), 0.87 (3H, t, J = 6.8 Hz, Me-18), 0.84 (3H, t, J = 6.0 Hz, Me-11′); ^{13}C NMR ($CDCl_3$): δ 171.63 (C-1), 65.03 (C-1′), 33.68 (CH_2), 31.93 (CH_2), 29.72 (17 x CH_2), 29.25 (CH_2), 29.18 (CH_2), 27.75 (CH_2), 25.23 (CH_2), 22.71 (CH_2), 14.14 (Me - 18), 14.11 (Me - 11′); TOF MS *m/z* (rel.int.): 438 $[M]^+$ ($C_{29}H_{58}O_2$) (6.3), 283 (14.6).

n-Pentacosane **(3)**

Elution of the column with petroleum ether – chloroform (1:1) furnished a colourless crystalline mass **3**, recrystallized from acetone - methanol (1:1), 1.19 g, m. p. 53 - 54 °C; UV λ_{max} (MeOH): 209 nm (log ε 3.5); IR υ_{max} (KBr): 2925, 2845, 1458, 1390, 1260, 1112, 725 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 2.33 (2H, m, CH_2), 1.69 (2H, m, CH_2), 1.54 (2H, m, CH_2), 1.26 (34H, brs, 17 x CH_2), 0.87 (3H, t, J = 6.6 Hz, Me-25), 0.84 (3H, t, J = 6.6 Hz, Me-1); ESI MS *m/z* (rel. int.): 352 $[M]^+$ ($C_{25}H_{52}$) (25.1).

n-Henetriacontane (**4**)

Elution of the column with petroleum ether – chloroform (1:3) furnished a colourless crystals of **4**, recrystallized from acetone - methanol (1:1), 1.88 mg, m. p. 67 – 68 °C; UV λ_{max} (MeOH): 206 nm (log ε 2.6); IR υ_{max} (KBr): 2916, 2847, 1462, 1377, 1120, 839, 719 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 2.19 (2H, m, CH_2), 1.65 (2H, m, CH_2), 1.29 (2H, m, CH_2), 1.27 (4H, m, 2 x CH_2), 1.25 (44H, brs, 22 x CH_2), 1.22 (4H, m, 2 x CH_2), 0.89 (3H, t, J = 6.8 Hz, Me-1), 0.86 (3H, t, J = 6.7 Hz, Me-31); ^{13}C NMR ($CDCl_3$): δ 31.92 (CH_2), 29.69 (CH_2), 29.69 (12 x CH_2), 29.66 (14 x CH_2), 29.31 (CH_2), 29..36 (CH_2), 24.37 (CH_2), 22.69 (CH_2), 14.13 (Me-35), 14.11 (Me-1); ESI MS *m/z* (rel. int.): 436 $[M]^+$ ($C_{31}H_{64}$) (12.8).

n-Pentatriacontane **(5)**

Further elution of the column with petroleum ether – chloroform (1:3) furnished a colourless crystals of **5**, recrystallized from acetone - methanol (1:1), 1.19 g, m. p. 73 -75 °C; UV λ_{max} (MeOH): 205 nm (log ε 2.9); IR υ_{max} (KBr): 2920, 2843, 1452, 1260, 1120, 722 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 2.36 (2H, m, CH_2), 2.18 (2H, m, CH_2), 1.62 (4H, m, 2 x CH_2), 1.29 (58H, brs, 29 x CH_2), 0.83 (3H, t, J = 6.2 Hz, Me-35), 0.80 (3H, t, J = 6.0 Hz, Me-1); ^{13}C NMR ($CDCl_3$): δ 31.92 (CH_2), 29.68 (CH_2), 29.35 (27 x CH_2), 29.35 (CH_2), 29.31 (CH_2), 27.32 (CH_2), 25.32 (CH_2), 24.86 (CH_2), 22.68 (CH_2), 14.12 (Me-35), 14.11 (Me-1); ESI MS *m/z* (rel. int.): 492 $[M]^+$ ($C_{35}H_{72}$) (22.3).

n-Tetracosanyl capriate **(6)**

Further elution of the column with chloroform furnished a colourless powder of **6**, yield 108 mg, m. p. 78 - 80 °C; IR γ_{max} (KBr) : 2917, 2848, 1735, 1643, 1467, 1376, 1170, 1112, 1029, 724 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 4.04 (2H, t, J = 6.4 Hz, H_2 -1′), 2.26 (2H, t, J = 7.2 Hz, H_2 -2), 1.64 (4H, m, 2 x CH_2), 1.44 (2H, m, CH_2), 1.26 (54H, brs, 27 × CH_2), 0.89 (3H, t, J = 6.6 Hz, Me-10), 0.84 (3H, t, J = 6.3 Hz, Me-24′); ^{13}C NMR ($CDCl_3$): δ 171.35 (C-1), 64.81 (C-1′), 33.69 - 22.71 (30 x CH_2), 14.16 (Me - 10, Me - 24′); ESI MS *m/z* (rel.int.): 508 $[M]^+$ ($C_{34}H_{68}O_2$) (2.8), 353 (11.5).

Lanostan-3-olyl (3′-eicosanyl) anthraquinone (7)

Elution of the column with chloroform – methanol (49 : 1) yielded a pale yellow crystals of **7**, yield 147 mg, m. p. 208 - 209 °C; UV λ_{max} (MeOH): 225, 253, 286, 303, 433 nm (log ε 6.8, 5.1, 3.2, 4.6, 2.9); IR γ_{max} (KBr) : 2925, 2856, 1701, 1687, 1609, 1516, 1467, 1376, 1274, 1236, 1164, 1106, 1032, 882, 727 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 3.96 (1H, dd, J = 5.6, 8.8 Hz, H -3α), 1.01 (3H, s, Me-19), 0.96 (3H, d, J = 6.3 Hz, Me-21), 0.91 (3H, d, J = 6.0 Hz, Me-26), 0.89 (3H, d, J = 6.0 Hz, Me-27), 0.87 (3H, s, Me-29), 0.82 (3H, brs, Me-28), 0.80 (3H, s, Me-30), 0.78 (3H, s, Me-18), 2.94 – 1.31 (28H, m, 11 x CH_2, 6 x CH), 7.96 (1H, d, J = 2.4 Hz, H -2′), 7.94 (1H, dd, J = 2.3, 8.0 Hz, H -8′), 7.69 (1H, m, H -9′), 7.54 (1H, m, H -1′), 7.23 (1H, d, J = 2.4 Hz, H -4′), 6.86 (1H, m, H -11′′), 2.34 (2H, t, J = 7.2 Hz, H_2-1′′), 1.52 (2H, m, H_2-2′′), 1,28 (34H, brs, 17 x CH_2), 0.84 (3H, t, J = 6.5 Hz, Me-20′′); ^{13}C NMR ($CDCl_3$): δ 34.33 (C-1), 27.29 (C-2), 79.15 (C-3), 38.87 (C-4), 50.56 (C-5), 18.29 (C-6), 38.36 (C-7), 42.43 (C-8), 49.25 (C-9), 37.16 (C-10), 22.87 (C-11), 30.75 (C-12), 40.71 (C-13), 56.35 (C-14), 31.49 (C-15), 32.18 (C-16), 55.34 (C-17), 15.34 (C-18), 19.16 (C-19), 34.10 (C-20), 19.39 (C-21), 38.73 (C-22), 24.76 (C-23), 28.93 (C-24), 31.27 (C-25), 20.83 (C-26), 16.13 (C-27), 19.08 (C-28), 25.41 (C-29), 16.04 (C-30), 150.48 (C-1′), 115.36 (C-2′), 133.21 (C-3′), 114.25 (C-4′), 129.13 (C-5′), 181.95 (C-6′), 132.52 (C-7′), 112.11 (C-8′), 130.25 (C-9′), 109.75 (C-10′), 127.67 (C-11′), 125.09 (C-12′), 183.06 (C-13′), 128.85 (C-14′), 30.56 (C-1′′), 29.71 – 29.11 (C-2′′ to C-18′′), 22.71 (C-19′′), 14.16 (C-20′′); ESI MS *m/z* (rel. int.): 916 $[M]^+$ ($C_{64}H_{100}O_3$) (2.3), 429 (4.1), 412 (12.5).

Lanostan-3-olyl (4′-arachidyloxy)-xanthone (**8**)

Elution of the column with chloroform – methanol (49 : 1) yielded a pale yellow crystals of **8**, yield 118 mg, m. p. 183 - 184 °C; UV λ_{max} (MeOH): 234, 255, 278, 330 nm (log ε 4.8, 5.5, 3.2, 1.3); IR γ_{max} (KBr) : 2920, 2851, 1730, 1689, 1637, 1514, 1460, 1376, 1274, 1236, 1164, 1106, 1032, 882, 727 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ δ 3.94 (1H, dd, J = 5.5, 9.1 Hz, H -3α), 0.99 (3H, s, Me-19), 0.94 (3H, d, J = 6.5 Hz, Me-21), 0.94 (3H, s, Me-30), 0.89 (3H, s, Me-28), 0.89 (3H, d, J = 6.5 Hz, Me-26), 0.86 (3H, d, J = 6.5 Hz, Me-27), 0.79 (3H, s, Me-29), 0.77 (3H, s, Me-18), 2.94 – 1.31 (28H, m, 11 x CH_2, 6 x CH), 7.97 (1H, dd, J = 2.4, 8.1 Hz, H -7′), 7.70 (1H, dd, J = 1.3, 8.3 Hz, H -10′), 7.67 (1H, d, J = 9.5 Hz, H -2′), 7.52 (1H, d, J = 9.5 Hz, H -3′), 7.24 (1H, m, H -9′), 6.97 (1H, m, H -8′), 2.36 (2H, t, J = 7.6 Hz, H_2-2″), 1.52 (2H, m, H_2-3″), 1.25 (12H, brs, 6 x CH_2), 1.23 (20H, brs, 10 x CH_2), 0.82 (3H, d, J = 6.5 Hz, Me-20″); ^{13}C NMR ($CDCl_3$): δ 34.31 (C-1), 27.98 (C-2), 79.11 (C-3), 38.37 (C-4), 50.51 (C-5), 18.28 (C-6), 27.28 (C-7), 42.45 (C-8), 49.22 (C-9), 37.18 (C-10), 22.71 (C-11), 30.56 (C-12), 40.68 (C-13), 56.37 (C-14), 31.53 (C-15), 32.14 (C-16), 55.31 (C-17), 15.38 (C-18), 19.18 (C-19), 34.06 (C-20), 19.36 (C-21), 38.70 (C-22), 24.73 (C-23), 28.89 (C-24), 31.84 (C-25), 20.85 (C-26), 19.03 (C-27), 22.71 (C-28), 25.49 (C-29), 16.12 (C-30), 152.08 (C-1′), 129.28 (C-2′), 127.67 (C-3′), 150.35 (C-4′), 150.44 (C-5′), 147.63 (C-6′), 115.38 (C-7′), 128.85 (C-8′), 130.95 (C-9′), 112.11 (C-10′), 114.25 (C-11′), 181.98 (C-12′), 109.70 (C-13′), 169.06 (C-1″), 56.09 (C-2″), 29.71 – 29.10 (C-3′ to C-18″), 22.69 (C-19″), 14.51 (C-20″), ESI MS *m/z* (rel. int.): 934 $[M]^+$ ($C_{63}H_{98}O_5$) (3.8), 429 (12.6).

Oleanolic acid 3β-O-α-D-glucosyl 2′-arachidate (**9**)

Elution of the column with chloroform - methanol (19:1) furnished colourless crystal of **9**, recrystallized from methanol, 221; m. p. 168 - 169 °C; UV λ_{max} (MeOH): 2214 nm (log ε 4.8); IR ν_{max} (KBr): 3452, 3386, 3360, 3255, 2918, 2849, 1735, 1686, 1635, 1463, 1376, 1234, 1165, 1106, 1037, 884, 793, 723 cm^{-1}; ^{1}H NMR (DMSO-d_6)**:** δ 5.87 (1H, d, J = 5.3 Hz, H-12), 3.65 (1H, dd, J = 3.2, 10.0 Hz, H-3α), 2.24 (1H, dd, J = 2.8, 18.4 Hz, H-18β), 0.98 (3H, brs, Me-23), 0.96 (3H, brs, Me-25), 0.94 (3H, brs, Me-26), 0.91 (3H, brs, Me-27), 0.88 (3H, brs, Me-24), 0.81 (3H, brs, Me-29), 0.75 (3H, brs, Me-30), 2.63 – 1.36 (23H, m, 10 x CH_2 , 3 x CH), 4.96 (1H, d, J = 6.6 Hz, H-1′α), 4.65 (1H, m, H-5′), 4.41 (1H, m, H-2′), 3.83 (1H, m, H-3′), 3.76 (1H, m, H-4′), 3.16 (2H, d, J = 5.8 Hz, H_2-6′), 2.14 (2H, t, J = 8.3 Hz, H_2-2″), 1.54 (2H, m, H_2-3″), 1.25 (32H, brs, 16 x CH_2), 0.84 (1H, d, J = 6.3 Hz, H-20″); ^{13}C NMR ($CDCl_3$): δ 38.35 (C-1), 22.29 (C-2), 79.86 (C-3), 37.32 (C-4), 55.31 (C-5), 18.73 (C-6), 33.16 (C-7), 39.92 (C-8), 47.64 (C-9), 36.93 (C-10), 23.51 (C-11), 121.73 (C-12), 145.41 (C-13), 41.82 (C-14), 26.31 (C-15), 26.28 (C-16), 32.52 (C-17), 47.39 (C-18), 46.91 (C-19), 31.28 (C-20), 34.83 (C-21), 37.23 (C-22), 16.81 (C-23), 28.51 (C-24), 15.65 (C-25), 16.31 (C-26), 25.97 (C-27), 23.41 (C-28), 33.43 (C-29), 19.51 (C-30), 103.82 (C-1′), 81.69 (C-2′), 65.18 (C-3′), 70.08 (C-4′), 77.02 (C-5′), 61.07 (C-6′), 172.21 (C-1″), 34.39 (C-2″), 31.89 (CH_2), 29.73 (13 x CH_2), 27.87 (CH_2), 25.94 (CH_2), 22.71 (CH_2), 14.30 (C-20″); +ve FAB MS *m/z* (rel. int.): 912 $[M]^+$ ($C_{56}H_{96}O_9$) (2.3), 473 (12.6), 457 (15.4), 455 (81.2), 311 (23/5), 179 (5.8).

Betulinic acid 3-O-β-D- glucosyl 2′-lignocerate (**10**)

Elution of the column with chloroform - methanol (9:1) offered colourless crystals of **10**, yield 117 mg, m. p. 202-203 °C; UV λmax (MeOH): 211 nm; IR γmax (KBr): 3452, 3380, 3260, 2918, 2849, 1721, 1687, 1642, 1463, 1376, 1235, 1167, 1106, 1038, 884, 723 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 4.74 (1H, s, H_2-29a), 4.66 (1H, s, H_2-29b), 3.65 (1H, dd, J = 5.9, 9.6 Hz, H-3α), 1.65 (3H, brs, Me-30), 1.17 (3H, brs, Me-23), 1.05 (3H, brs, Me-25), 0.96 (3H, s, Me-24), 0.79 (3H, brs, Me-26), 0.75 (3H, brs, Me-27), 2.25 - 1.39 (25 H, m, 10 × CH_2, 5 x CH), 5.87 (1H, d, J = 7.2 Hz, H-1′), 4.20 (1H, m, H-5′), 4.11 (1H, m, H-2′), 3.80 (1H, m, H-3′), 3.74 (1H, m, H-4′), 3.16 (2H, d, J = 6.6 Hz, H_2-6′), 2.19 (2H, t, J = 7.2 Hz, H-2″), 1.25 (42H, m, 21 x CH_2), 0.85 (3H, t, J = 6.6 Hz, Me--24″); ^{13}C NMR ($CDCl_3$): δ 38.74 (C-1), 29.69 (C-2), 79.06 (C-3), 39.83 (C-4), 55.34 (C-5), 18.29 (C-6), 33.98 (C-7), 40.79 (C-8), 50.41 (C-9), 37.35 (C-10), 21.09 (C-11), 26.21 (C-12), 38.11 (C-13), 42.79 (C-14), 31.47 (C-15), 32.61 (C-16), 56.12 (C-17), 48.38 (C-18), 49.05 (C-19), 150.91 (C-20), 29.88 (C-21), 37.11 (C-22), 27.92 (C-23), 15.42 (C-24), 16.21 (C-25), 16.04 (C-26), 14.65 (C-27), 178.90 (C-28), 109.37 (C-29), 19.36 (C-30), 103.69 (C-1′), 79.64 (C-2′), 73.85 (C-3′), 67.51 (C-4′), 76.73 (C-5′), 60.18 (C-6′), 172.15 (C-1″), 38.51 (C-2″), 29.70 – 29.12 (18 x

CH_2), 27.22 (CH_2), 25.64 (CH_2), 22.70 (CH_2), 14.16 (C-24″); ESI MS *m/z* (rel. int.): 968 $[M]^+$ ($C_{60}H_{104}O_9$) (2.5), 456 (12.1), 367 (8.7), 351 (15.2), 179 (10.4).

Results and Discussion

Compounds **1, 2** and **6** were the fatty esters characterized as *n*-undecanyl oleate, *n*-undecanyl stearate and *n*-tetracosanyl capriate, respectively. The compounds **3** – **5** were the long chain alkanes identified correspondingly as *n*-pentacosane, *n*-henetriacontane and *n*-pentatriacontane.

Compound **7**, a lanostan-3-olyl alkyl anthraquinone , displayed UV absorption maxima at 225, 253, 286, 303, 433 nm for anthraquinone unit and IR absorption bands for carbonyl functions (1701, 1609 cm^{-1}), unsaturation (1609 cm^{-1}), aromatic ring (1516, 1032 cm^{-1}) and long aliphatic chain (727 cm^{-1}). On the basis of mass and ^{13}C NMR spectra the molecular ion peak of **7** was established at *m/z* 916 corresponding to a molecular formula of a lanostanyl alkyl anthraquinone, $C_{64}H_{100}O_3$. An ion peaks arising at *m/z* 429 $[C_{1'}$ - O fission$]^+$ and 412 $[C_3$ – O fission] indicated that a tetracyclic triterpenoid was linked to the alkyl anthraquinone unit. The ^1H NMR spectrum of **7** exhibited a one-proton double doublet at δ 3.96 (J = 5.6, 8.0 Hz), assigned to oxymethine H-3α proton, five three – proton singlets at δ 1.01, 0.87, 0.82, 0.80, 0.78, three doublets at δ 0.97 (J = 6.3 Hz), 0.91 (J = 6.0 Hz) and 0.89 (J = 6.0 Hz) and a triplet at δ 0.84 (J = 6.5 Hz) were associated correspondingly with tertiary C-19, C-29, C-28, C-30 and C-18, secondary C-21, C-26 and C-27 and primary C-20″ methyl protons, all attached to saturated carbons, a two – proton triplets at δ 2.34 (J = 7.2 Hz) accounted to methylene H_2-2″ adjacent to the ester function, other methylene and methine protons as multiplets between δ 2.94 – 1.31 and as broad signals at δ 1.28 (34H), two one – proton doublets in the deshielded region at δ 7.96 (J = 2.4 Hz) and 7.23 (J = 2.4 Hz), a one – proton double doublet at δ 7.94 (J = 2.3, 8.0 Hz) and three one - proton multiplets at δ 7.69, 7.54 and 6.86 ascribed to aromatic meta-coupled H -2′ and H -4′, ortho-, meta-coupled H-8′, respectively, and other aromatic protons. The ^{13}C NMR spectrum of **7** displayed signals for carbonyl carbons at δ 181.95 (C-6′) and 183.06 (C-13′), aromatic carbons from δ 150.48 to 109.75, oxymethine carbon at δ 79.15 (C-3) and methyl carbons between δ 25.41 – 14.16. The ^1H and ^{13}C NMR spectral data of the triterpenoid unit of **7** were compared with the reported data of similar lanosteroids [24 – 26]. The spectral data of the anthraquinone moiety were also compared with the similar compounds [27]. On the basis of spectral data analysis the structure of **7** was formulated as lanostan-3-olyl 1′-(3′- eicosanyl) anthraquinone, a new alkylated anthraquinone lanostene triterpene.

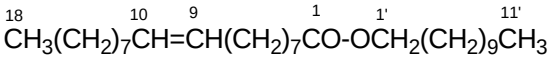
Compound **8**, a lanostan-3-olyl acyl xanthone, showed UV absorption maxima at 234, 255, 278, 330 nm for xanthone unit and IR absorption bands for ester group (1730 cm^{-1}), carbonyl function (1689 cm^{-1}), unsaturation (1637 cm^{-1}), aromatic ring (1514, 1032 cm^{-1}) and long aliphatic chain (727 cm^{-1}). On the basis of mass and ^{13}C NMR spectra the molecular ion peak of **8** was determined at *m/z* 934 corresponding to a molecular formula of a lanostanyl acyl xanthone, $C_{63}H_{98}O_5$. An ion peaks arising at *m/z* 429 $[C_{1'}$ - O fission, $C_{29}H_{53}O]^+$ indicated that a tetracyclic triterpenoid was linked to the acyl xanthone unit. The ^1H NMR spectrum of **8** exhibited a one-proton double doublet at δ 3.94 (J = 5.5, 9.1 Hz), assigned to oxymethine H-3α proton, five three – proton singlets at δ 0.99, 0.94, 0.91, 0.79 and 0.77, three doublets at δ 0.96 (J = 6.5 Hz), 0.89 (J = 6.5 Hz) and 0.86 (J = 6.6 Hz) and a triplet at δ 0.82 (J = 6.5 Hz) associated correspondingly with tertiary C-19, C-30, C-28, C-29 and C-18, secondary C-21, C-26 and C-27 and primary C-20″ methyl protons, all attached to saturated carbons. A three – proton triplet at δ 2.36 (J = 7.6 Hz) was accounted to methylene H_2-2′ adjacent to the ester function of the arachidyloxy chain located on the aromatic ring. The other methylene and methine protons resonated as multiplets between δ 2.94 – 1.31 and as broad signals at δ 1.25 (12H) and 1.23 (20H). Two one – proton doublets in the deshielded region at δ 7.67 (J = 9.5 Hz) and 7.52 (J = 9.5 Hz), two double doublets at δ 7.97 (J = 2.4, 8.1 Hz) and 7.70 (J = 1.3, 8.3 Hz), and three at δ 7.24 and 6.97 were ascribed to aromatic ortho-coupled H -2′ and H -3′, ortho-, meta-coupled H-7′ and H -10′, respectively, and other xanthone protons. The ^{13}C NMR spectrum of **8** displayed signals for ester carbon at δ 169.06 (C-1″), carbonyl carbon at δ 181.98 (C-12′), aromatic carbons from δ 152.08 to 109.70, oxymethine carbon at δ 79.11 (C-3) and methyl carbons between δ 25.49 – 15.38. The ^1H and ^{13}C NMR spectral data of the triterpenoid unit of **8** were compared with the reported data of similar lanostanoids [24 – 26]. The spectral data of

the xanthone moiety were also compared with the similar compounds [28]. These evidences led to the establishment of the structure of **8** as lanostan-3-olyl 1′-(4′-arachidyloxy)-xanthone, a new xanthonyl arachidioxy lanostane triterpenoid.

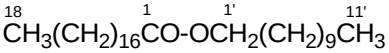
Compound **9**, named oleanolic acid 3β-O-α-D-glucosyl 2′-arachidate, responded triterpenic glycosidal tests positively and showed distinctive IR absorption bands for hydroxyl groups (3452, 3386, cm^{-1}), ester function (1735 cm^{-1}), carboxylic group (3255, 1686 cm^{-1}), unsaturation (1635 cm^{-1}) and long aliphatic chain (723 cm^{-1}). Its molecular ion peak was established at *m/z* 912 on the basis of mass and ^{13}C NMR spectra consistent with the molecular formula of an triterpenic glycosidic ester, $C_{56}H_{96}O_9$. The prominent ion peaks generating at *m/z* 455 [M - glycoside unit, $C_{30}H_{47}O_3]^+$, 473 [M - 455, $C_6H_{10}O_6$-CO-$(CH_2)_{18}CH_3$]$^+$, 457 [$C_6H_{10}O_5$-CO-$(CH_2)_{18}CH_3$]$^+$, 179 $[C_6H_{11}O_6]^+$ and 311 [OOC-$(CH_2)_{18}CH_3$]$^+$ indicated that arachidoic glycoside was linked with a pentacyclic triterpenic acid. The ^1H NMR spectrum of **9** displayed a one-proton doublet at δ 5.87 (J = 6.6 Hz) assigned to vinylic H-12 proton, a one-proton double doublet at δ 3.65 (J = 3.2, 10.0 Hz) ascribed to α-oriented oxymethine H-3 proton, a one-proton doublet at δ 4.96 (J = 6.6 Hz) attributed to anomeric H-1′ proton, four one-proton multiplets between δ 4.65 – 3.76 and a two-proton doublet at δ 3.16 (J = 5.8 Hz) accounted to other sugar protons, seven three-proton singlets from δ 0.98 to 0.73 due to tertiary methyl protons of n oleanoic acid and a three-proton triplet at δ 0.84 (J = 6.3 Hz) dipicted to primary C-20″ primary methyl protons of the ester linkage. The other methylene and methine protons resonated as multiplets between δ 2.63 – 1.36 and as a broad signal at δ 1.25 (32H). The ^{13}C NMR spectrum of **9** showed signals for ester carbon at δ 172.21 (C-1″), vinylic carbons at δ 121.73 (C-12) and 145.41 (C-13), oxymethine carbon at δ 79.86 C-3), carboxylic carbon at δ 180.41(C-28), anomeric carbon at δ 103.82 (C-1′), hydroxymethylene carbon at δ 61.07 (C-6 ′), other sugar carbons between δ 81.69 - 65.18 and methyl carbons in the range of δ 33.43 – 14.30. The presence of ^1H NMR signal at δ 4.41 (H-2′), and ^{13}C NMR signal at δ 81.69 (C-2′) in deshielded regions indicated the attachment of the ester linkage at C-2′ of the sugar moiety. The ^1H and ^{13}C NMR signals of the triterpenic unit **7** were compared with the reported spectral values of oleanolic acid [29,30]. Acid hydrolysis of **9** yielded oleanolic acid, m. p. 298 - 300 ^oC, R_f 0.41 (hexane: ethyl acetate: methanol, 8.2:1.8:0.5); D-glucose, R_f 0.41 (ethyl acetate : formic acid : water : methanol; 6:1:1:2) and arachidic acid, m. p. 73 - 75 ^oC, R_f 0.28 (*n*-hexane). On the basis of the foregoing discussion the structure of **9** has been established as oleanolic acid 3-O-α-D-glucopyranosyl-2′-arachidate, a new oleanolic acid glycosidic ester .

Compound **10**, named betulinic acid 3-O-β-D- glucosyl 2′-lignocerate, gave positive tests for triterpenic glycosides and showed IR absorption bands for ester group (1721 cm^{-1}), hydroxyl groups (3452, 3380 cm^{-1}), carboxylic function (3260, 1687 cm^{-1}), unsaturation (1642 cm^{-1}) and long aliphatic chain (723 cm^{-1}). Its molecular ion peak was determined at *m/z* 968 on the basis of mass and ^{13}C NMR spectra consistent with a molecular formula of a pentacyclic triterpenic glycosidic ester, $C_{60}H_{104}O_9$. The predominent ion peaks arising at *m/z* 456 [$C_{1'}$ - O fission, $C_{30}H_{48}O_3]^+$, 367 [OOC-$(CH_2)_{22}CH_3]^+$, 351 [OC-$(CH_2)_{22}CH_3]^+$ and 179 $[C_6H_{11}O_6]^+$ indicated that a pentacyclic triterpenic acid was linked with an acylated glycoside. Its ^1H NMR spectrum showed two one-proton singlets at δ 4.74 and 4.66 assigned to vinylic methylene H_2-29 protons. A one - proton double doublet at δ 3.65 (J = 5.9, 9.6 Hz) was ascribed to α- oriented oxymethine H-3 proton. A three-proton singlet at δ 1.65 was attributed to C-30 methyl protons linked to the vinylic C-20 carbon. The other methyl protons appeared as three-proton singlets from δ 1.17 to 0.75. A one – proton doublet at δ 5.87 (J = 7.2 Hz) was accounted to anomeric H-1′ proton. The other sugar protons resonated between δ 4.20 – 3.16. A two – proton triplet at δ 2.19 (J = 7.2 Hz), a broad singlet at δ 1.25 (42H) and a three – proton triplet at δ 0.85 (J = 6.6 Hz) were associated with the methylene and methyl protons of the acyl chain. The ^{13}C NMR spectrum of **10** exhibited the presence of important signals for vinylic carbons at δ 150.91 (C-20) and 109.37 (C-29), oxymethine carbon at δ 79.06 (C-3), methyl carbons from δ 27.92 to 14.16, anomeric carbon at δ 103.69 (C-1′), other sugar carbon signals between δ 79.64 – 60.18 and ester carbon at δ 172.15 (C-1″). The presence of H-2′ signal in the deshielded region at δ 4.11 and carbon C-2′ signal at δ 79.64 suggested that the acyl chain was linked at C-2′ of the sugar unit. The ^1H and ^{13}C NMR spectral values of the triterpenic unit were compared with reported NMR data of lupene-type triterpenoids [31]. Acid hydrolysis of 10 yielded betulinic acid, m. p. 316 – 318 °C, lignoceric acid, m. p. 83 – 84 °C and D-glucose, R_f 0.55 (*n*-butanol -

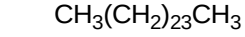
acetic acid - water, 2 : 1 : 1).. On the basis of these evidences the structure of **10** has been elucidated as betulinic acid 3β-O-α-D-glucopyranosyl-2′-lignocerate, a new lupenic glycosidic ester.



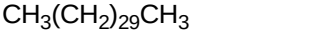
n-Undecanyl oleate **(1)**



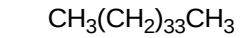
n-Undecanyl stearate **(2)**



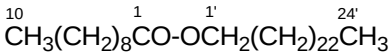
n-Pentacosane **(3)**



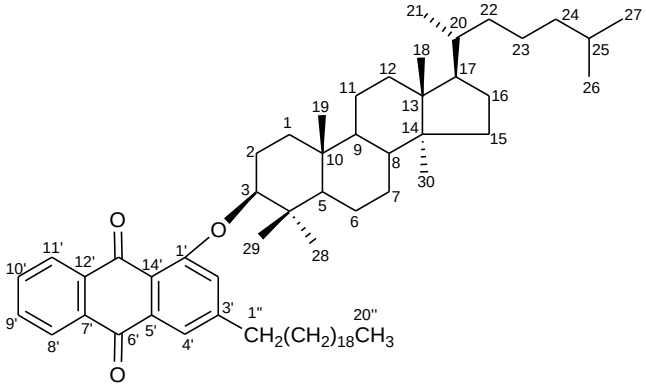
n-Henetriacontane **(4)**



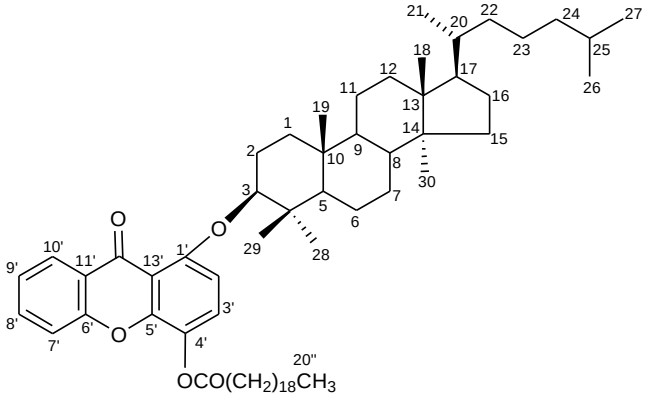
n-Pentatriacontane **(5)**



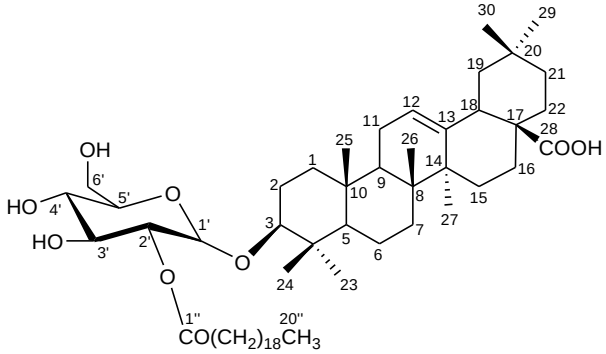
n-Tetracosanyl capriate **(6)**



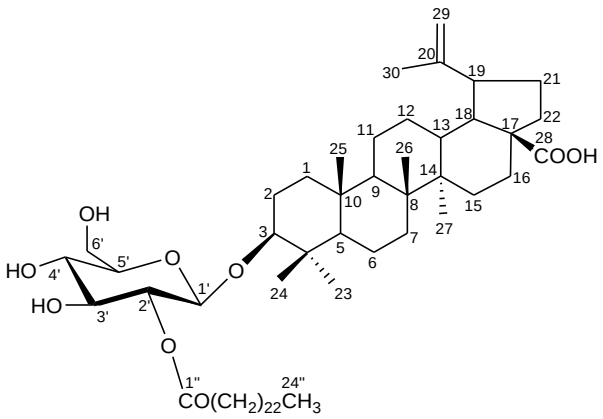
Lanostan-3-olyl-1′-(3′-eicosanyl) anthraquinone **(7)**



Lanostan-3-olyl-1′-(4′-arachidoxy) xanthone **(8)**



Oleanolic acid-3-O-β-L-glucosyl2′-arachidate **(9)**



Betulinic acid-3-*O*-β-D-glucosyl 2′-lignocerate **(10)**

Conclusion

Phytochemical investigation of a methanolic extract of the stem bark of *V. negundo* gave fatty esters, alkanes, lanostene alkyl anthraquinone, lanostene xanthone arachidate, oleanolic acid glucosidic esters and betulinic glucosidic ester. This work has enhanced understanding about the phytoconstituents of the plant. These secondary metabolites can be used as analytical markers for quality control of this plant.

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