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CHEMICAL CONSTITUENTS FROM THE LEAVES OF *EUCALYPTUS TERETICORNIS* SM. AND *VITIS VINIFERA* L.

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ABSTRACT

Eucalyptus tereticornis Sm. (family Myrtaceae), is a native to eastern Australia and southern New Guinea, planted all over India after teak. A leaf decoction is drunk to cure asthma, burns, body pain, fever, influenza, diarrhoea, pulmonary problems and rheumatism. *Vitis vinifera* L. (family Vitaceae), is a long-stemmed, woody vine found in Mediterranean region, central Europe, and southwestern Asia. Its leaves are used to treat boils, diarrhoea and toothache. Our study was planned to isolate chemical constituents from methanolic extracts of the leaves of *E. tereticornis* and *V. vinifera* and to characterize their structures on the basis spectral data analysis. The methanolic extract of leaves of *E. tereticornis* afforded a known fatty acid ester identified as *n*-tricosanyl *n*-octadec-9-en-1-oate (*n*-tricosanyl oleate, **1**), a new unsaturated aliphatic keto alcohol characterized as *n*- tricos-3β-ol-16-one-17(Z)-ene (**2**) and two new acyl coumarins and their structures have been established as 6-hexacosanoxycoumarin **(**6-cerotinoxycoumarin, **3**) and *n*-(21′α-hydroxyoctacosanoxy) coumarin or (6-(21′α-hydroxymontanoxy) coumarin (**4**). The methanolic extract of the leaves of *V. vinifera* on subjection to silica gel column furnished steroidal esters characterized as stigmast-5-en-3 β-yl *n*-octadec-9, 12-dienoate (β- sitosterol linoleate, **5)** and stigmast-5-en-3β-ol-3β-olyl n-docos-11-enoate (β-sitosterol 3β- cetoleate, **6**).

KEYWORDS: *Eucalyptus tereticornis*, *Vitis vinifera*, leaves, phytoconstituents, isolation, characterization.

INTRODUCTION

Eucalyptus tereticornis Sm., Syn. *Eucalyptus insignis* Naudin, *Eucalyptus populifolia* Desf., *Eucalyptus subulata* A. Cunn. ex Schauer, *Leptospermum umbellatum* Gaertn. (family Myrtaceae), commonly known as Forest red gum, Blue gum, Red iron gum, Eucalyptus, Safeda and Nilgiri, is a species of tree that is native to eastern Australia and southern New Guinea. It is planted all over India after teak except North-eastern states in agricultural lands, wasteland and along roadsides and many other countries. It is a 20-60 m tall tree, trunk is straight, unbranched for half of the total height of the tree. The bark is smooth, shed in irregular sheets, leaves lanceolate, tapering at the base to a 13–30 mm long petiole; the flower buds are arranged in leaf axils in groups of seven, flower buds in seven groups in leaf axils, flowers white, fruits hemispherical, woody capsules with protruding valves. Its resin is astringent and taken internally to treat dysentery. A leaf decoction is drunk to cure asthma, burns, body pain, fever, influenza, diarrhoea, pulmonary problems and rheumatism. The leaf essential oil showed analgesic, antibacterial, antifungal, anti-inflammatory and muscle-relaxant effects in rats and mice. Eucalyptus oil is used as an insect repellent, in flavouring and fragrance products and to manufacture cineole.[1,2]

The leaves of *E. tereticornis* contained ursolic acid and ursolic acid lactone,[3,4] flavonoids sideroxylin, 8-demethylsideroxylin and 7-methoxy-aromadendrin,[5] phenolic compounds, saponins, steroids and flavonoids,[6,7] lignins,[8] acyl phloroglucinols euglobal T1 and euglobal II c,[9] triterpene ester tereticornate A and B and betulonic acid. [10] Leaf essential oil was composed of α-pinene, 1,8-cineole, β-citronellal, (-)-isopulegol, β-citronellol,[11–17] sesquiterpenes (caryophyllene, eudesmol, globulol, spathulenol and virdiflorol) and monoterpenes (1, 8-cineole, citronellal, citronellol, limonene, pinenes, *trans*-pinocarveol, terpinolene and thujene).[15] The leaf oil from Guangxi province contained eucalyptol, alpha-pinene, isopinocarveol; the fruit oil possessed alpha-pinene, eucalyptol, and D-limonene.[18] The bark yielded betulinic acid, saponins, tannins, steroids and flavonoids,[18] hexadecanoic acid, methyl ester, quercetin and rhamnazin.[19]

Vitis vinifera L., syn. *Cissus vinifera* (L.) Kuntze (family Vitaceae), known as grape vine, is a native to the

Mediterranean region, central Europe, and southwestern Asia including Morocco, Iran and India. It is a liana growing 12–15 m tall at a fast rate, with a flaky bark, leaves alternate, palmately lobed, deciduous, supported by branched tendrils; flowers numerous; fruit is an ovoid or globular, dark blue or greenish berry, known as a grape. The leaves and tendrils are chewed to relieve toothache. Mustard oil is spread on the leaf, warmed and applied to cure boils. Stem mixed with *Solanum nigrum* and *Cestrum paraqui* is applied to reduce inflammation.[20] The leaves are astringent and taken orally to cure diarrhoea. Dried fruits are demulcent, laxative, refrigerant, stomachic and tonic, ingested to relieve allergies, anaemia, bronchitis, coughs, flu, tuberculosis and thirst. Juice of unripe fruits is astringent and used to prevent throat infections. Sap of the young branches is applied to subside skin diseases. The fruit preparations are effective against cancers, liver disorders and uterine tumours.[21]

Grape roots contained stilbenoid compounds, resveratrol, vitisins A and B, and picaetannol, and miyabenol C.[22] Other stilbenoid compounds in the grape root are trans-piecid, *cis*-piecid, vitisinol B, viniferether A, viniferether B, ampelopsin C, ampelopsin E, hopeaphenol, and isohopeaphenol.[23] Grape leaves afforded aromatic acids, coumarins, dihydrochalcone, stilbenes, flavan-3-ols, flavonols, flavones, flavanones, anthocyanins,,[23] condensed tannins,[24] resveratrol, ε-viniferins, balanocarpol, and balanocarpol glycoside,[25] stearic acid, α-amyrin, lupeol and squalene.[26] Grape seeds furnished procyanidin, gallic acid, epicatechin, catechin, and quercetin,[27] flavonol glycosides, resveratrol, and anthocyanidins,[28,29] phenolic compounds,[30] linoleic, primaric, caffeic, p-hydroxy-phenylacetic, and gallic acids.[31] Grape skin possessed flavonols, anthocyanins, flavan-3-ols, stilbenes and phenolic acid,[32,33] quercetin, vanillic acid, kaempferol, syringic acid, and gallic acid,[34] caffeic acid, coumaric acid, ferulic acid, caftaric acid, coutaric acid, fertaric acid, (-)-epicatechin, (+) catechin, resveratrol, procyanidin, and flavonols.[35, 36] Grape stem yielded phenolic acids, anthocyanins, catechins, flavanone, flavone, flavonols, stilbenes vitisins A, B, and C, miyabenol C, resveratrol, ε-viniferins, balanocarpol, and balanocarpol glycoside.[23,25] Grapevine canes gave phenolic acids, ferulic acid, flavonoids, stilbenes, *trans*-resveratrol-2-C-glucoside, *trans*-resveratrol, and ampelopsins A and D.[23] The presence of herbal chemical constituents vary due to many factors such as geographic regions, soils, seasonal changes, plant species and application of fertilizers. Keeping in views the various therapeutic values and variation aspects of chemical constituents of the plants and development of ecofriendly, biodegradable and safer herbal preparations, it has been aimed to establish chemical structures of phytoconstituents isolated from the leaves of *Eucalyptus tereticornis* Sm. and *Vitis vinifera* L.

MATERIALS AND METHODS

The protocols of all methodologies (procedures, experimental designs and spectral data analysis) were adopted from the earlier published work.[37,38,43,44]

Collection and authentication of plant materials

The leaves of *Eucalyptus tereticornis* and *Vitis vinifera* were collected from Delhi. The plant materials were identified and authenticated by Prof. M. P. Sharma, Taxonomist, Department of Botany, Jamia Hamdard, New Delhi. Voucher specimens of the plant materials were preserved in the herbarium of the Department of Pharmacognosy and Phytochemistry, Jamia Hamdard, New Delhi.

Extraction and isolation

The leaves of *E. tereticornis* and *V. vinifera* (1 kg each) were dried in air, coarsely powdered and extracted separately and exhaustively with methanol in a Soxhlet apparatus. The extracts were concentrated under reduced pressure to get dark brown masses, 132.7 g, and 121.4 g, respectively. The dried residues (100 g each) were dissolved in minimum amounts of methanol separately and adsorbed on silica gel columns grade (60-120 mesh) individually to obtain slurries. Each slurry was air-dried and chromatographed over silica gel columns loaded in petroleum ether (b. p. 60 – 80 °C). The columns were eluted with petroleum ether, petroleum ether - chloroform (9:1, 3:1, 1:1, 1:3, *v/v*), chloroform and chloroform - methanol (99:1, 49:1, 19:1, 9:1, *v/v*) mixtures. Various fractions were collected separately and matched by TLC to check homogeneity. Similar fractions having the same R_f values were combined and crystallized with solvents. The isolated compounds were recrystallized to get pure compounds.

Isolation of phytoconstituents from the leaves of *Eucalyptus tereticornis*

n-Tricosanyl oleate (1)

Elution of the column with petroleum ether – chloroform (1:3) gave a pale yellow mass of **1**, recrystallized from acetone-methanol (1:1), yield 102 mg, R_f: 0.69 ($CHCl_3$); m. p. 79-80 ^{o} C; UV λmax (MeOH): 217 nm (log ε 4.1); IR υ_{max} (KBr): 2949, 2845, 1725, 1645, 1435, 1362, 1210, 1005, 725 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 5.25 (1H, m, H-9), 5.22 (1H, m, H-10), 4.04 (2H, t, J = 6.9 Hz, H_2 -1′), 2.21 (2H, t, J = 7.2 Hz, H_2 -2), 2.15 (2H, m, H_2 -8), 2.08 (2H, m, H_2 -11), 1.73 (2H, m, H_2-3), 1.54 (4H, m, H_2-7, H_2-12), 1.32 (4H, brs, H_2-2′, H_2-3′), 1.29 (34H, brs, 17 x CH_2), 0.79 (3H, t, J = 6.8 Hz, Me-18), 0.76 (3H, t, J = 6.9 Hz, Me-23′); ^{13}C NMR ($CDCl_3$): δ 173.12 (C-1), 34.01 (C-2), 29.49 (C-3), 29.47 (C-4), 29.45 (C-5), 29.45 (C-6), 29.42 (C-7), 32.36 (C-8), 129.59 (C-9), 128.78 (C-10), 32.36 (C-11), 29.42 (C-12), 29.46 (C-13), 29.17 (C-14), 29.13 (C-15), 29.11 (C-16), 22.45 (C-17), 13.94 (C-18), 60.01 (C-1′), 329.49 (C-2′), 29.49 (C-3′), 29.47 (C-4′), 29.47 (C-5′), 29.45 (C-6′ to C-14′), 29.48 (C-15′), 29.48 (C-16′), 29.45 (C-17′), 29.17 (C-18′), 28.95 (C-19′), 28.95 (C-20′), 26.95 (C-21′), 25.37 (C-22′), 13.79

(C-23′); +ve FAB MS *m/z* (rel.int.): 604 $[M]^+$ ($C_{41}H_{80}O_2$) (3.1), 339 (11.2), 265 (12.8).

***n*- Tricos-3β-ol-16-one-17(Z)-ene (2)**

Elution of the column with chloroform yielded a colourless mass of **2,** yield 412 mg, purified by preparative TLC using chloroform-methanol (9.5 : 0.5, v/v), m. p. 54 - 55 ^{o} C; IR υ_{max} (KBr): 3390, 2928, 2850, 1707, 1619, 1448, 1384, 1219, 1040, 768 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 6.73 (1H, d, J = 8.9 Hz, H-17), 5.25 (1H, m, $w_{1/2}$ = 9.3 Hz, H-18), 3.65 (1H, m, $w_{1/2}$ = 18.5 Hz, H-3α), 2.25 (2H, m, H_2-15), 1.94 (2H, m, H_2-19), 1.52 (2H, m, H_2-14), 1.22 (28 H, brs, 14 × CH_2), 0.80 (3H, t, J = 7.2 Hz, Me-23), 0.75 (3H, t, J = 7.1 Hz, Me-1); ^{13}C NMR ($CDCl_3$): δ 13.68 (C-1), 31.93 (C-2), 74.16 (C-3), 29.72 (C-4), 22.71 (C-5 to C-14), 55.27 (C-15), 202.13 (C-16), 123.55 (C-17), 127.26 (C-18), 29.72 (C-19), 29.70 (C-20), 29.68 (C-21), 29.65 (C-22), 14.15 (C-23); +ve FAB MS *m/z* (rel. int.): 352 $[M]^+$ ($C_{23}H_{44}O_2$) (2.1), 125 (13.6), 97 (20.7), 73 (15.3), 71 (13.6), 59 (100).

6-Cerotinoxycoumarin (3)

Elution of the column with chloroform furnished light brown crystals of **3**, recrystallized from ethyl acetate, yield 252 mg; m. p. 147 - 149 ^{o} C; UV λ max (MeOH): 215, 285, 301 nm (log ε 5.1, 4.8, 2.1); IR υmax (KBr): 2928, 2845, 1735, 1710, 1635, 1565, 1480, 1438, 1395, 1220, 1005, 790 cm^{-1}; ^{1}H NMR ($CDCl_3$) : δ 7.04 (1H, m, H-7), 6.98 (1H, m, H-9), 6.57 (1H, m, H-8), 6.51 (1H, d, J = 7.5 Hz, H-4), 5.34 (1H, d, J = 7.5 Hz, H-3), 2.33 (2H, t, J = 7.2 Hz, H_2-2′), 2.01 (2H, m, H_2-3′), 1.82 (2H, m, H_2-4′), 1.62 (2H, m, H_2-5′), 1.25 (40H, brs, 20 x CH_2), 0.85 (3H, t, J = 7.1 Hz, Me-26′); ^{13}C NMR ($CDCl_3$) : δ 179.16 (C-2), 125.60 (C-3), 127.56 (C-4), 135.13 (C-5), 145.28 (C-6), 129.97 (C-7), 127.53 (C-8), 127.92 (C-9), 145.17 (C-10), 170.23 (C-1′), 56.03 (C-2′), 45.81 (C-3′), 40.36 (C-4′), 34.01 (C-5′), 31.89 (C-6′), 29.64 (C-7′ to C-13′), 29.09 (C-14′ to C-18′), 28.21 (C-19′), 27.17 (C-20′), 26.06 (C-21′), 24.72 (C-22′), 24.28 (C-23′), 23.65 (C-24′), 18.78 (C-25′), 14.07 (C-26′); +ve FAB MS *m/z* (rel. int.) : 540 $[M]^+$ ($C_{35}H_{56}O_4$) (6.8), 379 (5.3), 161 (11.6).

6-(21′α-Hydroxymontanoxy) coumarin (4)

Further elution of the column with chloroform afforded pale yellow mass of **4**, purified by preparative TLC using ethyl acetate - methanol (49:1, v/v), yield 190 mg; m. p. 58-58 ^{o} C; UV λ max (MeOH): 215, 283, 297 nm (log ε 3.8, 4.3, 1.9); IR υmax (KBr): 3497, 2921, 2850, 1725, 1707, 1641, 1520, 1462, 1240, 1007, 789 cm^{-1}; ^{1}H NMR ($CDCl_3$) : δ 7.10 (1H, m, H-7), 6.51 (1H, m, H-9), 6.58 (1H, m, H-8), 6.51 (1H, d, J = 5.4 Hz, H-4), 5.36 (1H, d, J = 5.4 Hz, H-3), 4.13 (1H, m, $w_{1/2}$ = 6.9 Hz, H-21′β), 2.34 (2H, t, J = 7.5 Hz, H_2-2′), 2.03 (2H, m, H_2-3′), 1.63 (2H, m, H_2-4′), 1.27 (44H, brs, 22 x CH_2), 0.82 (3H, t, J = 6.6 Hz, Me-28′); ^{13}C NMR ($CDCl_3$) : δ 179.53 (C-2), 128.01 (C-3), 127.78 (C-4), 136.11 (C-5), 145.13 (C-6), 130.56 (C-7), 128.55 (C-8), 129.63 (C-9), 145.23 (C-10), 170.16 (C-1′), 52.19 (C-2′), 45.67 (C-3′), 40.91 (C-4′), 33.90 (C-5′), 31.92 (C-6′), 29.65 (C-7′ to C-20′), 66.27 (C-21′), 29.67 (C-22′), 27.18 (C-23′), 24.71 (C-24′), 22.67 (C-25′), 14.09 (C-28′); +ve FAB MS *m/z* (rel. int.) : 584 $[M]^+$ ($C_{37}H_{60}O_5$) (3.2), 455 (10.1), 423 (3.2), 189 (61.3), 161 (38.7), 129 (47.5).

Isolation of phytoconstituents from the leaves of *Vitis vinifera*

β-Sitosterol linoleate (5)

Elution of the column with petroleum ether – chloroform (1:3) produced a colourless crystalline mass **5**, recrystallized from chloroform – methanol (1:1), yield 117 mg, m. p. 95 - 96 °C; UV λmax (MeOH): 221 nm (log ε 3.8); IR υ_{max} (KBr): 2929, 2851, 1738, 1639, 1463, 1378, 1243, 1177, 1116, 1037, 882, 721 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 5.36 (1H, m, H-6), 4.12 (1H, brm, $w_{1/2}$ = 16.5 Hz, H-3α), 2.39 (2H, m, H_2-1), 2.14 (2H, m, H_2-7), 1.90 (2H, m, H_2-4), 1.79 (2H, m, H_2-2), 1.01 (3H, brs, Me-19), 0.93 (3H, d, J = 6.3 Hz Me-21), 0.87 (3H, d, J = 6.6 Hz, Me-26), 0.80 (3H, d, J= 6.1 Hz, Me-27), 0.83 (3H, d, J = 6.1 Hz Me-29), 0.67 (3H, brs, Me-18), 5.32 (1H, m, H-9′), 5.30 (1H, m, H-12′), 5.15 (1H, m, H-10′), 5.12 (1H, m, H-13′), 2.82 (2H, m, H_2-11′), 2.29 (2H, t, J = 8.8 Hz, H_2-2′), 2.05 (2H, m, H_2-8′), 2.06 (2H, m, H_2-14′), 0.85 (3H, t, J= 6.5 Hz, Me-18′), 1.68 - 1.30 (23H, m, 8 x CH_2, 7 x CH), 1.29 (8H, brs, 4 x CH_2), 1.23 (6H, brs, 3 x CH_2); ^{13}C NMR ($CDCl_3$): δ 36.68 (C-1), 31.08 (C-2), 78.24 (C-3), 41.90 (C-4), 141.07 (C-5), 123.72 (C-6), 31.13 (C-7), 31.07 (C-8), 49.52 (C-9), 38.02 (C-10), 21.07 (C-11), 39.76 (C-12), 41.88 (C-13), 55.43 (C-14), 24.17 (C-15), 28.67 (C-16), 55.36 (C-17), 11.29 (C-18), 19.21 (C-19), 36.68 (C-20), 18.27 (C-21), 33.25 (C-22), 25.74 (C-23), 45.10 (C-24), 27.28 (C-25), 20.31 (C-26), 18.67 (C-27), 23.48 (C-28), 11.25 (C-29), 174.16 (C-1′), 59.91 (C-2′), 29.38 (C-3′), 29.27 (C-4′), 29.22 (C-5′), 29.08 (C-6′), 28.96 (C-7′), 50.02 (C-8′), 129.53 (C-9′), 127.71 (C-10′), 52.13 (C-11′), 128.61 (C-12′), 126.31 (C-13′), 26.78 (C-14′), 26.74 (C-15′), 24.77 (C-16′), 22.23 (C-17′), 13.86 (C-18′); +ve FAB MS *m/z* (rel. int.): 676 $[M]^+$ ($C_{47}H_{80}O_2$) (19.6), 413 (12.3), 397 (8.9), 386 (8.7), 279 (8.2), 271 (15.3), 263 (6.1).

β- Sitosterol cetoleate (6)

Elution of the column with chloroform afforded a colourless crystalline mass of **6**, recystallized by (chloroform-methanol, 1:1), 221 mg, R_f 0.42 (petroleum ether – chloroform, 1:3), UV λmax (MeOH): 216 nm; m. p. 75 - 77 °C; IR υ_{max} (KBr): 2925, 2853, 1736, 1643, 1462, 1378, 1237, 1039, 971, 883, 723 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 5.37 (1H, m, H-6), 4.15 (1H, brm, $w_{1/2}$ = 16.5 Hz, H-3α), 2.27 (2H, m, H_2-1), 2.31 (2H, m, H_2-7), 1.84 (2H, m, H_2-4), 2.01 (2H, m, H_2-2), 1.03 (3H, brs, Me-19), 0.92 (3H, d, J = 6.3 Hz Me-21), 0.86 (3H, d, J = 6.4 Hz, Me-26), 0.81 (3H, d, J= 6.1 Hz, Me-27), 0.82 (3H, d, J = 6.0 Hz Me-29), 0.68 (3H, brs, Me-18), 5.34 (1H, m, H-11′), 5.31 (1H, m, H-12′), 2.75 (2H, t, J = 6.4 Hz, H_2-2′), 2.05 (2H, m, H_2-10′), 1.67 (2H, m, H_2-13′), 0.84 (3H, t, J= 6.1 Hz, Me-22′), 1.63 - 1.30 (25H, m, 9 x CH_2, 7 x CH), 1.25 (16H, brs, 8 x CH_2), 1.23 (10H, brs, 5 x CH_2); ^{13}C NMR ($CDCl_3$): δ 36.82 (C-1), 32.76 (C-2), 78.16 (C-3), 41.95 (C-4), 140.08 (C-5), 122.15 (C-6), 31.23 (C-7), 31.05 (C-8), 49.53 (C-9), 38.37 (C-10), 23.56 (C-11),

39.77 (C-12), 41.87 (C-13), 55.99 (C-14), 24.09 (C-15), 28.87 (C-16), 55.35 (C-17), 11.37 (C-18), 19.25 (C-19), 36.72 (C-20), 18.39 (C-21), 33.19 (C-22), 25.67 (C-23), 45.08 (C-24), 27.83 (C-25), 20.36 (C-26), 22.50 (C-27), 23.91 (C-28), 11.59 (C-29), 171.97 (C-1′), 40.12 (C-2′), 30.16 (C-3′), 30.93 (C-4′), 30.82 (C-5′), 30.87 (C-6′), 30.71 (C-7′), 30.63 (C-8′), 30.54 (C-9′), 30.47 (C-10′), 129.15 (C-11′), 130.81 (C-12′), 30.34 (C-13′), 30.33 (C-14′), 28.77 (C-15′), 28.73 (C-16′), 28.24 (C-17′), 28.07 (C-18′), 26.63 (C-19′), 24.57 (C-20′), 22.27 (C-21′), 14.61 (C-22′); +ve FAB MS *m/z* (rel. int.): 734 $[M]^+$ ($C_{51}H_{90}O_2$) (11.5), 413 (31.8), 337 (45.1), 321 (18.2).

RESULTS AND DISCUSSION

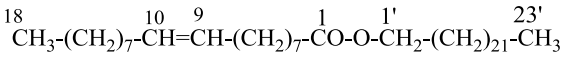
Compound **1** was a known fatty acid ester identified as *n*-tricosanyl *n*-octadec-9-en-1-oate (*n*-tricosanyl oleate) (Fig.1).[37,38]

Compound **2** decolourized bromine water suggesting unsaturated nature of the molecule. Its IR spectrum showed characteristic absorption bands for a hydroxyl group (3390 cm^{-1}), carbonyl group (1707 cm^{-1}), unsaturation (1619 cm^{-1}) and long aliphatic chain (768 cm^{-1}). Its molecular weight was established at *m/z* 352 on the basis of mass spectrum consistent with a molecular formula of an unsaturated aliphatic alcohol with one vinylic linkage, $C_{23}H_{44}O_2$. The ion peaks arising at *m/z* 59 [$C_3 - C_4$ fission, -CH(OH)-CH_2-CH_3, $C_3H_7O]^+$ and 73 [$C_4 - C_5$ fission, CH_2-CH(OH)-CH_2-CH_3, $C_4H_9O]^+$ suggested the location of the hydroxyl group C-3 carbon position. The ion fragments produced at *m/z* 71 [$C_{18} - C_{19}$ fission, CH_3-$(CH_2)_4$ -, $C_5H_{11}]^+$, 97 [$C_{16} - C_{17}$ fission, CH_3-$(CH_2)_4$ -CH=CH-, $C_7H_{13}]^+$ and 125 [$C_{15} - C_{16}$ fission, CH_3-$(CH_2)_4$ -CH=CH-CO, $C_8H_{13}O]^+$ indicated the presence of the vinylic linkage at C-17, C-18 carbon positions and carbonyl group at C-16 carbon. The ^1H NMR spectrum of **2** showed a one-proton doublet at δ 6.73 (J = 8.9 Hz, H-17) and a one-proton multiplet at δ 5.25 with half-width of 9.3 Hz assigned to *cis*- vinylic H-17 and H-18 protons, respectively. A one-proton multiplet at δ 3.65 ($w_{1/2}$ = 18.5 Hz) was ascribed to alpha-oriented carbinol H-3 proton. The methylene protons appeared as two-proton multiplets at δ 2.25, 1.94 and 1.52 and as a singlet at δ 1.22 (28H). Two three-proton triplets at δ 0.80 (J = 7.2 Hz) and 0.75 (J = 7.1 Hz) were accounted correspondingly to C-23 and C-1 primary methyl protons. The ^{13}C NMR spectrum of **2** exhibited signals for the vinylic carbons at δ 123.55 (C-17) and 127.26 (C-18), carbinol carbon at δ 74.16 (C-3), carbonyl carbon at δ 202.13 (C-16), methylene carbons between δ 55.27 – 22.71 and methyl carbons at δ 13.68 (C-1) and 14.15 (C-23). On the basis of spectral data analysis, the structure of **2** has been elucidated as *n*-tricos-3β-ol-16-one-17(Z)-ene, a new unsaturated aliphatic keto alcohol (Fig.1).

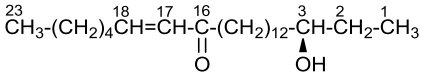
Compound **3** showed UV absorption maxima at 285 and 312 nm for coumarins[39,40] and IR absorption bands for an ester function (1735 cm^{-1}), lactone carbonyl (1710 cm^{-1}), unsaturation (1635 cm^{-1}), aromatic ring (1565 cm^{-1}) and long aliphatic chain (790 cm^{-1}). On the basis of mass and ^{13}C NMR spectra its molecular ion peak was determined at *m/z* 540 consistent with a molecular formula of an acyl coumarin, $C_{35}H_{56}O_4$. The ion peaks arising at *m/z* 379 [$C_{1'}$ - O fission, CH_3-$(CH_2)_{24}$-$CO]^+$ and 161 [M - 379, $C_9H_5O_3]^+$ indicated that the hydroxycoumarin was esterified with cerotic acid. The ^1H NMR spectrum of **3** displayed three one-proton multiplets at δ 7.04, 6.98 and 6.57 assigned to aromatic H-7, H-9 and H-8 protons, respectively, two one-proton doublets at δ 6.51 (J = 7.5 Hz) and 5.34 (J = 7.5 Hz) ascribed correspondingly to coumarin vinylic H-4 and H-3 protons, a two-proton triplet at δ 2.33 (J = 7.2 Hz) attributed to methylene H_2-2′ protons adjacent to the ester function, other methylene protons as two-proton multiplets at δ 2.01, 1.82 and 1.62 and a singlet at δ 1.25 (40H) and a three-proton triplet δ 0.85 (J = 7.1 Hz) accounted to terminal C-26′ primary methyl protons. The ^{13}C NMR spectrum of **3** exhibited signals for lactone carbonyl carbon at δ 179.16 (C-2), ester carbon at δ 170.23 (C-1′), aromatic carbons between δ 145.28 – 125.60, methylene carbons from δ 56.03 to 19.78 and methyl carbon at δ 14.07 (C-26′). On the basis of the aforementioned spectral data analysis the structure of **3** has been established as 6-hexacosanoxycoumarin **(**6-cerotinoxycoumarin), a new acyl coumarin (Fig. 1).

Compound **4** had UV absorption maxima at 283 and 297 nm for coumarins[39,40] and IR absorption bands for an ester function (1725 cm^{-1}), lactone carbonyl (1707 cm^{-1}), unsaturation (1641 cm^{-1}), aromatic ring (1520 cm^{-1}) and long aliphatic chain (789 cm^{-1}). Its molecular ion peak was found at *m/z* 540 on the basis of mass and ^{13}C NMR spectra consistent with a molecular formula of a hydroxyacyl coumarin, $C_{37}H_{60}O_5$. The ion peaks arising at *m/z* 161 [$C_{1'}$ - O fission, $C_9H_5O_3]^+$ and 423 [M - 161, $C_{28}H_{55}O_2]^+$ suggested that the hydroxycoumarin was esterified with hydroxyoctacosanoic acid. An ion fragment produced at m/z 129 [$C_{20'}$ - $C_{21'}$, CH(OH)-$(CH_2)_6$-CH_3 $]^+$ supported the existence of the hydroxy group at C-21′ carbon position. The ^1H NMR spectrum of **4** exhibited three one-proton multiplets at δ 7.10, 6.51 and 6.58 assigned to aromatic H-7, H-9 and H-8 protons, respectively, two one-proton doublets at δ 6.51 (J = 5.4 Hz) and 5.36 (J = 5.4 Hz) ascribed correspondingly to coumarin vinylic H-4 and H-3 protons, a one-proton multiplet at δ 4.13 with half-width of 6.9 Hz accounted to beta-oriented carbinol H-21′ proton, a two-proton triplet at δ 2.34 (J = 7.5 Hz) attributed to methylene H_2-2′ protons adjacent to the ester function, other methylene protons as two-proton multiplets at δ 2.03 and 1.63 and a singlet at δ 1.27 (44H) and a three-proton triplet δ 0.82 (J = 6.6 Hz) accounted to terminal C-28′ primary methyl protons. The ^{13}C NMR spectrum of **4** showed signals for the lactone carbonyl carbon at δ 179.53 (C-2), ester carbon at δ 170.16 (C-1′), aromatic carbons between δ 145.13 – 127.78, carbinol carbon at δ 66.27 (C-21′), methylene carbons from δ 52.19 to 22.67 and methyl carbon at δ 14.09 (C-28′). On the basis of the aforementioned spectral data analysis the structure of **4**

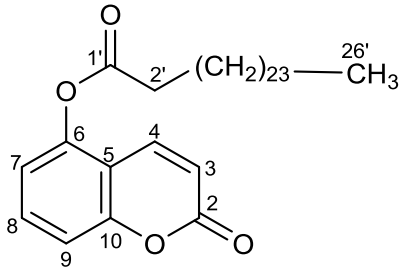
has been formulated as *n*-(21′α-hydroxyoctacosanoxy) coumarin, (6-(21′α-hydroxymontanoxy) coumarin, a new acyl coumarin (Fig. 1).



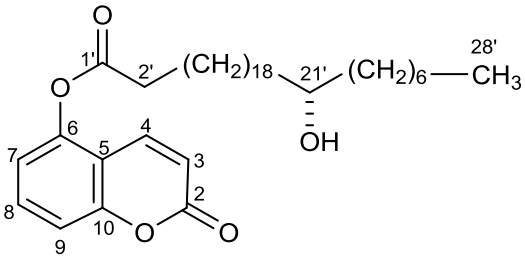
n-Tricosanyl oleate **(1)**



(Z) n-Tricosan-3β-ol-16-one **(2)**



6-Cerotinoxycoumarin **(3)**

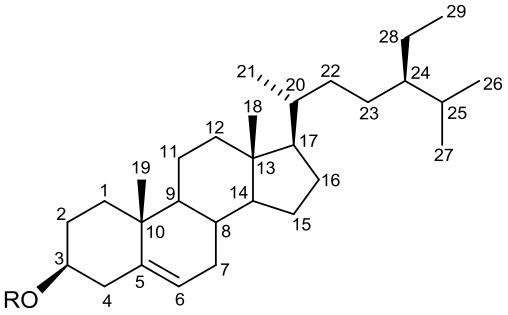


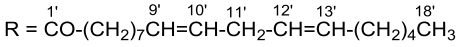
6-(21′α-Hydroxymontanoxy)-coumarin **(4)**

Fig. 1: Chemical constituents 1 to 4 isolated from the leaves of *Eucalyptus tereticornis*.

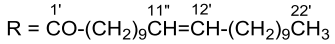
Compound **5** was a known steroidal ester characterized as stigmast-5-en-3 β-yl *n*-octadec-9, 12-dienoate (β-sitosterol linoleate) (Fig. 2).[41,42]

Compound **6** showed characteristics IR absorption bands for an ester function (1736 cm^{-1}), unsaturation (1643 cm^{-1}) and a long aliphatic chain (723 cm^{-1}). Its molecular ion peak was established at *m/z* 734 on the basis of mass and ^{13}C NMR spectra consistent with a molecular formula of a steroidal ester, $C_{51}H_{90}O_2$. The prominent ion peaks produced at *m/z* 413 [$C_{1'}$ - O fission, $C_{29}H_{49}O]^+$, 321 [M – 413, CH_3 -$(CH_2)_9$ -CH=CH-$(CH_2)_9$-$CO]^+$ and 337 [C_3 - O fission, CH_3 -$(CH_2)_9$ -CH=CH-$(CH_2)_9$- $COO]^+$ indicated that beta-sitosterol was esterified with cetoleic acid. The ^1H NMR spectrum of **6** showed three one-proton multiplets at δ 5.37, 5.34 and 5.31 assigned to vinylic H-6, H-11′ and H-12′ protons, respectively. A one-proton broad multiplet at δ 4.15 with half-with of 16.5 Hz was attributed to oxygenated α-oriented H-3 methine proton. Two three-proton singlets at δ 1.03 and 0.68, and four three-proton doublets at δ 0.92 (J = 6.3 Hz), 0.86 (J = 6.4 Hz), 0.81 (J = 6.1 Hz) and 0.82 (J = 6.0 Hz) were associated with the tertiary C-19 and C-18, secondary C-21, C-26 and C-27 and primary C-29 methyl protons, respectively. A three- proton triplet at δ 0.84 (J = 6.1 Hz) was accommodated to primary C-22′ methyl protons. The remaining methine and methylene protons resonated from δ 2.75 to 1.23. The ^{13}C NMR spectrum of **6** displayed important signals for steroidal vinylic carbons at δ 140.08 (C-5) and 122.15 (C-6), oxymethine carbon at δ 78.16 (C-3), methyl carbons at δ 11.37 (C-18), 19.25 (C-19), 18.39 (C-21), 20.36 (C-26), 22.50 (C-27) and 11.59 (C-29), and acyl signals for the ester carbon at δ 171.97 (C-1′), vinylic carbons at δ 129.15 (C-11′) and 130.81 (C-12′), and methyl carbon at δ 14.61 (C-22′). The ^1H NMR and ^{13}C NMR spectral data of the steroidal nucleus were compared with other stigmastene-type molecules.[43,44] On the basis of spectral data analysis and chemical reactions, the structure of **6** has been determined as stigmast-5-en-3β-ol-3β-olyl n-docos-11-enoate (β-sitosterol 3β- cetoleate), a new steroid ester (Fig. 2).





β-Sitosterol linoleate **(5)**



β-Sitosterol cetoleate **(6)**

Fig. 2: Chemical constituents 5 and 6 isolated from the leaves of *Vitis vinifera*.

CONCLUSION

The methanolic extract of leaves of *E. tereticornis* afforded a known fatty acid ester identified as *n*-tricosanyl *n*-octadec-9-en-1-oate (*n*-tricosanyl oleate, **1**), a new unsaturated aliphatic keto alcohol characterized as *n*- tricos-3β-ol-16-one-17(Z)-ene (**2**) and two new acyl coumarins and their structures have been established as 6-hexacosanoxycoumarin **(**6-cerotinoxycoumarin, **3**) and *n*-(21′α-hydroxyoctacosanoxy) coumarin or (6-(21′α-hydroxymontanoxy) coumarin (**4**). The methanol extract of the leaves of *V. vinifera* on subjection to silica gel column furnished steroidal esters identified as stigmast-5-en-3 β-yl *n*-octadec-9, 12-dienoate (β- sitosterol linoleate, **5**) and stigmast-5-en-3β-ol-3β-olyl *n*-docos-11-enoate (β-sitosterol 3β- cetoleate, **6**). This work has enhanced understanding about the chemical constituents of the undertaken plants. Further research is recommended to screen bioactivities of the isolated phytoconstituents with a view for supplementing conventional drug development especially in developing countries.

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