ejpmr, 2020,7(6), 692-701

SJIF Impact Factor 6.222

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH CHEMICAL CONSTITUENTS FROM THE LEAVES OF *CALLISTEMON LANCEOLATUS* (SM.) SWEET

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Article Received on 07/04/2020 **Article Revised on 28/04/2020** **Article Accepted on 18/05/2020**

ABSTRACT

Callistemon lanceolatus (Sm.) Sweet (Myrtaceae) grows all over the world and is used as a tea substitute with a delightfully refreshing flavor and to treat bronchitis and cough. This research work was undertaken to characterize structures of chemical constituents isolated from the plant leaves. A methanol extract of the leaves was adsorbed on silica gel (60-120 mesh) and subjected to silica gel column packed in petroleum ether. The column was eluted with petroleum ether, chloroform and methanol, successively, in order of increasing polarity to isolate the compounds. The isolated chemical constituents were characterized as 4-methoxyphenyl 4′-octanyl ether (**1**), 5,7-dihydroxy-4′-methoxy-8-ethyl flavone (8-ethyl 4′-methoxyapigenin, **2**), 5,7,4′-trimethoxy-6-methyl-8-isopropanolyl apigenin (**3**), lanostan-3β,19-, 18,21-diolide (callistelanostanediolide, **6**), 4′-methoxy-8-(2′′-propanolyl) apigenin (**7**), 4-hydroxyphenyl propanoloxycerotate (**8**), 4-hydroxyphenethyl tetratriacontanoate (tyrosolylgheddate, **9**), 4-hydroxyphenyl 1-propanyl tetratriacontanoate (4-hydroxyphenyl propanyloxygheddate, **10**) along with the known constituents 1-heptacosanol (**4**), 1-octacosanol (**5**) and α-amyrin (**11**). Their structures were established on the basis of spectral data analysis and chemical reactions.

KEYWORDS: *Callistemon lanceolatus*, leaves, extraction, phytoconstituents, isolation, spectral data, characterization.

INTRODUCTION

Global estimate indicates that 80% of about four billion populations are using traditional medicines, which are mainly derived from medicinal plants listing over 20,000 species.[1] Presently about 25% of pharmaceutical prescriptions in the United States contain at least one plant-derived ingredient. Most of the vegetable plants contained dietary antioxidants which are useful to reduce the risk of several diseases. Many food constituents play a vital role as essential nutrients in preventing and delaying the premature onset of chronic disease late in life. Consumption of fruits and vegetables reduces the risk of cancer, cardiovascular diseases, cerebro-vascular disorders and mortality by 15 -30 %.[2,3] Phytochemicals are natural, bioactive, plant products which provide health for human beings, protection to plants cells from disease, damage, stress, drought, light exposure and pathogenic attack and contribute their color, aroma and flavor. Their dietary intake as neutraceuticals is significant. Among 4,000 characterized phytochemicals, about 150 chemical compounds have been studied in detail. These phytoconstituents are present in fruits, vegetables, legumes, whole grains, nuts, seeds, fungi, herbs and spices.[4] They accumulate in different parts of the plants, such as in the roots, stems, leaves, flowers, fruits or seeds. The pigment molecules are often concentrated in the outer layers of the various plant tissues. These compounds are known as secondary plant metabolites and have biological properties such as antioxidant activity, antimicrobial effect, modulation of detoxification enzymes, stimulation of the immune system, decrease of platelet aggregation and modulation of hormone metabolism and anticancer property.[5,6] Phytochemicals are essential nutrients required by the human body for sustaining life and they prevent common diseases.[7]

Callistemon lanceolatus (Sm.) Sweet, syn. *C. citrinus* (Curtis) Skeels, *C. flavescens* Regel, *C. lilacinus* Cheel, *C. longifolius* (Dum.Cours.) auct., *C. pendulus* Regel, *Metrosideros lanceolata* Sm. (family Myrtaceae), known as cheel, lemon bottle brush and red bottle brush, is originally native to Australia and widely planted as an ornamental plant all over the world including India. It is a medium sized evergreen, around 8 m tall tree; leaves simple, alternate, narrow, lanceolate,

with prominent vein, midrib and oil gland, arranged spirally along loose hanging stems; bark dark grey, fissured; flowers are glowing red born in circular manner along these stems. Each flower head produces a profusion of triple-celled seed capsules around a stem.[8] The leaves are used as a tea substitute with a delightfully refreshing flavor. Egyptians utilize the plant volatile oils as antimicrobial and insecticidal agents and for the treatment of bronchitis and cough.[9,10]

The leaves of *C. lanceolatus* contained neolignans, named callislignans A and B, C-methyl-flavonoids, flavones, flavonol glycosides, polyphenols, phloroglucinol derivatives, blumenol A, tetratriacontanol, benzoic acid derivatives, 2,6,10-bisabolatriene, β-sitosterol-3-O-β-D-glucoside and pentacyclic triterpenoids.[11-17] The fruits possessed saponins, tannins, carbohydrates, steroids, proteins, amino acids, phenolic compounds and anthraquinone glycosides. [18] The leaf essential oil was mainly composed of 1,8-cineole, *α*-phellandrene, α-pinene, limonene and *α*-terpineol. [19-25] The aerial parts afforded 5,7-dihydroxy-6,8-dimethyl- 4′ -methoxy flavone , 8-(2-hydroxy- propan-2-yl)-5-hydroxy-7-methoxy-6-methyl-4′-methoxy flavone, α-amyrin, β-sitosterol, 3-epiursolic acid acetate, urs-12-en-3β-ol-β-D-glucopyranoside, betulinic acid, oleanolic acid and kaempferol,[26] 8-(1″-hydroxyiso- pranyl)-5,6-dihydroxy-7,4′-dimethoxy flavone, 2,3,4-trihydroxyphenethyl tetracontanoate and 2,3,4-trihydroxyphenethyl tetracontanoate-4-β-xylopyranoside,[25] 1-triacosanol, fatty esters, 4-hydroxyphenethyl carbocerate, 4-hydroxyphenethyl gheddate, urs-12-en-3α-acetoxy-28-oic acid and β-sitosterol 3β-D-glucuronoside.[26] In the present study, plant leaves collected from South Delhi were extracted with methanol. The concentrated methanolic extract was used for the isolation of chemical constituents. Structures of the isolated hytoconstituents were established using detailed spectral studies.

MATERIALS AND METHODS

The protocols of all methodologies (procedures, experimental designs and analysis assays) were adopted from the earlier published work.[27-29]

General procedures: The melting points were determined in one end open capillary tubes on a melting point M-560 apparatus (Perfit, India) heated thermoelectrically. UV spectra were determined with Lambda Bio 20 spectrophotometer (Perkin Elmer, Schwerzenbach, Switzerland) in methanol. The IR spectra were recorded by using KBr pellets with Jasco FT/IR-5000 Spectrometer (FTS 135, Hong Kong). The ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR spectra were recorded on Bruker DRX Spectrometer (Rheinstetten, 2 Germany) using $CDCl_3$ as a solvent and TMS (Fluka analytical, Sigma-Aldrich, Netherland) as an internal standard. Mass spectra were recorded on a Jeol JMS-D 300 instrument using Argon/Xenon gas as the FAB. Petroleum ether, chloroform, methanol and other solvents of analytical grade were purchased from E. Merck(India) Ltd , New Delhi. Silica gel with 60-120 mesh particle size was procured from Qualigens, Mumbai, India) and used for column chromatography. The purity of the isolated compounds was checked on precoated TLC plates with Silica gel 60 F_{254} (Merck, 0.25 mm) and the spots were visualized by exposure to iodine vapors or under UV radiations and spraying with ceric sulfate solution.

Plant material: The leaves of *C. lanceolatus* were collected from the Jahan Panah forest garden, New Delhi and authenticated by Prof. M. P. Sharma, Taxonomist, Department of Botany, Jamia Hamdard, New Delhi. A voucher specimen of the leaves is preserved in the herbarium of the Department of Pharmacognosy and Phytochemistry, Jamia Hamdard, New Delhi.

Extraction and isolation: The air dried leaf powder of *C. lanceolatus* (2 kg) was extracted with methanol in a Soxhlet apparatus. The alcoholic extract was concentrated under reduced pressure to yield a dark brown viscous mass (425 g). Small portion of each extract was analyzed chemically to determine the presence of different chemical constituents. A slurry of silica gel (60-120 mesh) was prepared by adsorbing the dried extract (200 g) in a small amount of methanol. It was dried in air and chromatographed over silica gel columns (1.6 m x 16 mm x 2 mm) packed in petroleum ether. Various solvent mixtures of increasing polarity, viz., petroleum ether, petroleum ether - chloroform (9:1, 3:1, 1:1, 1:3, *v/v*), chloroform, chloroform - methanol (19.9: 0.1; 99: 1; 97: 3; 19: 1; 93: 7; 9: 1; 17: 3; 4:1; 3: 1; 3: 2; 2: 3, v/v) and methanol were used to eluted the column. The 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 recrystallized to get the following pure compounds:

4-Methoxyphenyl 4′-octanyl ether (1)

Elution of the column with petroleum ether – chloroform (1:1) gave pale yellow crystals of **1**, recrystallized from chloroform-methanol (1:1), yield 214 mg; m. p. 127 - 128 °C, R_f : 0.33 (*n*-hexane - ethyl acetate, 7:3); UV λmax (MeOH): 279 nm; IR γ max (KBr) : 2917, 2841, 1646, 1525, 1493, 1376, 1134, 1063, 945, 826 cm^{-1}; ^{1}H NMR ($CDCl_3$) : δ 7.87 (2H, d, J = 8.4 Hz, H-3, H-5), 7.03 (2H, d, J = 8.4 Hz, H-2, H-6), 3.93 (1H, m, $w_{1/2}$ = 15.2 Hz, H-4′), 3.89 (3H,brs, OMe), 2.01 (2H, m, H_2-3′), 1.97 (2H, m, H_2-5′), 1.25 (2H, m, H_2-2′), 1.23 (4 H, m, H_2-6′, H_2-7′), 0.96 (3H, t, J = 6.5 Hz, Me-1′), 0.87 (3H, t, J = 6.3 Hz, Me-8′); ^{13}C NMR ($CDCl_3$) : δ 162.51 (C-4), 153.17 (C-1), 130.03 (C-3), 127.96 (C-5), 114.51 (C-2), 114.47 (C-6), 89.34 (C-4′), 55.52 (OMe), 34.37 (C-3′, C-5′), 29.71 (C-6′), 29.47 (C-2′, C-7′), 14.13 (C-1′), 7.29 (C-8′); +ve FAB MS *m/*z (rel. int.): 236 $[M]^+$ ($C_{15}H_{24}O_2$) (4.9), 123 (6.2), 108 (26.3).

8-Ethyl-4′-methoxyapigenin (2)

Elution of column with chloroform afforded a pale yellow amorphous mass of compound **2**, recrystallized from acetone, yield 123 mg, m. p. 225- 226 °C; R_f : 0.47 (*n*-hexane - ethyl acetate, 3:2); UV λmax (MeOH): 267, 324 nm; IR γ_{max} (KBr): 3510, 2925, 2846, 1698, 1517, 1463, 1035, 828 cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.88 (1H, d, J = 8.8 Hz, H-2′), 7.85 (1H, d, J = 8.8 Hz, H-6′), 7.25 (2H, d, J = 8.8 Hz, H-3′, H-5′), 7.04 (1H, s, H-6), 6.59 (1H, s, H-3), 3.90 (3H, brs, OMe), 2.36 (1H, m, H_2-1″a), 2.18 (1H, m, H_2-1″b), 1.25 (3H, t, J = 6.3 Hz, Me-2″); ^{13}C NMR ($CDCl_3$): δ 161.88 (C-2), 102.86 (C-3), 181.93 (C-4), 159.56 (C-5), 101.56 (C-6), 162.61 (C-7), 109.53 (C-8), 156.03 (C-9), 103.57 (C-10), 123.21 (C-1′), 114.15 (C-2′), 127.56 (C-3′), 152.31 (C-4′), 114.15 (C-5′), 106.81 (C-6′), 28.98 (C-1″), 8.06 (C-2″), 55.10 (OMe); +ve ion FAB MS *m*/z (rel. int.): 312 $[M]^+$ ($C_{18}H_{16}O_5$) (20.7).

5,7,4′-Trimethoxy-6-methyl-8-isopropanolyl apigenin (3)

Further elution of the column with chloroform furnished yellow crystals of **3**, recrystallized from acetone, yield 108 mg, m. p. 132 – 134 °C; R_f : 0.90 (*n*-hexane - ethyl acetate, 7:3); UV λmax (MeOH): 278, 321 nm; IR γ_{max} (KBr): 3508, 2927, 2842, 1703, 1657, 1507, 1465, 1258, 1013 cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.79 (1H, d, J = 9.3 Hz, H-3′), 7.75 (1H, d, J = 8.7 Hz, H-5′), 6.96 (1H, d, J = 9.3 Hz, H-2′), 6.92 (1H, d, J = 8.7 Hz, H-6′), 6.53 (1H, s, H-3), 3.83 (3H, brs, OMe), 3.81 (3H, brs, OMe), 3.72 (3H, brs, OMe), 2.31 (3H, brs, Me-1″), 2.13 (3H, brs, Me-2‴), 2.03 (3H, brs, Me-3‴); ^{13}C NMR ($CDCl_3$): δ 162.33 (C-2), 103.01 (C-3), 182.21 (C-4), 161.55 (C-5), 107.99 (C-6), 162.81 (C-7), 109.76 (C-8), 157.42 (C-9), 104.27 (C-10), 122.81 (C-1′), 126.94 (C-2′), 113.52 (C-3′), 154.89 (C-4′), 113.71 (C-5′), 122.69 (C-6′), 7.54 (C-1″), 88.29 (C-1‴), 7.24 (C-2‴), 6.25 (C-3‴), 59.48 (OMe), 54.81 (OMe), 54.49 (OMe); +ve ion FAB MS *m*/z (rel. int.): 384 $[M]^+$ ($C_{22}H_{24}O_6$) (1.7), 369 (25.3), 353 (11.9), 325 (18.5), 277 (63.1), 249 (25.3), 135 (8.2), 107 (31.8).

1-Heptacosanol (4)

Further elution of the column with chloroform yielded colourless flakes of **4**, recrystallized from chloroform-methanol (1:1), yield 109 mg; R_f: 0.33 (chloroform), m. p. 80 - 81°C; UV λmax (MeOH): 203 nm; IR υ_{max} (KBr): 3410, 2911, 2851, 1462, 1372, 1061, 721 cm^{-1}; ^1H NMR ($CDCl_3$): δ 3.65 (2H, t, J = 6.8 Hz, H_2-1), 1.57 (2H, m, CH_2), 1.53 (2H, m, CH_2), 1.28 (8H, brs, 4 × CH_2), 1.25 (38H, brs, 19 × CH_2), 0.87 (3H, t, J = 6.5 Hz, Me-27); ^{13}C NMR ($CDCl_3$): δ 63.13 (C-1), 32.84 (C-2), 31.94 (C-3), 29.71 (20 × CH_2), 29.37 (CH_2), 25.75 (C- 25), 22.76 (C-26), 14.18 (Me-27); +ve FAB MS *m/z* (rel. int.): 396 $[M]^+$ ($C_{27}H_{56}O$) (26.2).

1-Octacosanol (5)

Elution of the column with chloroform-methanol (99:1) yielded colourless crystals of **5**, recrystallized from chloroform-methanol (9:1), yield 105 mg; R_f : 0.71 (petroleum ether – chloroform - methanol; 2:7:1); m. p. 82 - 83 °C; UV λ max (MeOH): 205 nm (log ε 5.3); IR υmax (KBr): 3310, 2921, 2848, 1463, 1373, 1061, 722 cm^{-1} ; ^1H NMR ($CDCl_3$): δ 3.64 (2H, t, J = 5.1 Hz, H_2-1), 2.03 (2H, m, CH_2), 1.83 (2H, m, CH_2), 1.57 (2H, m, CH_2), 1.25 (46H, brs, 23 × CH_2), 0.87 (3H, t, J = 6.2 Hz, Me-28); ^{13}C NMR ($CDCl_3$): δ 61.90 (C-1), 53.21 (C-2), 40.62 (C-3), 32.91 (C-4), 31.80 (C-5), 29.55 (19 × CH_2), 29.22 (C-25), 25.90 (C-26), 22.57 (C-27), 14.16 (Me-28); +ve FAB MS *m/z* (rel. int.): 410 $[M]^+$ ($C_{28}H_{58}O$) (48.6).

Callistelanostanediolide (6)

Further elution of the column with chloroform - methanol (99 : 1) afforded a colourless amorphous powder of **6**, recrystallized from ethyl acetate, yield 97 mg, m. p. 208 - 210 ° C; UV λ max (MeOH): 209 nm (log ε 3.7); IR υmax (KBr): 2932, 2871, 1725, 1636, 1458, 1381, 1234, 1188, 1034, 879 cm^{-1}; ^1H NMR ($CDCl_3$): δ 4.48 (1H, d, J = 5.6, 8.9 Hz, H-3α), 4.02 (1H, d, J = 7.2 Hz, H_2- 21a), 3.97 (1H, d, J = 7.2 Hz, H-21b), 1.19 (3H, brs, Me-29), 0.89 (3H, d, J = 6.5 Hz, Me- 26), 0.84 (3H, d, J = 6.1 Hz, Me-27), 0.74 (3H, brs, Me-28), 0.68 (3H, brs, Me-30), 2.08 - 1.23 (28H, m, 11 x CH_2, 6 x CH); ^{13}C NMR ($CDCI_3$): δ 35.78 (C-1), 28.17 (C-2), 81.43 (C-3), 39.79 (C-4), 50.46 (C-5), 37.23 (C-6), 28.74 (C-7), 38.29 (C-8), 49.82 (C-9), 37.46 (C-10), 22.47 (C-11), 34.08 (C-12), 47.72 (C-13), 48.45 (C-14), 31.89 (C-15), 32.45 (C-16), 52.18 (C-17), 173.46 (C-18), 169.74 (C-19), 36.54 (C-20), 65.83 (C-21), 38.14 (C-22), 25.01 (C-23), 30.39 (C-24), 31.28 (C-25), 27.40 (C-26), 26.07 (C-27), 24.85 (C-28), 26.72 (C-29), 16.41 (C-30); +ve ion FAB MS *m*/z (rel.int.): 471 $[M+H]^+$ ($C_{30}H_{47}O_4$) (19.3), 455 (22.5), 439 (25.1), 236 (12.6), 234 (18.4), 152 (9.3).

4′-Methoxy-8-(2″-propanolyl) apigenin (7)

Elution of the column with chloroform - methanol (48:1) produced a pale yellow amorphous powder of **7**, recrystallized from ethyl acetate, yield 123 mg, m. p. 144 -145 °C; R_f 0.80 (*n*-hexane - ethyl acetate, 3:2); UV λ max (MeOH): 269, 335 nm; IR γmax (KBr): 3510, 3479, 2925, 2847, 1698, 1635, 1541, 1417, 1339, 1176, 1042 cm^{-1}; ^1H NMR ($CDCl_3$) : δ 7.84 (1H, dd, J = 2.7, 8.8 Hz, H-3′), 7.82 (1H, dd, J = 2.7, 8.8 Hz, H-5′), 7.25 (1H, s, H-6), 7.02 (1H, dd, J = 2.9, 8.8 Hz, H-2′), 6.99 (1H, dd, J = 2.9, 8.8 Hz, H-6′), 6.61 (1H, s, H-3), 3.89 (3H, brs, OMe), 3.80 (1H, m, $w_{1/2}$ = 8.2 Hz, H-2″), 2.39 (1H, d, J = 6.9 Hz, H_2-1″a), 2.21 (1H, d, J = 6.9 Hz, H_2-1″b), 1.49 (3H, d, J = 7.1 Hz, H_3-3″); ^{13}C NMR ($CDCl_3$) : δ 163.37 (C-2), 104.46 (C-3), 182.40 (C-4), 162.46 (C-5), 104.01 (C-6), 163.85 (C-7), 114.10 (C-8), 158.45 (C-9), 105.36 (C-10), 123.71 (C-1′), 127.98 (C-2′), 114.56 (C-3′), 152.98 (C-4′), 114.45 (C-5′), 109.03 (C-6′), 34.16 (C-1″), 89.33 (C-2″), 7.29 (C-3″), 55.53 (OMe); +ve FAB MS *m*/z (rel. int.): 342 $[M]^+$ ($C_{19}H_{18}O_6$) (3.8).

4-Hydroxyphenyl propanoloxycerotate (8)

Further elution of the column with chloroform - methanol (48:1) gave colourless granules of **8**, recrystallized from ethyl acetate, yield 152 mg; m. p. 87 -

88 ^{o} C; R_f 0.84 (*n*-hexane - ethyl acetate, 1:1), UV λ max (MeOH): 267 nm; IR υmax (KBr): 3450, 2928, 2846, 1725, 1647, 1507, 1457, 1360, 1256, 925, 720 cm^{-1}; ^{1}H NMR ($CDCl_3$) : δ 7.07 (2H, d, J = 8.6 Hz, H-3, H-5), 6.85 (2H, d, J = 8.6 Hz, H-2, H-6), 4.21 (2H, t, J = 6.7 Hz, H_2-9), 2.87 (2H, t, J = 7.0 Hz, H_2-7), 2.27 (2H, t, J = 7.5 Hz, H_2-2′), 2.24 (2H, m, H_2-8), 1.41 (2H, m, CH_2), 1.28 (8H, brs, 4 x CH_2), 1.24 (36 H, brs, 18 × CH_2), 0.87 (3H, t, J = 6.5 Hz, Me-26′); ^{13}C NMR ($CDCl_3$) : δ 171.63 (C-1′), 159.77 (C-4), 138.37 (C-1), 129.89 (C-3, C-5), 115.47 (C-2, C-6), 64.95 (C-9), 34.52 (C-7), 34.36 (C-8), 31.48 (CH_2), 29.90 (19 x CH_2), 29.49 (CH_2), 29.31 (CH_2), 28.21 (CH_2), 22.53 (CH_2), 14.16 (Me-26′); +ve FAB MS *m/z* (rel. int.) : 530 $[M]^+$ ($C_{35}H_{62}O_3$) (91.8), 395 (11.6), 379 (18.3), 151 (6.1).

Tyrosolyl gheddate (9)

Elution of the column with chloroform-methanol (97 : 3) yielded lustrous silver granules of **9**, recrystallized from ethyl acetate, yield 145 mg; m. p. 87-88 ºC; UV λmax (MeOH): 277 nm; R_f 0.83 (*n*-hexane-ethyl acetate, 4 : 1); IR γmax (KBr): 3503, 2927, 2851, 1735, 1647, 1542, 1456, 1351, 1263, 982, 723 cm^{-1}; ^{1}H NMR ($CDCl_3$) : δ 10.89 (1H, s, 4-OH), 7.09 (2H, d, J = 8.4 Hz, H-3, H-5), 6.77 (2H, d, J = 8.4 Hz, H-2, H-6), 4.25 (2H, t, J = 7.1 Hz, H_2-8), 2.87 (2H, t, J = 7.5 Hz, H_2-7), 2.29 (2H, t, J = 7.4 Hz, H_2-2′), 1.57 (2H, m, CH_2), 1.29 (4H, m, 2 x CH_2), 1.25 (36 H, brs, 18 × CH_2), 1.23 (24 H, brs, 12 × CH_2), 0.87 (3H, t, J = 6.5 Hz, Me-34′); ^{13}C NMR ($CDCl_3$) : δ 171.82 (C-1′), 159.83 (C-4), 138.45 (C-1), 130.08 (C-3, C-5), 115.37 (C-2, C-6), 64.97 (C-8), 34.54 (C-7), 34.42 (C-2′), 31.92 (CH_2), 29.74 (23 x CH_2), 29.65 (CH_2), 29.41 (CH_2), 29.39 (CH_2), 29.31 (CH_2), 29.18 (CH_2), 24.93 (CH_2), 22.67 (C-33), 14.18 (Me-34′); +ve FAB MS *m/z* (rel. int.) : 628 $[M]^+$ ($C_{42}H_{76}O_3$) (97.8), 507 (9.8), 491 (22.5), 137 15.6).

4-Hydroxyphenyl propanyloxygheddate (10)

Further elution of the column with chloroform-methanol (97:3) produced lustrous silver granules of **10**, recrystallized from chloroform-methanol (9:1), yield 211 mg; m. p. 101 - 103 ºC; UV λmax (MeOH): 276 nm; R_f 0.85 (*n*-hexane - ethyl acetate, 4 : 1); IR γmax (KBr): 3515, 2915, 2842, 1733, 1650, 1522, 1455, 1356, 1261, 971, 721 cm^{-1}; ^{1}H NMR ($CDCl_3$) : δ 10.78 (1H, s, 4-OH), 7.11 (2H, d, J = 8.4 Hz, H-3, H-5), 6.79 (2H, d, J = 8.4 Hz, H-2, H-6), 4.23 (2H, t, J = 7.1 Hz, H_2-9), 2.86 (2H, t, J = 7.3 Hz, H_2-7), 2.27 (2H, t, J = 7.4 Hz, H_2-2′), 1.57 (2H, m, CH_2), 1.27 (16 H, m, 8 x CH_2), 1.22 (44 H, brs, 22 × CH_2), 0.87 (3H, t, J = 6.9 Hz, Me-34′); ^{13}C NMR ($CDCl_3$) : δ 171.25 (C-1′), 159.61 (C-4), 137.86 (C-1), 129.75 (C-3, C-5), 115.18 (C-2, C-6), 65.01 (C-9), 34.54 (C-7), 34.22 (C-7), 31.83 (CH_2), 29.59 (25 x CH_2), 29.52 (CH_2), 29.37 (CH_2), 29.26 (CH_2), 29.13 (CH_2), 29.05 (CH_2), 24.87 (CH_2), 22.61 (C-33), 14.16 (Me-34′); +ve FAB MS *m/z* (rel. int.) : 642 $[M]^+$ ($C_{43}H_{78}O_3$) (6.2), 507 (3.8), 491 (17.6), 151 (8.7).

α-Amyrin (11)

Elution of the column with chloroform – methanol (19 : 1) provided colourless needles of **11**, recrystallized from chloroform-methanol (9:1), yield 236 mg, m. p. 185-187 ° C, R_f : 0.82 (*n*-hexane - ethyl acetate, 3 : 2); UV λmax (MeOH): 205 nm (log ε 4.1); IR γmax (KBr) : 3410, 2922, 2845, 1652, 1457, 1365, 1260, 1022, 991 cm^{-1}; ^{1}H NMR ($CDCl_3$) : δ 5.31 (1H, m, H-12), 3.96 (1H, dd, J = 5.2, 9.1 Hz, H-3α), 2.19 (1H, d, J = 5.3 Hz, H-18β), 2.18-1.29 (23H, m, 9 x CH_2; 5 x CH), 1.01 (3H, brs, Me-23), 0.94 (3H, brs, Me-25), 0.90 (3H, brs, Me-26), 0.90 (3H, brs, Me-26), 0.87 (3H, brs, Me-27), 0.80 (3H, d, J = 6.5 Hz, Me-29), 0.72 (3H, d, J = 6.3 Hz, Me-30), 0.66 (3H, brs, Me-24); ^{13}C NMR ($CDCl_3$) : δ 39.06 (C-1), 35.67 (C-2), 77.57 (C-3), 41.28 (C-4), 55.69 (C-5), 18.23 (C-6), 32.87 (C-7), 39.40 (C-8), 47.42 (C-9), 37.75 (C-10), 23.42 (C-11), 121.65 (C-12), 141.26 (C-13), 39.46 (C-14), 25.85 (C-15), 27.40 (C-16), 50.49 (C-17), 55.22 (C-18), 39.31 (C-19), 40.37 (C-20), 30.81 (C-21), 38.95 (C-22), 28.51 (C-23), 14.86 (C-24), 16.23 (C-25), 16.86 (C-26), 23.42 (C-27), 15.82 (C-28), 27.60 (C-29), 21.64 (C-30); +ve FAB MS *m/z* (rel. int.): 426 $[M]^+$ ($C_{30}H_{50}O$) (10.4).

RESULTS AND DISCUSSION

Compound **1** was obtained as a pale yellow crystalline mass from petroleum ether – chloroform (1:1) eluants. Compounds **2**, **3** and **4** were isolated as a pale yellow amorphous mass, yellow crystals and colourless flakes, respectively, when the column was eluted with chloroform. Colourless compounds **5** and **6** were separated with chloroform-methanol (99:1) eluants of the column. Increment of polarity of the column eluants to chloroform - methanol (48:1) produced a pale yellow amorphous powder of **7** and granules of **8**. Lustrous silver granules of **9** and **10** were secured with chloroform-methanol (97 : 3) eluants of the column. Elution of the column with chloroform – methanol (19 : 1) provided colourless needles of **11**. The chemical constituents **1**, **4**, **5**, **10** and **11** were recrystallized from various combinations of chloroform – methanol mixtures. Acetone was used to recrystallize the apigenin derivatives **2** and **3**. The isolates **6** to **9** were purified by recrystallization with ethyl acetate. The purity of each phytoconstituent was checked by silica gel TLC developed in a suitable solvent system. All NMR measurements were carried out on a Bruker DRX spectrometer (Rheinstetten, 2 Germany) using $CDCl_3$ as a solvent. Two higher aliphatic alcohols **4** and **5**, a lanostane-type triterpene **6** and a pentacyclic triterpene **11** (α-amyrin) were also isolated from the methanolic extract of the leaves of *C. lanceolatus* (Fig. 1).

The majority of the isolated phytoconstituents **1**, **2**, **3**, **7**, **8**, **9** and **10** were the aromatic compounds and their UV absorption maxima were in the range of 267 – 335 nm. The compounds **2**, **3** and **7** were the flavone derivatives and their UV spectra showed a prominent absorption maxima near 270 nm (band I) and another small absorption maxima near 330 nm (band II) characteristic of flavone-type flavonoids. Compounds 4 and 5 were the aliphatic higher alcohols and their UV absorption maxima near 205 nm indicated the absence of conjugated

vinylic linkage or their saturated nature. Two compounds **6** and **11** were the triterpenic constituents devoid of conjugated double bonds.

Compound **1** exhibited UV absorption maximum at 279 nm for an aromatic compound and IR absorption bands for aromatic ring (1646, 1525, 1063 cm^{-1}). Its mass spectrum displayed a molecular ion peak at *m/*z 236 consistent with a molecular formula of an alkyl phenyl ether, $C_{15}H_{24}O_2$. The ion fragments arising at *m/*z 123 [$C_{4'}$ - O fission, MeO-C_6H_4-O]$^+$ and 108 [123 – Me]$^+$ indicated that 4-methoxyphenol was involved to form the ether with 4-octanol. The ^{1}H NMR spectrum of **1** showed two doublets at δ 7.87 (J = 8.4 Hz) and δ 7.03 (J = 8.4 Hz) integrating for two-protons each assigned to aromatic H-3, H-5 and H-2, H-6 protons, respectively, a one-proton multiplet at δ 3.93 with half-width of 15.2 Hz ascribed to α-oriented oxymethine H-4′ proton, a three-proton singlet attributed to the methoxy protons, three two-proton multiplets at δ 2.01 (H_2-3′), 1.97 (H_2-5′) and 1.25 (H_2-2′) and a four-proton multiplet at δ 1.23 (H_2-6′, H_2-7′) accounted to methylene protons and two three-proton triplets at δ 0.96 (J = 6.5 Hz) and 0.87 (J = 6.3 Hz) associated correspondingly with primary C-1′ and C-8′ methyl protons. The ^{13}C NMR spectrum of **1** displayed signals for aromatic carbons between δ 162.51 – 114.47, oxymethine carbon at δ 89.34 (C-4′), methoxy carbon at δ 55.52, methylene carbons from δ 34.37 to 29.47 and methyl carbons at δ 14.13 (C-1′) and 7.29 (C-8′). On the basis of these evidences, the structure of **1** has been formulated as 4-methoxyphenyl 4′-octanyl ether, a new aromatic ether (Fig. 1). This is the first report of isolation of an aromatic ether from *C. lanceolatus.*

Compound **2,** named 8-ethyl-4′-methoxyapigenin, responded Shinoda test for flavonoids positively, showed UV absorption maxima at 267 and 324 nm and IR absorption bands for hydroxyl groups (3510 cm^{-1}), carbonyl function (1698 cm^{-1}) and aromaticity (1517, 1035 cm^{-1}) suggesting flavone-type skeleton. Shifting of UV absorption band I to + 48 nm with sodium methoxide indicated the presence of a free hydroxy group. There was a shift of bands with sodium acetate solution supporting free nature of the 7-hydroxyl group. There was no significant shift in band I with sodium acetate and boric acid ruling out the existence of B-ring dihydroxy groups. Measuring of UV spectrum with aluminum chloride displayed no shift of band I suggesting the absence of free 4′-hydroxyl group.[30-32] On the basis of its mass and ^{13}C NMR spectra the molecular ion peak of **2** was determined at *m/*z 312 consistent with the molecular formula of an alkyl flavone, $C_{18}H_{16}O_5$. The ^{1}H NMR spectrum of **2** showed two one-proton doublets at δ 7.88 (J = 8.8 Hz) and 7.85 (J = 8.8 Hz) and a two-proton doublet at δ 7.25 (J = 8.8 Hz) assigned to B-ring H-2′, H-6′, H-3′ and H-5′ protons, respectively, and two one-proton singlets at δ 7.04 and 6.59 ascribed correspondingly to flavone H-6 and H-3 protons. Two one-proton multiplets at δ 2.36 and 2.18 were accounted to methylene H_2-1″ attached to the aromatic ring. A three-proton triplet at δ 1.25 (J = 6.3 Hz) was attributed to primary C-2″ methyl protons. A three-proton singlet at δ 3.90 was due to the methoxy protons. The ^{13}C NMR spectrum of **2** exhibited signals for carbonyl carbon at δ 181.93 (C-4) and methine carbon at δ 102.86 (C-3) supporting the flavone-type carbon framework of the molecule, other flavone carbons between δ 161.88 - 101.56, ethyl carbons at δ 28.98 (C-1″) and 8.06 (C-2″) and methoxy carbon at δ 55.10. The absence of C-8 carbon signal near δ 94.2 indicated the attachment of the ethyl group at C-8 (22). These evidences led to established the structure of **2** as 5,7-dihydroxy-4′-methoxy-8-ethyl flavone (8-ethyl 4′-methoxyapigenin), a new flavone derivative from a plant source (Fig. 1). The flavones have been earlier reported from the aerial parts of *C. lanceolatus.*[24.25]

Compound **3** was isolated as a yellow crystalline mass, gave positive Shinoda test for flavonoids and showed UV absorption maxima at 278 and 321 nm typical of substituted flavones [30-32]. The IR spectrum of **3** exhibited absorption bands for a hydroxyl group (3508 cm^{-1}), carbonyl function (1703 cm^{-1}) and aromatic rings (1657, 1507, 1013 cm^{-1}). The +ve FAB mass spectrum had a molecular ion peak at *m/*z 384, supported with ^{13}C NMR spectrum, consistent with the molecular formula of a flavone, $C_{22}H_{24}O_6$. The ion peaks generating at *m/*z 369 [M- Me]$^+$, 353 [M - OMe]$^+$ and 325 [M – $CH_3CH(OH)CH_3$]$^+$indicated that methyl, methoxy and propanolyl groups were attached to the flavonoid skeleton. The ion fragments arising at *m*/z 277, 107 [C_2 – O, C_3- C_4fission]$^+$ and 249, 135 [C_2 – O, C_4 – C_{10} fission]$^+$ supported that one of the methoxy group was located in ring B and one methyl, two methoxy and a propanolyl functions were present in the ring A of the flavone moiety. The ^{1}H NMR spectrum of **3** showed the presence of four one-proton doublets at δ 7.79 (J = 9.3 Hz, H-3′), 7.75 (J = 8.7 Hz, H-5′), 6.96 (J = 9.3 Hz, H-2′), 6.92 (J = 8.7 Hz, H-6′) and a one-proton singlet at δ 6.53 assigned to aromatic H-3′, H-5′, H-2′ and H-6′ and flavone H-3 protons, respectively. Three broad singlets at δ 3.83, 3.81 and 3.72 integrating for three-protons each were associated with the methoxy protons. Three three-proton broad singlets at δ 2.31, 2.13 and 2.03 were due to C-1″ methyl protons attached to aromatic C-6 position, and C-2‴ and C-3‴ tertiary methyl protons located at C-1‴ carbon of the isopropanolyl unit. The ^{13}C NMR spectrum of **3** displayed signals for the carbonyl carbon at δ 182.21 (C-4), oxycarbon at δ 162.33 (C-2) and methine carbon at δ 103.01 (C-3) suggesting flavone nature of the molecule, hydroxycarbon at δ 88.29 (C-1‴), methoxy carbons between δ 59.48 – 54.49 and methyl carbons at δ 7.54 (C-1″), 7.24 (C-2‴) and 6.25 (C-3‴). The absence of C-8 carbon signal near δ 94.2 indicated the attachment of the isopropanolyl group at C-8. These data led to establish the structure of **3** as 5,7,4′-trimethoxy-6-methyl-8-isopropanolyl apigenin, a new apigenin derivative (Fig. 1). The flavones have been reported previously from the aerial parts of *C. lanceolatus.*[24,25]

Compounds **4** and **5** were the known aliphatic alcohols identified as 1-heptacosanol [33-35] and 1-octacosanol.[36,37]

Compound **6**, named callistelanostanediolide, showed IR absorption bands for lactone rings (1725 cm^{-1}). Its molecular ion peak was determined at *m/*z 471 $[M+H]^+$ on the basis of FAB mass and ^{13}C NMR spectra relating to a lanostane-type triterpenic dilactone, $C_{30}H_{47}O_4$. The ion fragments generating at *m/*z 152 [$C_{5,6} - C_{9,10}$ fission, $C_9H_{12}O_2]^+$, 234 [$C_{8,14} - C_{12,13}$ fission, $C_{155}H_{22}O_2$]$^+$, 236 [M - 234]$^+$, 455 [M – Me]$^+$ and 439 [455 – Me]$^+$ suggested the presence of one of the lactone ring in ring A and another lactone between C-18 and C-21 positions. The ^1H NMR spectrum of **6** exhibited a one-proton double doublet at δ 4.48 (J = 5.6, 8.9 Hz) assigned to α-oriented oxymethine H-3, two one-proton doublets at δ 4.02 (J = 7.2 Hz) and 3.97 (J = 7.2 Hz) ascribed to oxymethylene H_2-21 protons, three broad singlets at δ 1.19, 0.74 and 0.68 integrating for three-protons each accounted to tertiary C-29, C-28 and C-30 methyl protons, respectively, and two three-proton doublets at δ 0.89 (J = 6.5 Hz, Me- 26) and 0.84 (J = 6.1 Hz, Me-27) attributed correspondingly to secondary C-26 and C-27 methyl protons of lanostene-type triterpenoids. The remaining methine and methylene protons resonated as multiplets from δ 2.08 to 1.23. The ^{13}C NMR spectrum of **6** displayed signals for lactone carbons at δ 173.46 (C-18) and 169.74 (C-19), oxymethine carbon at δ 81.43 (C-3), oxymethylene carbon at δ 65.83 (C-21) and methyl carbons from δ 27.40 to 16.41. The ^1H and ^{13}C NMR spectral data of the triterpenic unit of **6** were compared with the reported spectral data of lanostene-type triterpenoids.[38-42] On the basis of these evidences the structure of **6** was established as lanostan-3β,19-, 18,21-diolide, a new lanostenic dilactone (Fig. 1).

Compound **7** was obtained as a pale yellow amorphous mass and gave positive Shinoda test for flavonoids. Its UV absorption maxima at 262 and 359 nm were typically of flavones.[30-32] It had IR absorption bands for hydroxyl groups (3510 cm^{-1}), carbonyl function (1698 cm^{-1}) and aromatic rings (1635, 1541, 1042 cm^{-1}). Its mass spectrum displayed a molecular ion peak at *m/*z 342 consistent with molecular formula of a propanolyl flavone $C_{19}H_{18}O_6$ that was supported by its ^{13}C NMR spectral data. The ^1H NMR of **7** showed four one-proton double doublets in the deshielded region at δ 7.84 (J = 2.7, 8.8 Hz), 7.82 (J = 2.7, 8.8 Hz), 7.02 (J = 2.9, 8.8 Hz) and 6.99 (J = 2.9, 8.8 Hz) assigned to meta-, ortho-coupled aromatic H-3′, H-5′, H-2′ and H-6′ protons, respectively, two one-proton singlets at δ 7.25 and 6.61 ascribed correspondingly to H-6 and -3 protons and a three-proton broad singlet at δ 3.89 due to methoxy protons. The signals as a one-proton multiplet at δ 3.80 with half width of 8.2 Hz was due to carbinol H-2″, two one-proton doublets at δ 2.39 (J = 6.9 Hz, H_2-1″a) and 2.21 (J = 6.9 Hz, H_2-1″b) were associated with the methylene protons linked to the aromatic ring and a three-proton doublet at δ 1.49 (J = 7.1 Hz) was accounted to C-3″ primary methyl protons suggesting that an isopropanolyl group was linked to the flavone moiety. The ^{13}C NMR of **7** showed the presence of nineteen carbon signals and the signals at δ 163.37 (C-2), 104.46 (C-3) and 182.40 (C-4) supported the flavone skeleton of the molecule[32]. The signals for methylene carbon at δ 34.16 (C-1″), carbinol carbon at δ 89.33 (C-2″) and methyl carbon at δ 7.29 (C-3″) indicated the attachment of the 2″-propanolyl group to the flavone carbon skeleton. The absence of C-8 carbon signal near δ 94.2 indicated the attachment of the 2″-propanolyl group at C-8.[42] On the basis of these evidences the structure of **7** was characterized as 4′-methoxy-8-(2″-propanolyl) apigenin, a new apigenin derivative (Fig. 1). Earlier the flavones have been reported from the aerial parts of *C. lanceolatus*.[24,25]

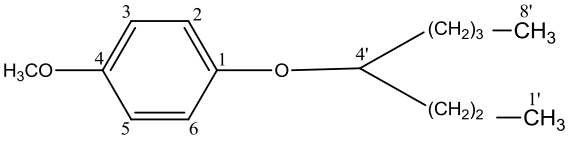
Compound **8** gave positive tests of phenols, showed UV absorption maximum at 267 nm for aromatic ring and IR absorption bands for a hydroxyl group (3450 cm^{-1}), ester function (1725 cm^{-1}), aromatic ring (1647, 1507 cm^{-1}) and long aliphatic chain (720 cm^{-1}). On the basis of mass and ^{13}C NMR spectra its molecular ion peak was determined at *m/*z 530 consistent with a molecular formula of a phenylpropanol ester, $C_{35}H_{62}O_3$. The ion peaks arising at *m/*z 395 [C_9- O fission, CH_3-$(CH_2)_{24}$-CO-O]$^+$, 379 [$C_{1'}$ - O fission, CH_3-$(CH_2)_{24}$-CO]$^+$ and 151 [M - 379, HO-C_6H_4-$(CH_2)_3$-O]$^+$ indicated that hydroxyphenylpropanol was esterified with cerotic acid. The ^1H NMR spectrum of **8** displayed two two-proton doublets at δ 7.07 (J = 8.6 Hz) and 6.85 (J = 8.6 Hz) assigned to aromatic H-3, H-5 and H-2 and H-6 protons, respectively, a two-proton triplet at δ 4.21 (J = 6.7 Hz) ascribed to oxymethylene H_2-9 protons, two two-proton triplets at δ 2.87 (J = 7.0 Hz) and 2.27 (J = 7.5 Hz) attributed to methylene H_2-7 attached to the aromatic ring and H_2-2′ protons adjacent to the ester function, other methylene protons as two-proton multiplets at δ 2.24 and 1.41 and two singlets at δ 1.28 (8H) and 1.24 (36 H) and a three-proton triplet δ 0.87 (J = 6.5 Hz) accounted to terminal C-26′ primary methyl protons. The ^{13}C NMR spectrum of **8** exhibited signals for ester carbon at δ 171.63 (C-1′), aromatic carbons between δ 156.77 – 115.47, oxymethylene carbon at δ 64.95 (C-9), other methylene carbons from δ 34.52 to 22.53 and methyl carbon at δ 14.16 (C-26′). On the basis of the aforementioned spectral data analysis the structure of **8** has been elucidated as 4-hydroxyphenyl propanoloxycerotate, a new aromatic ester (Fig. 1). Earlier the phenolic esters have been reported from the aerial parts of *C. lanceolatus*.[25]

Compound **9**, named tyrosolylgheddate, responded phenolic tests positively, had UV absorption maximum at 277 nm for an aromatic compound and IR absorption bands for hydroxyl group (3503 cm^{-1}), ester function (1735 cm^{-1}), aromatic ring (1647, 1542 cm^{-1}) and a long aliphatic chain (723 cm^{-1}). Its mass spectrum displayed a molecular ion peak at *m/*z 628 consistent with a molecular formula of a fatty acid ester with phenolic alcohol, $C_{42}H_{76}O_3$. The ion peaks arising at *m/*z 507 [C_8-

O fission, $OCO(CH_2)_{32}CH_3]^+$, 491 [$C_{1'}$- O fission, $CO(CH_2)_{32}CH_3]^+$ and 137 [M - 491]$^+$suggested that gheddic acid (tetratriacontanoic acid) was esterified withtyrosol (4-hydroxyphenethyl alcohol). The ^{1}H NMR spectrum of **9** displayed two two-proton doublets δ 7.09 (J = 8.4 Hz) and 6.77 (J = 8.4 Hz) assigned to aromatic H-3, H-5 and H-2, H-6 protons, respectively, three two-proton triplets at δ 4.25 (J = 7.1 Hz), 2.87 (J = 7.5 Hz) and 2.29 (J =7.4 Hz) ascribed correspondingly oxymethylene H_2-8 and methylene H_2-7 linked to the aromatic ring and H_2-2′ protons adjacent to the ester group, other methylene protons as multiplets at δ 1.57 (2H) and 1.29 (4H) and as a broad singlet at δ 1.23 (56 H) and a three-proton triplet at δ 0.87 (J = 6.5 Hz) accounted to terminal primary C-34′ methyl protons. The ^{13}C NMR spectrum of **9** exhibited signals for ester carbon at δ 171.82 (C-1′), aromatic carbons between δ 159.83 - 115.37, oxymethylene carbon at δ 64.97 (C-8), other methylene carbons in the range of δ 34.54 – 22.67 and methyl carbon at δ 14.18 (Me-34′). Acid hydrolysis of **9** yielded 4-hydroxyphenethyl alcohol (tyrosol), m. p. 90 - 92 °C, $[M]^+$*m/z* at 138 ($C_8H_{10}O_2$) and gheddic acid (tetratriacontanoic acid), m. p. 93 - 94 °C; $[M]^+$*m/z* at 508 ($C_{34}H_{68}O_2$). On the basis of spectral data evidences and chemical reactions, the structure of **9** has been elucidated as 4-hydroxyphenethyl tetratriacontanoate, a new aromatic ester (Fig. 1).

Compound **10**, named 4-hydroxyphenyl propanyloxygheddate, responded to phenolic tests positively and showed UV absorption maximum at 276 nm for an aromatic compound and IR absorption bands for a hydroxyl group (3515 cm^{-1}), ester function (1735 cm^{-1}), aromatic ring (1650, 1522 cm^{-1}) and a long aliphatic chain (721 cm^{-1}). Its mass spectrum displayed a molecular ion peak at *m/z* 642 consistent with a molecular formula of a fatty acid ester with an aromatic alcohol, $C_{43}H_{78}O_3$. The ion peaks generating at *m/z* 507 [C_9 - O fission, $OCO(CH_2)_{32}CH_3]^+$, 491 [$C_{1'}$ – O fission, $CO(CH_2)_{32}CH_3]^+$ and 151 [M - 491]$^+$ suggested that gheddic acid (tetratriacontanoic acid) was esterified with 4-hydroxyphenyl propanol. The ^{1}H NMR spectrum of **10** displayed two two-proton doublets δ 7.11 (J = 8.4 Hz) and 6.79 (J = 8.4 Hz) assigned to aromatic H-3, H-5 and H-2, H-6 protons, respectively, three two-proton triplets at δ 4.23 (J = 7.1 Hz), 2.86 (J = 7.3 Hz) and 2.27 (J = 7.4 Hz) ascribed correspondingly oxymethylene H_2-9 and methylene H_2-7 linked to the aromatic ring and H_2-2′ protons adjacent to the ester group, other methylene protons as a two-proton multiplet at δ 1.57 and as broad singlets at δ 1.27 (16H) and 1.22 (44 H) and a three-proton triplet at δ 0.87 (J = 6.9 Hz) accounted to terminal primary C-34′ methyl protons. The ^{13}C NMR spectrum of **10** exhibited signals for the ester carbon at δ 171.25 (C-1′), aromatic carbons between δ 159.61 - 115.18, oxymethylene carbon at δ 65.01 (C-9), other methylene carbons in the range of δ 34.54 – 22.61 and methyl carbon at δ 14.16 (Me-34′). Acid hydrolysis of **10** yielded 3-(4-hydroxyphenyl)-1-propanol, m. p. 51 - 54 ° C; $[M]^+$*m/z* at 152 ($C_9H_{12}O_2$) and gheddic acid (tetratriacontanoic acid), m. p. 93 - 94 ° C; $[M]^+$*m/z* at 508 ($C_{34}H_{68}O_2$). On the basis of these evidences, the structure of **10** was formulated 4-hydroxyphenyl 1-propanyl tetratriacontanoate, a new aromatic ester (Fig. 1).

Compound **11**, $[M]^+$ at *m/z* 426 ($C_{30}H_{50}O$) was characterized as α-amyrin on the basis of spectral data analysis and comparison of the physical parameters with the reported data.[43,44]



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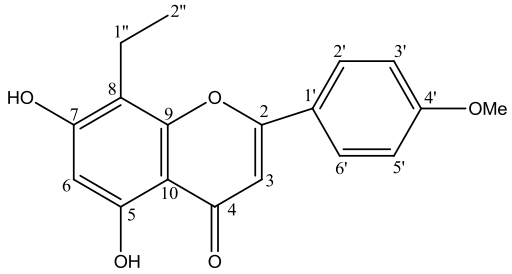
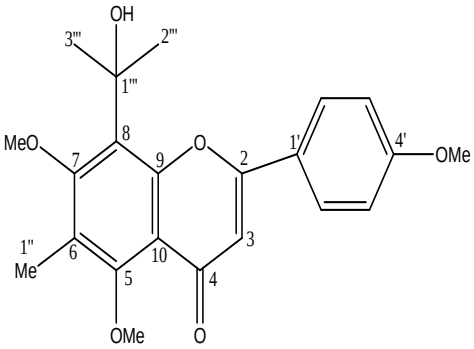
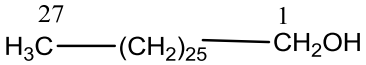


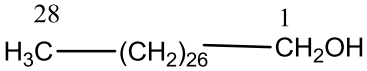
8-Ethyl 4′-methoxy apigenin (2)



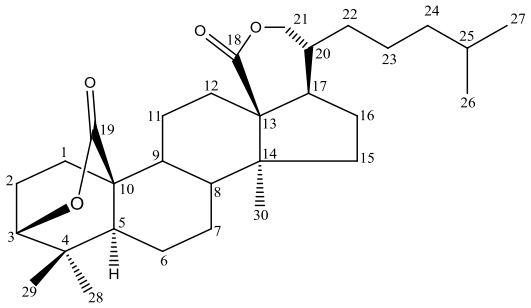
5,7,4′-Trimethoxy-6-methyl-8-isopropanolyl apigenin (3)



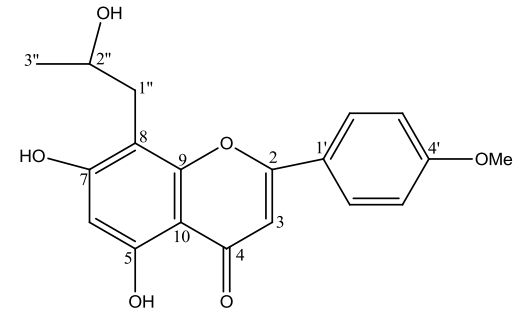
1-Heptacosanol (4)



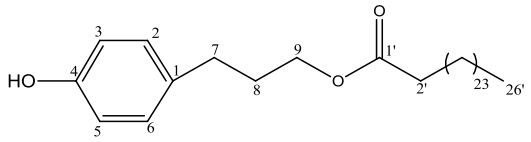
1-Octacosanol (5)



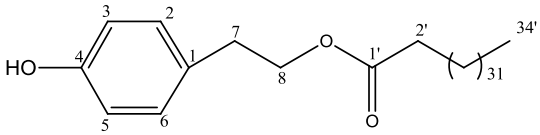
Callistelanostandiolide (6)



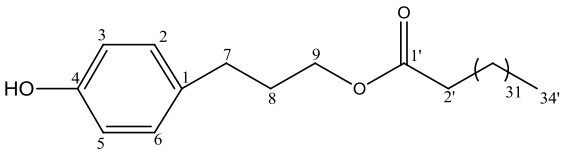
4′-Methoxy-8-(2′′-propanolyl) apigenin (7)



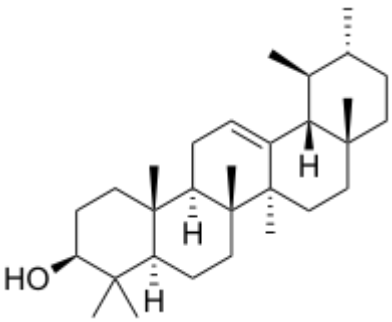
4-Hydroxyphenyl propanyloxycerotate (8)



4-Hydroxyphenethyl gheddate (9)



4-Hydroxyphenyl propanyloxygheddate (10)



α-Amyrin (11)

Figure 1: Chemical constituents 1 – 11 isolated from the leaves of *Callistemon lanceolatus.*

CONCLUSION

Phytochemical investigation of the leaves parts of *Callistemon lanceolatus* led to the isolation of p-methoxyphenyl 4′-octanyl ether (**1**), three apigenin derivatives (**2,3,7**), two higher alcohols (**4** and **5**), a lanostenediolide (**6**), three 4-hydroxyphenyl alkyl esters (**8** – **10**) and α-amyrin (**11**). This study discovers the isolation of eight new phytoconstituents including one aromatic ether (**1**), three apigenin derivatives (**2**, **3** and **7**), a lanostenic dilactone (**6**) and three aromatic esters (**8**, **9** and **10**) from the leaves of *C. lanceolatus.* This work has enhanced understanding about the phytoconstituents of the undertaken plant. These secondary metabolites can be used as analytical markers for quality control of the *C. lanceolatus*. All these phytoconstituents are reported for the first time from this plant and can be used for quality control of the plant.

ACKNOWLEDGEMENTS

The authors are thankful to the Heads, Advanced Instrumentation Research Facility, Jawaharlal Nehru University, New Delhi and Saphosticated Instrumentation Analytical Facility, Central Drug Research Institute, Lucknow for recording spectral data of the compounds.

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