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Isolation of Apigenin Derivatives from the Leaves of *Chorisia speciosa, Cordia dichotoma, Mentha piperita* and Roots of *Pluchea lanceolata*

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ABSTRACT

Apigenin (4′,5,7-trihydroxyflavone) is a plant-derived skin cancer chemopreventive flavonoid that has anti-allergic, anti-depressant, anti-inflammatory, antimetastatic, antioxidant and anti-cancer properties. Our study was planned to isolate the apigenin derivatives from the leaves of *Chorisia speciosa* A. St. Hil.(Bombacaceae, Malvaceae), *Cordia dichotoma* G. Forst. (Boraginaceae) and *Mentha piperita* L. (Lamiaceae) and the roots of *Pluchea lanceolata* (DC.) C. B. Clarke (Asteraceae). The air-dried plant materials were exhaustively extracted with methanol in a Soxhlet apparatus. The concentrated methanol extracts were adsorbed on silica gel (60-120 mesh) for the preparation of slurries. The dried slurries were chromatographed over silica gel columns individually. The columns were eluted with petroleum ether, chloroform and methanol, successively, in order of increasing polarity to isolate the apigenin derivatives. Phytochemical investigation of the methanol extract of the leaves of *C. speciosa* afforded *β*-sitosterol 3-β-L-glucopyranoside (**1**) and apigenin 4′-O-*α*-L-glucopyranosyl-(6″→1‴)-*α*-L-rhamnopyranoside (**2**). Column chromatography of the methanol extract of the leaves of *C. dichotoma* gave *α*-L-arabinose (**3**), *β*-D-arabinose (**4**) and acacetin-7-O-*β*-D-glucopyranosyl-(6a→1b)-O-*β*-D-glucopyranosyl-(6b→1c)-O-*β*-D-glucopyranosyl-2c-linolenate (**5**). The methanol extract of the leaves of *M. piperita* on subjection to silica gel column furnished 5-hydroxy-6, 7, 3′, 4′-tetramethoxy-8-(1″-gerananyl)-flavone or 8-(1″-gerananyl)-5-demethylsinensetin (**6**) and 3′,4′-dihydroxy-*β*-phenyl ethyl caffeate-4-(3‴-menthyl)-4′-β-D-glucopyranoside or 4-(3″-menthyl) teucrol 4′-O-*β*-D-glucoside**, 7).** The chemical constituents isolated from the methanol extract of the roots of *P. lanceolata* included *n*-tridecyl stearate (**8**), *n*-nonadecanol (**9**) and 8-isobutyl apigenin (**10**). Apigenin derivatives were isolated from the investigated medicinal plants. Their structures were established on the basis of spectral data analysis and chemical reactions.

Keywords: Apigenin derivatives, Herbal drugs, Isolation, Characterization

Introduction

Apigenin (4′,5,7-trihydroxyflavone) belongs to the flavone class of flavonoid secondary metabolites, it is a common component of many fruits and vegetables including parsley, celery, celeriac and chamomile tea.[1] It has antioxidant, anti-inflammatory, anti-allergic, anti-depressant and anti-tumour properties.[2] It stimulates adult neurogenesis, readily crosses the blood-brain barrier and has not demonstrated toxicity at high doses.[3]

Chorisia speciosa A. St. Hil., syn. *Ceiba speciosa* (A. St.-Hil.) Ravenna (Bombacaceae, Malvaceae), known as kapok, floss silk tree, Mexican silk-cotton tree and chorisia, is a native to Brazil and Argentina and cultivated in many tropical areas like southern California, Bolivia, Paraguay, Uruguay and India. It occurs as a 9 – 20 m tall tree with palmately green leaves, pink to purple five-petaled flowers and pear-shaped capsules. Its wood is used as a source of cellulose. The plant extracts have been reported to possess antimicrobial, anti-inflammatory, antioxidant and antipyretic activities.[4] Its flowers contained steroids, furanoids, phenolic acids and esters, flavonoids, mono-octyl phthalate and succinic acid.[5]

Cordia dichotoma G. Forst., syn. *C. indica* Lam., *C. latifolia* Roxb., *C. wallichii* G. Don (Boraginaceae), known as fragrant manjack, glue berry, pink pearl, bird lime tree, Indian cherry and lasoda, is distributed in northern Australia, India, Philippines, southern China, Taiwan and Formosa.[6] It is a medium-sized deciduous tree with a short crooked trunk and spreading crown. Its fruits are edible and have antidiabetic, anthelmintic, demulcent, diuretic, hepatoprotective, immune-modulatory activities, and used to treat biliousness, blood disorders, colds, colic pain, coryza, diseases of the lungs, uterus and urethra, fever, haemorrhage, seminal debility and skin diseases.[6] The plant bark is utilized as a mild tonic and to relieve boils, colic, catarrh, calculus infections, diarrhoea, dysentery, dyspepsia, fatigue, fevers, insect bites, itchy skin patches, strangury, tumours, mouth ulcers, intestinal worms, wounds and to strengthen the teeth. A leaf decoction is useful to cure headache and ulcers.[6] The fruits contained sugars, gum, *β*-sitosterol, fatty acids, sugars, latifolinal, latifolidin, latifolicinins A-D, cordicinol and amino acids.[7-9] The bark contained allantoin, tannin, cathartin, gallic acid, flavanone 7-L-rhamnoside and *β*-sitosterol. The compounds reported from the leaves included *β*-sitosterol, its 3-glucoside, flavonol glycosides, quercetin, quercitrin, cinnamate ester, latifolinal, latifolidin, phenolics and chlorophyll.[8-10] The twig has linolenoylglycerol.[6] The seeds have been

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reported to contain *α*-amyrin, dirhamnoside, betulin, octacosanol, lupeol-3-rhamnoside, *β*-sitosterol, its glucoside, hentricontanol, hentricontane, taxifolin-3-5-dirhamnoside, hesperitin-7-rhamnoside, fatty acids, caffeic acid, flavonoid glycosides dihydrorobinetin and chlorogenic acid.[10-12]

Mentha piperita L. (Lamiaceae) or peppermint is a cultivated natural hybrid of *M. aquatica* L. and *M. spicata* L. The genus is native to the Mediterranean region, now it has been spread all over the world for its use in flavour, fragrance, medicinal and pharmaceutical applications. The plant grows up to 90 cm tall with dark green and opposite leaves and white flowers. Peppermint has astringent, antiseptic, antipruritic, antispasmodic, antiemetic, analgesic, anticatarrhal, antimicrobial, carminative, diaphoretic, emmenagogue, rubefacient and stimulant properties.[13,14] Peppermint oil vapours are inhaled for respiratory congestion. Peppermint oil is a remedy to alleviate allergic rashes, anorexia, bacterial infections, bronchitis, chicken pox, colitis, coughs, dandruff, diarrhea, inflammation of the oral mucosa and throat, colic in infants, Crohn's disease, flatulence, headaches, indigestion, nausea, neuralgia, morning sickness, irritable bowel syndrome, biliary tract disorders, liver complaints, migraines, menstrual cramps, muscular pains, myalgia, toothaches and vomiting.[14, 15] It has insect repellent activity.[13-15] The major constituents of peppermint oil are limonene, cineole, menthone, menthofuran, isomenthone, menthyl acetate, menthol, pulegone and carvone.[16-21] Other bioactive components of the plant include caffeic acid, flavonoids, polymerized polyphenols, carotenes, tocopherols, betaine, choline and tannins.[22,23]

Pluchea lanceolata (DC.) C. B. Clarke, syn. *Berthelotia lanceolata* DC. (Asteraceae), known as rasna, Indian camphor weed and Indian fleabane, is found in a saline or sandy soil in semiarid regions of India, Afghanistan, Bangladesh, Nepal, China, Pakistan and North Africa. It is an erect, perennial under-shrub, 30-100 cm high, with stem and branches terete, erect, ashy and pubescent; leaves sessile, coriaceous, oblong or lanceolate, obtuse apiculate arranged at the base.[24,25] The plant is used as an analgesic, febrifuge, laxative and nervine tonic and to treat disorders of digestive, circulatory, nervous, respiratory, urinary and reproductive systems, arthritis, skin diseases, inflammations, psoriasis, piles and rheumatism. The roots are bitter, it has anticonvulsant, febrifuge, laxative, thermogenic activities and used for the treatment of scorpion stings, epilepsy, hysteria, syncope and mental fatigue. The leaves are taken as a laxative, analgesic and antipyretic. Its rhizome is used as antiepileptic, antispasmodic, laxative, stimulant and as a tonic, it is also used as an aromatic adjunct to prepare medicinal oils applied to promote hair growth and to blacken them.[26] The plant contained quercetin, isorhamnetin, daidzein, taraxasterol acetate, pluchine, moretenol, neolupenol, lignoceric and cerotic acids, triacontanol, stigmasterol and *β*-sitosterol D-glucoside.[27-30] The present study reported the isolation and characterization of apigenin derivatives from the leaves of *Chorisia speciosa*, *Cordia dichotoma* and *Mentha piperita* and the roots of *Pluchea lanceolata*.

Materials and Methods

General procedures

Melting points were determined on a Perfit melting point apparatus and are uncorrected. UV spectra were determined on Shimadzu-120 double beam spectrophotometer with methanol as a solvent. IR spectra were recorded in KBr pellet on a Shimadzu FTIR-8400 spectrophotometer. The ^{1}H- and ^{13}C-NMR spectra were scanned on a Bruker DRX (300 MHz) instrument using TMS as an internal standard and coupling constants (*J* values) is expressed in Hertz (Hz). Mass spectra were recorded by Fast Atom Bombardment (FAB) ionization technique. The *m/z* values of the more intense peaks are mentioned and the figures in bracket attached to each *m/z* values indicated relative intensities with respect to the base peak. Column chromatography was performed on silica gel (60-120 mesh; Qualigen, Mumbai, India). TLC was run on silica gel G 60 F_{254} pre-coated TLC plates (Merck, Mumbai, India). Spots were visualised by exposing to iodine vapours and UV radiations (254 and 366 nm) and spraying with ceric sulphate solution.

Plant material

The leaves of *Chorisia speciosa*, *Cordia dichotoma* and *Mentha piperita* and roots of *Pluchea lanceolata* were collected locally from Delhi and authenticated by Prof. M. P. Sharma, a taxonomist, of the Department of Botany, Jamia Hamdard, New Delhi. The voucher specimens *C. speciosa* (No. PRL/JH/12/05), *C. dichotoma* (No. PRL/JH/12/06), *M. piperita* (No. PRL/JH/12/0) and *P. lanceolata* (No. PRL/JH/12/08) are preserved in the herbarium of the Department of Pharmacognosy and Phytochemistry, Jamia Hamdard, New Delhi.

Extraction and isolation

One kilogramme (1 kg) each of the leaves of *C. speciosa*, *C. dichotoma* and *M. piperita* and roots of *P. lanceolata* were 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, 113.2 g, 127.4 g, 116.8 g, and 107.8 g, respectively. The dried residue (100 g each) was dissolved in minimum amount of methanol and adsorbed on silica gel column grade (60-120 mesh) separately to obtain slurries. Each slurry was air-dried and chromatographed over silica gel columns loaded in petroleum ether (b. p. 60 - 80°C) individually. Each column was 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, 19:5, 9:1, 17:3, 4:1 7:3, 1:1, *v/v*). 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 Chorisia speciosa (*Ceiba speciosa*)

β-Sitosterol 3-β-L-glucopyranoside (1)

Elution of the column with chloroform-methanol (9:1) furnished a colourless amorphous powder, recrystallization from methanol gave compound **1**, yield 206 mg; m. p. 265- 267 °C, UV λ max (MeOH): 214 nm (log ε 2.9); IR (KBr) *V*max : 3411, 3391, 3248, 2952, 2866, 1641, 1464, 1377, 1261, 1073, 1023 cm^{-1}; ^{1}H-NMR ($CDCl_3$): *δ* 5.33 (1H, m, H-6), 3.45 (1H, brs, *w* ½ = 18.5 Hz, H-3α), 0.97 (3H, brs, Me-19), 0.90 (3H, d, *J* = 6.2 Hz, Me- 21), 0.83 (3H, d, *J* = 6.7 Hz, Me-27), 0.80 (3H, d, *J* = 6.6 Hz, Me- 26), 0.77 (3H, d, *J* = 5.9 Hz, Me-29), 0.67 (3H, brs, Me-18), 5.09 (1H, d, *J* = 4.6 Hz, H- 1′), 4.37 (1H, m, H- 5′), 4.23 (1H, m, H-2′), 4.16 (1H, m, H-3′), 3.91 (1H, m, H-4′), 3.08 (2H, d, *J* = 7.6 Hz, H-6′), 2.91 – 1.12 (29H, m, 7 x CH, 11 x CH_2); ^{13}C-NMR ($CDCl_3$): *δ* 36.84 (C- 1), 29.25 (C- 2), 73.36 (C- 3), 41.13 (C- 4), 140.31 (C- 5), 121.16 (C- 6), 31.39 (C- 7), 35.49 (C- 8), 50.21 (C- 9), 36.21 (C- 10), 22.56 (C- 11), 38.89 (C- 12), 41.78 (C- 13), 56.18 (C- 14), 23.81 (C- 15), 28.22 (C- 16), 55.42 (C- 17), 11.75 (C- 18), 19.63 (C- 19), 38.27 (C- 20), 18.55 (C- 21), 31.39 (C- 22), 25.48 (C- 23), 45.18 (C- 24), 28.68 (C- 25), 19.07 (C- 26), 18.78 (C- 27), 22.54 (C- 28), 11.57 (C- 29), 101.20 (C-1′), 76.72 (C- 2′), 76.61 (C- 3′), 70.16 (C- 4′), 79.45 (C- 5′), 61.11 (C- 6′); FAB MS (+ve ion) *m/z* (rel. int.): 576 $[M]^+$ (41.3), 413 (10.1), 398 (100) for $C_{35}H_{60}O_6$.

Apigenin 4′-O-α-L-gluco-rhamnoside (2)

Elution of column with chloroform – methanol (3:1) yielded yellow crystalline solid of **2**, yield 187 mg, m. p. 292 – 294 °C, UV λmax (MeOH): 267, 296, 335 nm; IR (KBr) *V*max : 3426, 3378, 3219, 2923, 2837, 1659, 1608, 1527, 1499, 1450, 1376, 1287, 1127, 1086, 1042, 827 cm^{-1}; ^{1}H-NMR (DMSO-d_6): *δ* 7.83 (1H, d, *J* = 8.2 Hz, H-2′), 7.80 (1H, d, *J* = 8.2 Hz, H-6′), 6.94 (1H, d, *J* = 8.2 Hz, H-3′), 6.89 (1H, d, *J* = 8.2 Hz, H-5′), 6.75 (1H, s, H-3), 6.63 (1H, d, *J* = 1.9 Hz, H-8), 6.43 (1H, d, *J* = 1.9 Hz, H-6), 5.28 (1H, d, *J* = 2.7 Hz, H-1″α), 3.97 (1H, m, H-5″), 3.74 (1H, m, H-2″), 3.67 (1H, m, H-3″), 3.46 (1H, m, H-4″), 3.31 (2H, d, *J* = 7.6 Hz, H-6″), 5.19 (1H, d, *J* = 4.1 Hz, H-1‴α), 3.93 (1H, m, H-5‴), 3.68 (1H, m, H-2‴), 3.57 (1H, m, H-3‴), 3.39 (1H, m, H-4‴), 1.32 (2H, d, *J* = 6.2 Hz, Me-6‴); ^{13}C-NMR (DMSO-d_6): *δ* 162.87 (C-2), 103.81 (C-3), 183.62 (C-4), 164.31 (C-5), 101.03 (C-6), 166.65 (C-7), 95.91 (C-8), 158.88 (C-9), 105.62 (C-10), 129.59 (C-1′), 122.96 (C-2′), 117.07 (C-3′), 162.82 (C-4′), 117.05 (C-5′), 129.61 (C-6′), 102.54 (C-1″), 73.99 (C-2″), 72.16 (C-3″), 70.07 (C-4″), 79.06 (C-5″), 62.46 (C-6″), 99.75 (C-1‴), 73.97 (C-2‴), 71.38 (C-3‴), 69.85 (C-4‴), 78.27 (C-5‴), 18.27 (C-6‴); FAB MS (+ve ion) *m/z* (rel. int.): 578 $[M]^+$ ($C_{27}H_{30}O_{14}$) (28.3), 309 (12.3), 270 (9.7), 163 (14.6).

Isolation of phytoconstituents from Cordia dichotoma

α-L-Arabinose (3)

Elution of the column with chloroform – methanol (9:1) gave colourless crystals of **3**, yield 127 mg, m. p. 163 – 165 °C; $[\alpha]_D^{20}$ + 103° (*c* 4, H_2O); IR (KBr) *V*max: 3395, 3269, 3023, 2834, 1617, 1461, 1378, 1217, 1079 cm^{-1} ; ^{1}H NMR (DMSO-d_6): δ 5.51 (1H, d, *J* = 5.1 Hz, H-1), 4.42 (1H, m, H-4), 3.95 (1H, m, H-3), 3.56 (1H, m, H-4), 3.47 (2H, brs, H_2-5); FAB MS (+ve ion) *m/z* (rel. int.): 150 $[M]^+$ (1.3) for $C_5H_{10}O_5$.

β-D-Arabinose (4)

Further elution of the column with chloroform – methanol (9:1) afforded colourless crystals of **4**, yield 235 mg, m. p. 156 – 159°C; $[\alpha]_D^{20}$ - 103° (*c* = 10, H_2O); IR (KBr) *V*max: 3391, 3266, 3118, 2937, 2832, 1623, 1465, 1381, 1224, 1082 cm^{-1}; ^{1}H-NMR (DMSO-*d*$_6$): δ 5.32 (1H, d, *J* = 7.3 Hz, H-1), 4.39 (1H, m, H-4), 3.92 (1H, m, H-3), 3.61 (1H, m, H-4), 3.41 (2H, brs, H-5); FAB MS (+ve ion) *m/z* (rel. int.): 150 $[M]^+$ (2.6) for $C_5H_{10}O_5$.

Acacetin- 7-O-β-D-triglucosyl 2c-linolenate (5)

Elution of column with chloroform – methanol (1:1) produced reddish brown crystals of **5**, yield 187 mg, m. p. 172 – 174°C, UV λmax (MeOH): 271, 302, 319 nm; IR (KBr) *V*max: 3429, 3388, 3291, 3023, 2938, 2842, 1721, 1667, 1635, 1527, 1451, 1386, 1217, 1086, 753 cm^{-1}; ^{1}H- NMR (DMSO-*d*$_6$): *δ* 7.59 (1H, d, *J* = 8.9 Hz, H-2′), 7.50 (1H, d, *J* = 8.9 Hz, H-6′), 7.14 (1H, d, *J* = 8.9 Hz, H-3′), 6.98 (1H, d, *J* = 8.9 Hz, H-5′), 6.89 (1H, s, H-3), 6.65 (1H, d, *J* = 2.1 Hz, H-8), 6.34 (1H, d, *J* = 2.1 Hz, H-6), 3.45 (3H, s, OMe), 5.48 (1H, d, *J* = 7.4 Hz, H-1a), 4.19 (1H, m, H-5a), 3.97 (1H, m, H-2a), 3.87 (1H, m, H-3a), 3.64 (1H, m, H-4a), 3.39 (2H, d, *J* = 6.9 Hz, H-6a), 5.24 (1H, d, *J* = 7.5 Hz, H-1b), 4.13 (1H, m, H-5b), 3.89 (1H, m, H-2b), 3.77 (1H, m, H-3b), 3.61 (1H, m, H-4b), 3.32 (2H, d, *J* = 5.6 Hz, H-6b), 5.14 (1H, d, *J* = 7.3 Hz, H-1c), 4.03 (1H, m, H-5c), 4.28 (1H, m, H-2c), 3.70 (1H, m, H-3c), 3.56 (1H, m, H-4c), 3.13 (2H, d, *J* = 5.1 Hz, H_2-6c), 5.33 (1H, m, H-9″), 5.30 (2H, m, H-10″, H-12″), 5.27 (2H, m, H-13″, H-15″), 5.19 (1H, m, H-16″), 2.60 (2H, m, H_2-11″), 2.32 (2H, t, *J* = 7.2 Hz, H_2-2″), 2.03 (2H, m, H_2-14″), 1.98 (2H, m, H_2-8″), 1.63 (2H, m, H_2-17″), 1.56 (2H, m, CH_2), 1.26 (10H, brs, 5 x CH_2), 0.88 (3H, t, *J* = 6.5 Hz, Me-18″); ^{13}C-NMR (DMSO-*d*$_6$): *δ* 162.12 (C-2), 104.98 (C-3), 178.21 (C-4), 155.67 (C-5), 104.33 (C-6), 161.85 (C-7), 98.61 (C-8), 159.26 (C-9), 114.97 (C-10), 131.54 (C-1′), 121.96 (C-2′), 116.73 (C-3′), 161.29 (C-4′), 116.51 (C-5′), 122.16 (C-6′), 57.85 (OMe), 102.95 (C-1a), 75.93 (C-2a), 74.46 (C-3a), 73.97 (C-4a), 79.05 (C-5a), 65.36 (C-6a), 97.89 (C-1b), 75.87 (C-2b), 74.23 (C-3b), 73.56 (C-4b), 77.63 (C-5b), 64.71 (C-6b), 93.84 (C-1c), 82.43 (C-2c), 74.09 (C-3c), 73.15 (C-4c), 76.64 (C-5c), 61.96 (C-6c), 169.09 (C-1″), 41.59 (C-2″), 29.88 (C-3″), 30.42 (C-4″), 29.76 (C-5″), 29.63 (C-6″), 22.69 (C-7″), 40.38 (C-8″), 130.39 (C-9″), 129.81 (C-10″), 47.29 (C-11″), 123.19 (C-12″), 121.16 (C-13″), 37.68 (C-14″), 119.48 (C-15″), 117.45 (C-16″), 29.56 (C-17″), 15.48 (C-18″); FAB MS (+ve ion) *m/z* (rel. int.): 1030 $[M]^+$($C_{52}H_{70}O_{21}$) (1.3), 439 (11.8), 423 (9.1), 277 (11.9), 261 (10.9), 178 (12.3), 162 (44.6).

Isolation of phytoconstituents from Mentha piperita

8-(1″-Gerananyl)-5-demethylsinensetin (6)

Elution of the column with chloroform - methanol (19:1) afforded pale yellow crystals of **6**, recrystallized from acetone, yield 405 mg, R_f 0.53 (chloroform - methanol 19:1), m. p. 109 -110 ℃; UV λmax (MeOH): 273, 335 nm, UV λmax (MeOH + NaOMe): 281, 339 nm, UV λmax (MeOH + NaOAc): no shift, UV λmax (MeOH + NaOAc + H_3BO_3) : no shift, UV λ_{max} (MeOH + $AlCl_3$+ HCl): 273, 355 nm, IR (KBr) *V*max : 3329, 2933, 2872, 1685, 1602, 1513, 1445, 1376, 1275,1177, 1031 cm^{-1}; ^{1}H-NMR (DMSO-*d*$_6$): *δ* 7.43 (1H, d, *J* = 9.0 Hz, H-5′), 7.26 (1H, d, *J* = 3.0 Hz, H-2′), 7.01 (1H, dd, *J* = 9.0, 3.0 Hz, H-6′), 6.73 (1H, s, H-3), 3.92 (3H, brs, OMe), 3.91 (3H, brs, OMe), 3.89 (3H, brs, OMe), 3.40 (3H, brs, OMe), 2.47 (1H, m, H-2″), 2.24 (1H, m, H-6″), 2.19 (1H, d, *J* = 6.6 Hz, H-1″a) , 2.17 (1H, d, *J* = 7.2 Hz, H-1″b), 1.99 (2H, m, H_2-3″), 1.65 (2H, m, H_2-4″), 1.43 (2H, m, H_2-9″), 1.29 (2H, m, H_2-5″), 1.18 (3H, d, *J* = 6.9 Hz, Me-7″), 1.16 (3H, d, *J* = 5.8 Hz, Me-8″), 0.88 (3H, t, *J* = 6.7 Hz, Me-10″); ^{13}C-NMR (DMSO-*d*$_6$): *δ* 161.28 (C-2), 106.16 (C-3), 174.63 (C-4), 148.08 (C-5), 142.94 (C-6), 163.87 (C-7), 134.27 (C-8), 150.91 (C-9), 102.75 (C-10), 121.69 (C-1′), 120.28 (C-2′), 141.87 (C-3′), 143.16 (C-4′), 115.94 (C-5′), 121.69 (C-6′), 61.87, 61.02, 60.46, 55.82 (4 x OMe), 42.16 (C-1″), 33.70 (C-2″), 24.56 (C-3″), 28.19 (C-4″), 28.60 (C-5″), 31.32 (C-6″), 18.70 (C-7″), 18.75 (C-8″), 22.21 (C-9″), 13.95 (C-10″); FAB MS (+ve ion) *m/z* (ret.int): 498 $[M]^+$ ($C_{29}H_{38}O_7$) (2.1).

4-(3″-Menthyl) teucrol 4′-O-β-D-glucoside (7)

Elution of the column with chloroform - methanol (9:1) furnished light brown crystals of **7**, recrystallized from chloroform - methanol (1:1), yield 635 mg, R_f: 0.68 (chloroform - methanol, 9:1), m. p. 149-150 °C; UV λmax (MeOH): 250, 290, 332 nm (log ε 1.2, 6.3, 5.8); IR (KBr) *V*max: 3425, 3366, 3255, 2933, 2845, 1720, 1605, 1517, 1446, 1386, 1284, 1163, 1074 cm^{-1}; ^{1}H-NMR (MeOD): *δ* 7.55 (1H, d, *J* = 15.6 Hz, H-8), 7.05 (1H, d, *J* = 1.4 Hz, H-2), 6.93 (1H, dd, *J* = 1.4, 8.1 Hz, H-6), 6.81 (1H, d, *J* = 8.1 Hz, H-5), 6.28 (1H, d, *J* = 15.6 Hz, H-7), 6.77 (1H, dd, *J* = 1.5, 8.0 Hz, H-6′), 6.71 (1H, d, *J* = 8.0 Hz, H-5′), 6.63 (1H, d, *J* = 1.5 Hz, H-2′), 3.54 (2H, t, *J* = 11.6 Hz, H_2-8′), 2.28 (2H, t, *J* = 11.6 Hz, H-7′), 5.32 (1H, d, *J* = 7.2 Hz, H-1″), 4.34 (1H, m, H-5″), 3.76 (1H, m, H-2″), 3.70 (1H, m, H-3″), 3.62 (1H, dd, *J* = 7.2, 6.3 Hz, H-4″), 3.02 (2H, d, *J* = 7.2 Hz, H_2-6″), 3.86 (1H, m, $w_{1/2}$ = 7.8 Hz, H-3‴), 2.78 (1H, m, H-2‴a), 2.65 (1H, m, H-2‴b), 2.14 (1H, m, H-4‴), 2.02 (1H, m, H-1‴), 1.94 (1H, m, H-8‴), 1.77 (2H, m, H-5‴), 1.60 (1H, m, H-6‴a), 1.49 (1H, m, H-6‴b), 1.20 (3H, d, *J* = 6.1 Hz, Me-9‴), 1.17 (3H, d, *J* = 6.0 Hz, Me-10‴), 1.01 (3H, d, *J* = 6.5 Hz, Me-7‴); ^{13}C-NMR (MeOD): *δ* 126.31 (C-1), 115.01 (C-2), 144.69 (C-3), 148.26 (C-4), 116.29 (C-5), 121.86 (C-6), 146.24 (C-7), 113.94 (C-8), 167.30 (C-9), 126.24 (C-1′), 113.24 (C-2′), 143.76 (C-3′), 145.32 (C-4′), 113.22 (C-5′), 120.53 (C-6′), 36.67 (C-7′), 62.02 (C-8′), 102.01 (C-1″), 73.03 (C-2″), 72.46 (C-3″), 70.21 (C-4″), 76.69 (C-5″), 61.37 (C-6″), 29.36 (C-1‴), 22.44 (C-2‴), 76.42 (C-3‴), 34.37 (C-4‴), 20.18 (C-5‴), 18.65 (C-6‴), 15.02 (C-7‴), 26.56 (C-8‴), 12.93 (C-9″), 13.99 (C-10″); FAB MS (+ve ion) *m/z* (ret. Int.): 616 $[M]^+$ ($C_{33}H_{44}O_{11}$) (2.5), 163 (14.7), 139 (8.6).

Isolation of phytoconstituents from Pluchea lanceolata

n-Tridecyl stearate (8)

Elution of column with petroleum ether –chloroform (3:1) gave creamy white amorphous mass of compound **8**, yield 93 mg, R_f 0.38 (petroleum ether - chloroform, 3:2), m. p. 48 - 49 °C. IR (KBr) *V*max: 2925, 2842, 1738, 1461, 1376, 1175, 1070, 723 cm^{-1} ; ^{1}H-NMR ($CDCl_3$): *δ* 4.09 (2H, t, *J* = 8.6 Hz, H-1′), 2.31 (2H, t, *J* = 7.6 Hz, H_2-2), 1.59 (2H, m, CH_2), 1.23 (50H, br s, 25 × CH_2), 0.87 (3H, t, *J* = 6.3 Hz, Me-18), 0.84 (3H, t, *J* = 6.5 Hz, Me-13′); ^{13}C-NMR ($CDCl_3$): *δ* 171.63 (C-1), 62.16 (C-1′), 34.37 (CH_2), 31.75 (CH_2), 29.11 (16 × CH_2), 29.07 (CH_2), 28.54 (CH_2), 28.36 (CH_2), 28.25 (CH_2), 28.09 (CH_2), 27.15 (CH_2), 24.89 (CH_2), 22.69 (CH_2), 14.11 (Me-18, Me-13′); FAB MS (+ve ion) *m/z* (rel. int.): 466 $[M]^+$ ($C_{31}H_{62}O_2$) (25.3), 283 (78.1).

n-Nonadecanol (9)

Column chromatography with petroleum ether - chloroform (1:1) yielded colourless amorphous powder of compound **9**, yield 69 mg, R_f 0.57 (petroleum ether - chloroform, 2:3), m. p. 52 - 53 °C. IR (KBr) *V*max DCl_3): *δ* 3.61 (2H, t, *J* = 6.8 Hz, H_2-1), 1.56 (2H, m, CH_2), 1.23 (32H, br s, 16 × CH_2), 0.86 (3H, t, *J* = 6.0 Hz, Me-19); ^{13}C-NMR ($CDCl_3$): *δ* 63.07 (C-1), 32.81 (CH_2), 31.93 (CH_2), 29.70 (8 × CH_2), 29.67 (CH_2), 29.63 (CH_2), 29.62 (CH_2), 29.45 (CH_2), 29.37 (CH_2), 25.75 (CH_2), 22.69 (CH_2), 14.11 (Me-19); FAB MS (+ve ion) *m/z* (rel. int.): 284 $[M]^+$ (56.7) for $C_{19}H_{40}O$.

8-Isobutyl apigenin (10)

Elution of column with chloroform – methanol (19:1) afforded reddish brown amorphous mass of compound **10**, 123 mg, m. p. 73 – 74°C, UV λmax (MeOH): 275, 326 nm; IR (KBr) *V*max: 3349, 3150, 2927, 2856, 1675, 1537, 1453, 1257, 1175, 1116, 955, 891, 828 cm^{-1}; ^{1}H-NMR ($CDCl_3$): *δ* 7.98 (1H, d, *J* = 8.0 Hz, H-2′), 7.90 (1H, d, *J* = 8.0 Hz, H-6′), 7.48 (1H, d, *J* = 8.0 Hz, H-3′), 7.45 (1H, d, *J* = 8.0 Hz, H-5′), 6.64 (1H, s, H-3), 6.52 (1H, s, H-6), 2.42 (1H, d, *J* = 7.2 Hz, H-1″a), 2.39 (1H, d, *J* = 7.2 Hz, H_2-1″b), 1.66 (1H, m, H-2″), 1.23 (3H, d, *J* = 6.8 Hz, Me-3″), 1.20 (3H, d, *J* = 7.2 Hz, Me-4″); ^{13}C-NMR ($CDCl_3$): *δ* 161.79 (C-2), 108.68 (C-3), 178.11 (C-4), 157.05 (C-5), 109.96 (C-6), 161.05 (C-7), 142.01 (C-8), 152.60 (C-9), 112.47 (C-10), 130.76 (C-1′), 122.14 (C-2′), 121.57 (C-3′), 159.09 (C-4′), 124.42 (C-5′), 126.06 (C-6′), 45.08 (C-1″), 29.69 (C-2″), 12.49 (C-3″, 4″); FAB MS (+ve ion) *m/z* (rel. int.): 326 $[M]^+$ ($C_{19}H_{18}O_5$) (21.2).

Results and Discussion

Compound **1**, $[M]^+$ at *m/z* 576 ($C_{35}H_{60}O_6$), had similar spectral data to that of *β*-sitosterol-3*β*-O-glucoside,[31,32] except the anomeric proton as a one - proton doublet at *δ* 5.09 (*J* = 4.6 Hz) assigned to α-oriented H-1′. These evidence led to establishing the structure of **1** as *β*-sitosterol 3-*β*-L-glucopyranoside (Figure 1).

Compound **2,** named as apigenin 4′-O-*α*-L-gluco-rhamnoside, $[M]^+$ at *m/z* 578 ($C_{27}H_{30}O_{14}$), exhibited UV absorption maxima at 273 and 325 nm and

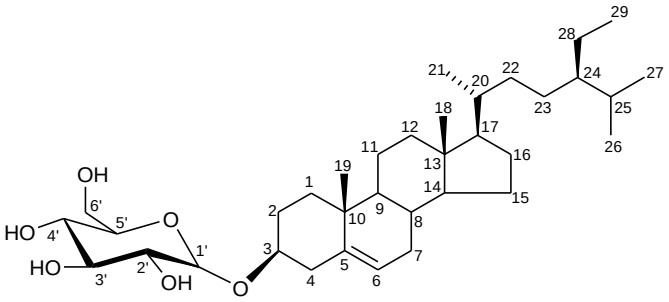
IR absorption bands for hydroxyl groups (3426, 3378 cm^{-1}), carbonyl group (1659 cm^{-1}), unsaturation (1608 cm^{-1}), aromaticity (1527, 1086 cm^{-1}). There was a shift of band I with sodium methoxide suggesting the presence of free hydroxyl groups, a shift of bands with sodium acetate solution indicating free nature of 7-hydroxyl group, a shift of band I with aluminum chloride suggesting the presence of free 5-hydroxyl group. There was no shift of band I with aluminum chloride and hydrochloric acid excluding the existence of B-ring *o*-dihydroxy functions.[33,34] The ion fragments arising at *m/z* 163 $[C_6H_{11}O_5]^+$, 309 $[C_6H_{11}O_5 - C_6H_{10}O_4]^+$ and 270 $[M - 308]^+$ suggested that a disaccharide unit was linked with apigenin. The ^{1}H-NMR spectrum of **2** displayed four one-proton doublets at δ 7.83, 7.80, 6.94 and 6.89 with coupling interactions of 8.2 Hz each assigned to B-ring H-2′, H-6′, H-3′ and H-5′ protons, respectively, a one-proton singlet at δ 6.75 due to flavone H-3 proton, two one-proton doublets at δ 6.63 and 6.43 with coupling constants of 1.9 Hz each accounted correspondingly to *meta*-coupled H-8 and H-6 protons. Two one-proton doublets at δ 5.28 (J = 2.7 Hz) and 5.19 (J = 4.1 Hz) were accounted to anomeric H-1″α and H-1‴α protons, respectively. The other sugar protons appeared from δ 3.97 to 1.32. A three-proton doublet at δ 1.32 (J = 6.2 Hz) was due to secondary C-6‴ methyl protons of the sugar unit. The ^{13}C-NMR spectrum of **2** exhibited signals for carbonyl carbon at δ 183.62 (C-4) and vinylic methine carbon at δ 103.81 (C-3) supporting the flavone-type carbon framework of the molecule, other flavone carbons between δ 162.87 – 95.91, anomeric carbons at δ 102.54 (C-1″) and 99.75 (C-1‴), remaining sugar carbons from δ 79.06 – 18.27. The presence of the oxymethylene protons in the deshielded region as a two-proton doublet at δ 3.31 (J = 7.6 Hz) and its respective carbon signal at δ 62.46 (C-6″) supported (6″→1‴) linkage of the sugar units. Acid hydrolysis of **3** yielded L-glucose, R_f 0.39 (water saturated phenol), L-rhamnose, R_f 0.59 (water saturated phenol) and apigenin , m. p. 345 - 348 °C. On the basis of the above mentioned spectral data and chemical reactions, the structure of compound **2** has been characterized as apigenin 4′-O-α-L-glucopyranosyl-(6″→1‴)-α-L-rhamnopyranoside, a new apigenin diglycoside isolated from a plant source (Figure 1).

Compounds **3** and **4** were the monosaccharides identified as α-L-arabinose and β-D-arabinose, respectively.

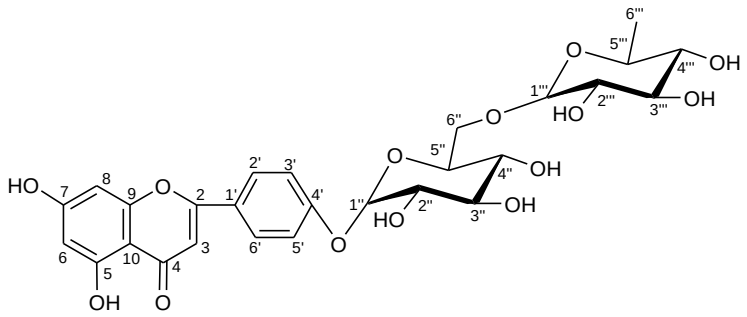
Compound **5,** named as acacetin 7-O-β-D-triglucosyl 2c-linolenate, displayed UV absorption maxima at 271, 302 and 319 nm, a one-proton singlet at δ 6.89 and the corresponding upfield vinylic carbon signal at δ 104.98 characteristic of H-3 and C-3, respectively, in a flavone structure. Shifting of the band I to + 48 nm with no decrease of intensity with sodium methoxide indicated the presence of a free hydroxyl group. The absence of any shift of bands with sodium acetate solution indicated bound nature of 7-hydroxyl group. There was no significant shift in band I with sodium acetate and boric acid ruling out the existence of B-ring dihydroxyl groups. There was a shift of band I with aluminum chloride suggesting the presence of a free 5-hydroxyl group. There was no shift of band I with aluminum chloride and hydrochloric acid excluding the existence of B-ring *ortho*-dihydroxy functions.[33,34] Its IR spectrum exhibited characteristic absorption bands for hydroxyl groups (3429, 3388, 3291, 3023 cm^{-1}), ester function (1721 cm^{-1}), carbonyl group (1667 cm^{-1}), unsaturation (1635 cm^{-1}), aromaticity (1527, 1085 cm^{-1}) and aliphatic chain (753 cm^{-1}). On the basis of its mass and ^{13}C-NMR spectra, the molecular ion peak of **5** was determined at *m/z* 1030 consistent with the molecular formula of a flavonoid triglycosidic ester, $C_{52}H_{70}O_{21}$. The important fragment ion peaks generated at *m/z* 261 $[OC(CH_2)_7\text{-}(CH{=}CH\text{-}CH_2)_3\text{-}CH_3]^+$, 277 $[OOC(CH_2)_7\text{-}(CH{=}CH\text{-}CH_2)_3\text{-}CH_3]^+$, 423 $[O_5H_{10}C_6\text{-}OC(CH_2)_7\text{-}(CH{=}CH\text{-}CH_2)_3\text{-}CH_3]^+$, 439 $[O_6H_{10}C_6\text{-}OC(CH_2)_7\text{-}(CH{=}CH\text{-}CH_2)_3\text{-}CH_3]^+$, 283 [$C_{1'}$ - O fission, $C_{16}H_{11}O_5]^+$, 162 $[C_6H_{10}O_5]^+$, and 178 $[C_6H_{10}O_6]^+$ supported that a trihexoside unit was linked with a methoxyapigenin moiety and linolenic acid was esterified with the sugar chain. The ^{1}H-NMR spectrum of **5** showed four one-proton doublets at δ 7.59, 7.50, 7.14, and 6.98 with coupling interactions of 8.9 Hz each assigned to B-ring H-2′, H-6′, H-3′ and H-5′ protons, respectively, a one-proton singlet at δ 6.89 due to flavone H-3 proton, two one-proton doublets at δ 6.65 and 6.34 with coupling constants of 2.1 Hz each accounted correspondingly to *meta*-coupled H-8 and H-6 protons and a three-proton singlet at δ 3.45 attributed to methoxy protons. Three one-proton doublets at δ 5.48 (J = 7.4 Hz), 5.24 (J = 7.5 Hz) and 5.14 (J = 7.3 Hz) were assigned to anomeric H-1a, H-1b and H-1c protons, respectively. The other sugar protons appeared from δ 4.28 to 3.13. Four multiplets in the range of δ 5.33 – 5.19 were associated with the vinylic protons of the acyl chain. A two-proton triplet at δ 2.32 (J = 7.2 Hz, H-2″), five two-proton multiplets between δ 2.60 - 1.56 and a broad 10-proton signal at δ 1.26 were accommodated in the methylene protons of the acyl chain. A three-proton triplet at δ 0.88 (J = 6.5 Hz) accounted for C-18″ primary methyl protons. The ^{13}C-NMR spectrum of **5** exhibited signals for carbonyl carbon at δ 178.21 (C-4) and methine carbon at δ 104.98 (C-3) supporting the flavone type carbon framework of the molecule, methoxy carbon at δ 57.85, other flavone carbons between δ 161.85 – 98.61, anomeric carbons at δ 102.95 (C-1a), 97.89 (C-1b) and 93.84 (C-1c), remaining sugar carbons from δ 79.05 to 61.96, ester carbon at δ 169.09 (C-1″), vinylic carbons in the range of δ 130.39 – 117.45 and methyl carbon at δ 15.48 (C-18″). The presence of the oxymethylene in the deshielded region as two-proton doublets at δ 3.39 (J = 6.9 Hz, H-6a) and 3.32 (J = 5.6 Hz, H-6b) and their respective carbon signals at δ 65.36 (C-6a) and 64.71 (C-6b) supported (6→1) linkages of the sugar units. The existence of the sugar H-2c proton in the downfield region at δ 4.28 and C-2c carbon at δ 82.43 suggested the presence of the ester linkage at C-2c. Acid hydrolysis of **5** yielded D-glucose, R_f 0.26 (*n*-butanol- acetic acid – water, 4: 1 : 5), acacetin (4′-methoxyapigenin, m. p. 260 – 263 °C) and linolenic acid, R_f 0.84 (gl. acetic acid, 85%). On the basis of above mentioned evidences, the structure of compound **5** has been characterized as acacetin 7-O-β-D-glucopyranosyl-(6a→1b)-O-β-D-glucopyranosyl-(6b→1c)-O-β-D-glucopyranosyl-2c-linolenate, a new apigenin triglycosidic ester isolated from a plant source (Figure 2).

Compound **6** displayed UV absorption bands at 273, 335 nm characteristic of flavones.[33, 34] The compound did not show any shift with sodium acetate and boric acid suggesting the presence of bound C-7 hydroxyl function and absence of *ortho*-dihydroxyl groups. The shifting of band I to 355 nm indicated free C-5 hydroxyl group. The compound gave a positive ferric chloride test due to the presence of C-5 phenolic group. Its IR spectrum exhibited characteristic absorption bands for hydroxyl group (3329 cm^{-1}) and keto group (1685 cm^{-1}). On the basis of mass and ^{13}C-NMR spectra the molecular ion peak of **6** was determined at *m/z* 498 consistent with a molecular formula of C_{10} substituted flavone, $C_{29}H_{38}O_7$. The ^{1}H-NMR spectrum of **6** showed a one-proton signal at δ 6.73 assigned to H-3. Two one - proton doublets at δ 7.26 (J = 3.0 Hz) and 7.43 (J = 9.0 Hz) and a one - proton double doublet at δ 7.01 (J = 9.0, 3.0 Hz) were assigned to meta-coupled H-2′, *ortho*-coupled H-5′ and *ortho*-, *meta*-coupled H-6′ protons, respectively, indicating ABX system of the ring B. Two three - proton doublets at δ 1.18 (J = 6.9 Hz) and 1.16 (J = 5.8 Hz) and a three - proton triplet at δ 0.88 (J = 6.9 Hz) were attributed to secondary C-7″ and C-8″ and primary C-10″ methyl protons, respectively, suggesting acyclic nature of the monoterpene unit. Two one proton doublet at δ 2.19 (J = 6.6 Hz) and 2.17 (J = 7.2 Hz) were due to methylene H-1″ protons linked to the aromatic ring. The other methine and methylene protons appeared from δ 247 to 1.29. The absence of two *meta*-coupled signals near δ 6.50 supported attachment of functional groups at C-6 and C-8. Four three – proton broad singlets at δ 3.92, 3.91, 3.89 and 3.40 were associated with the four methoxy protons. The ^{13}C-NMR spectrum of **6** exhibited signals for carbonyl carbon at δ 174.63 (C-4), flavones carbons from δ 163.87 to 102.75, methoxy carbons between δ 61.87 - 55.82, methyl carbons at δ 18.70 (6-7″), 18.75 (C-8″) and 13.95 (C-10″) and other methine and methylene carbons from δ 42.16 to 22.21. The absence of carbon signals near δ 98.0 for C-6 and 93.0 for C-8 suggested the location of one of the methoxy function at C-6 and monoterpene unit at C-8. The ^{13}C-NMR spectral data of **6** were compared with the values of the reported flavones.[35,36] On the basis of these evidence the structure of **6** was elucidated as 5-hydroxy-6.7, 3′, 4′-tetramethoxy-8-(1″-gerananyl)-flavone or 8-(1″-geranyl)-5-demethylsinensetin, a new flavones derivative isolated from a plant source (Figure 3).

Compound **7,** named as 4-(3″-menthyl) teucrol 4′-O-β-D-glucoside, gave positive tests for phenols and exhibited fluorescent blue spot on TLC under UV lamp changing to bright yellow colour on fuming with ammonia and UV absorption maxima at 253, 291 and 332 nm typical for caffeote ester.[37] Its IR spectrum showed characteristic absorption bands for hydroxyl groups (3425, 3366, 3255 cm^{-1}), ester function (1720 cm^{-1}) and aromatic ring (1605, 1517, 1074 cm^{-1}). On the basis of mass and ^{13}C-NMR spectra, the molecular ion peak of **7** was established at *m/z* 616 corresponding to a molecular formula of glucosidic menthyl teucrol, $C_{33}H_{44}O_{11}$. The fragment ion peaks generating at *m/z* 163 $[C_6H_{11}O_5]^+$ and 139 $[C_{10}H_{19}]^+$ indicated that a hexose unit and monocyclic monoterpenic unit were linked to teucrol. The ^{1}H-NMR spectrum of **7** exhibited four one-proton doublets at δ 6.81 (J = 8.1 Hz), 6.71 (J = 8.0 Hz), 7.05 (J = 1.4 Hz) and 6.63 (J = 1.5 Hz) assigned correspondingly to *ortho*-coupled H-5, H-5′ and *meta*-coupled H-2, H-2′ protons, two one-proton double doublets at δ 6.93 (J = 8.1, 1.4 Hz) and 6.77 (J = 8.0, 1.5 Hz) were ascribed to *ortho*-, *meta*-coupled H-6 and H-6′, respectively, suggesting ABX system of both the rings, two one - proton doublets at δ 6.28 and 7.55 with coupling interactions of 15.6 Hz were attributed to *trans*-oriented vinylic H-7 and H-8 protons, respectively, and a one-proton doublet at δ 5.32 (J = 7.2 Hz)

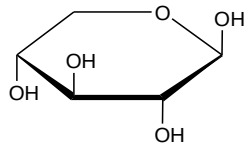


β-Sitosterol 3-*O*-*β*-L-glucopyranoside **(1)**

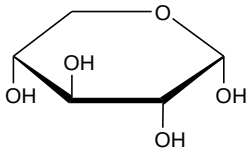


Apigenin-4′-O-*α*-L-glucorhamnoside **(2)**

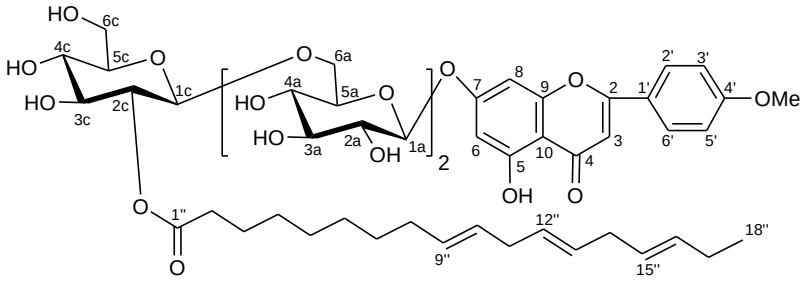
Figure 1: Structural formulae of compounds **1** and **2** isolated from *Chorisia speciosa* leaves.



α-L-Arabinose **(3)**

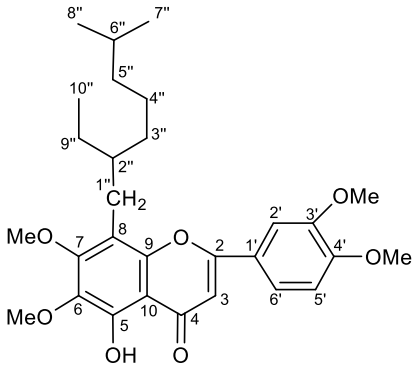


β-D-Arabinose **(4)**

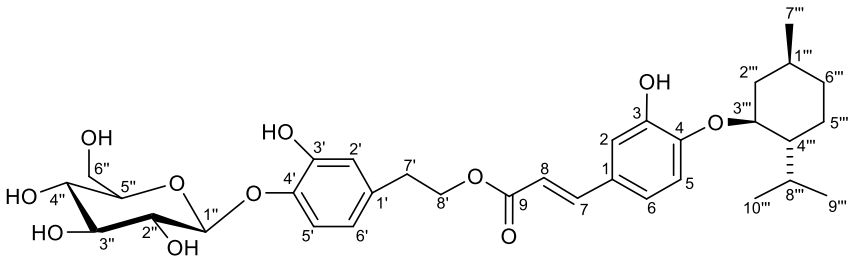


Acacetin 7-O-*β*-D-triglucosyl-2c-linolenate **(5)**

Figure 2: Structural formulae of compounds **3 - 5** isolated from *Cordia dichotoma* leaves.

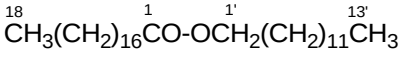


8-(1′′-Geranyl)-5-demethyl sinensetin (**6**)

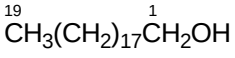


4-(3′′-Menthyl) teucrol 4′-O-*β*-D-glucoside (**7**)

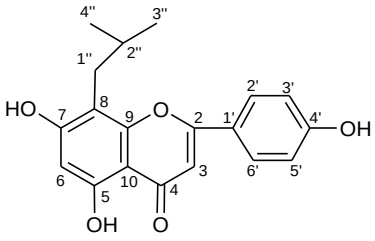
Figure 3: Structural formulae of compounds **6** and **7** isolated from *Mentha piperita* leaves.



n-Tridecyl stearate **(8)**



n-Nonadecanol **(9)**



8-Isobutyl apigenin **(10)**

Figure 4: Structural formulae of compounds **8, 9** and **10** isolated from *Pluchea lanceolata* roots.

accounted for anomeric H-1″ proton. The other sugar protons appeared as one – proton multiplets at δ 4.34 (H-5″), 3.76 (H-3″) and 3.70 (H-4″), as a one – proton double doublet at δ 6.62 (J = 7.2, 6.3 Hz, H-2″) and as a two – proton doublet at δ 6.62 (J = 7.2 Hz, H-2″). A one-proton multiplet at δ 3.86 ($w_{1/2}$ = 7.8 Hz) was accommodated to α-oriented oxymethine H-3‴ proton. Three doublets at δ 1.01 (J = 6.5 Hz), 1.20 (J = 6.1 Hz) and 1.17 (J = 6.0 Hz), integrated for three protons each, were associated with secondary C-7‴, C-9‴ and C-10‴ methyl protons, respectively. The remaining methine and methylene protons resonated from δ 2.78 to 1.49. These data supported the incorporation of glycosyl of 3, 4-dihydroxy-β-phenyl ethyl cafeate ester linked with menthol. The ^{13}C-NMR spectrum of **7** exhibited signals for ester carbon at δ 167.30 (C-9) aromatic and vinylic carbons between δ 148.26 - 113.22, anomeric carbon at δ 102.01 (C-1″), other sugar carbons from δ 76.69 to 61.37, oxymethine carbon at δ 76.42 (C-3‴), oxymethylene carbon at δ 62.02 (C-8′) and methyl carbons at δ 15.02 (C-7‴), 12.93 (C-9‴) and 13.99 (C-10‴). The ^{13}C-NMR spectral data were compared with rosemeric acid[38] and teucrol.[39] The carbon signals of the sugar unit were comparable to glucoside chain.[39, 40] The normal positions of the sugar carbons in the ^{13}C-NMR spectrum suggested that menthol was linked to the phenolic carbon and not to the sugar unit. Acid hydrolysis of compound **7** yielded cafferic acid, 3,4-dihydroxy-β-phenyl ethanol, glucose and menthol, co-TLC comparable. On the basis of the foregoing discussion the structure of compound **7** was elucidated as 3′,4′-dihydroxy-β-phenyl ethyl caffeate-4-(3‴-menthyl)-4′-β-D-glucopyranoside, a new decarboxyrosemarinic acid glucoside isolated from a plant source (Figure 3).

Compounds **8** and **9** were the aliphatic constituents identified as *n*-tridecyl stearate and *n*-nonadecanol, respectively.

Compound **10,** named 8-isobutyl apigenin, showed UV absorption maxima at 275 and 326 nm and IR absorption bands for hydroxyl groups (3349, 3150 cm^{-1}), carbonyl function (1675 cm^{-1}) and aromaticity (1537, 1089 cm^{-1}) suggesting flavone-type skeleton. Shifting of band I to + 48 nm with sodium methoxide indicated the presence of a free hydroxy groups. There was a shift of bands with sodium acetate solution which indicated the free nature of 7-hydroxyl group. There was no significant shift in the band I with sodium acetate and boric acid ruling out the existence of B-ring dihydroxy groups. There was a shift of band I with aluminium chloride suggesting the presence of free 5- and 4′-hydroxyl groups.[33-36] On the basis of its mass and ^{13}C-NMR spectra the molecular ion peak of **10** was determined at *m/z* 326 consistent with the molecular formula of an alkyl flavone, $C_{19}H_{18}O_5$. The ^{1}H-NMR spectrum of **10** showed four one-proton doublets at δ 7.98, 7.90, 7.48, and 7.45 with coupling constant of 8.0 Hz each assigned to B-ring H-2′, H-6′, H-3′ and H-5′ protons, respectively, and two one-proton singlets at δ 6.64 and 6.52 assigned correspondingly to flavone H-3 and H-6 protons. Two one-proton doublets at δ 2.42 (J = 7.2 Hz) and 2.39 (J = 7.2 Hz) were attributed to methylene H-1″ attached to the aromatic ring. A one-proton multiplet at δ 1.66 and two-three proton doublets at δ 1.23 (J = 6.8 Hz) and 1.20 (J = 7.2 Hz) were attributed to methine H-2″ and secondary methyl Me-3″ and Me-4″ protons, respectively. The ^{13}C-NMR spectrum of **10** exhibited signals for carbonyl carbon at δ 178.11 (C-4) and methine carbon at δ 108.68 (C-3) supporting the flavone-type carbon framework of the molecule, other flavone carbons between δ 161.79 - 108.68 and isobutyl carbons between δ 45.08 – 12.69. The absence of C-8 carbon signal near δ 94.2 indicated the attachment of the isobutyl unit at C-8. These evidence led to establish the structure of **10** as 5,7,4′-trihydroxy-8-isobutyl flavone (8-isobutyl apigenin), a new flavone derivative from a plant source (Figure 4).

Conclusion

Phytochemical investigation of the methanol extract of the leaves of *Chorisia speciosa* afforded β-sitosterol 3-O-β-L-glucoside and apigenin 4′-O-α-L-glucosyl-6″O-α-L-rhamnoside. The leaves of *Cordia dichotoma* gave α-L-arabinose, β-D-arabinose and acacetin 7-O-β-D-triglucosyl-2c-linolenate. *Mentha piperita* furnished 8-(1″-gerananyl)-5-demethylsinensetin and 4-(3″-menthyl) teucrol 4′-O-β-D-glucoside. From the roots of *Pluchea lanceolate*, *n*-tridecyl stearate, *n*-nonadecanol and 8-isobutyl apigenin were isolated. This work has enhanced understanding about the phytoconstituents of these plants. These secondary metabolites can be used as analytical markers for quality control of these herbal drugs.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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