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CHEMICAL CONSTITUENTS FROM THE SEEDS OF *CELASTRUS PANICULATUS* WILLD., LEAVES OF *CAESALPINIA BONDUC* (L.) ROXB. AND ROOT BARKS OF *PREMNA MOLLISSIMA* ROTH

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ABSTRACT

Celastrus paniculatus Willd. (Celastraceae) is a woody liana. Its seeds are used to treat abdominal disorders, arthritis, asthma, body ache, leprosy, dysmenorrhea, gout, menorrhea, paralysis, rheumatism, ulcers and skin diseases. The leaves of *Caesalpinia bonduc* (L.) Roxb. (family Caesalpinaceae) are beneficial to cure amenorrhea, asthma, body ache, chest pain, cough, diarrhoea, dysmenorrhoea, elephantiasis, fevers, headache, hepatomegaly, hydrocele, indigestion, intestinal worms, menstruation disorders, rheumatism, skin infections, smallpox and splenomegaly. *Premna mollissima* Roth (family Lamiaceae) is found in southern Asia. Its roots are useful to relieve abscess, asthma, bronchitis, cardiac disorders, cough, diabetes, diarrhoea, inflammations, neuralgia, obesity, rheumatoid arthritis, rhinitis, stomach disorders and as a post-delivery tonic for women. Our study was planned to isolate chemical constituents of the methanolic extracts obtained from the seeds of *C. paniculatus*, leaves of *C. bonduc* and root barks of *P. mollissima* and to characterize their structures. The air-dried plant materials were exhaustively extracted with methanol separately in a Soxhlet apparatus. Each concentrated methanolic extract was adsorbed on silica gel (60-120 mesh) one by one for the preparation of slurries. The dried slurries were chromatographed over silica gel columns individually packed in petroleum ether. The columns were eluted with petroleum ether, chloroform and methanol successively in order of increasing polarity to isolate a variety of phytoconstituents. Phytochemical investigation of the methanolic extract of the seeds of *C. paniculatus* gave a mixed glyceride identified as glycerol-1-linoleio-2-oleo-3-stearate (**1**). The leaf methanolic extract of *C. bonduc* afforded a new fatty acid characterized as *n*-triacont-5(Z),19(Z)-dienoic acid. The root bark methanolic extract of *P. mollissima* furnished the known phytoconstituents characterized as myristyl lignocerate (tetradecyl tetracosanoate, **3**), β-sitosteryl oleate (**4**), 1-hexacosanol (**5**) and new monoterpenic esters 1-benzoyloxy-10-plamityloxy geranilane (**6**), 1-benzoyloxy-10-(octadec-9"-enoyl) geranilane (**7**) and 1-benzoyloxy-10-octadecanyl geranilane (**8**). Their structures were established by analysis of spectral data analysis and chemical reactions.

KEYWORDS: *Celastrus paniculatus* seeds, *Caesalpinia bonduc* leaves, *Premna mollissima* root barks, Chemical constituents, isolation, characterization.

INTRODUCTION

Celastrus paniculatus Willd., syn, *C. dependens* Wall. (Celastraceae), is a woody liana commonly known as black oil plant, climbing staff tree, intellect tree, jyotishmati and mal-kangani. It is a deciduous climbing vine having simple, broad, oval, obovate or elliptic leaves with toothed margins. It grows throughout India up to 1,800 m altitude, in Australia, China, Taiwan, Cambodia, Indonesia, Laos, Malaysia, Myanmar, Nepal, Sri-Lanka, Thailand and Vietnam.[1] The whole plant is analgesic, antidysenteric, anti-inflammatory, antituberculosis, cytotoxic, diaphoretic, insecticidal and stimulant, used for blood clotting and to cure skin diseases. A leaf infusion with *Centella asiatica* juice is taken as a nervine tonic; a leaf paste is applied to subdue sores caused by *Schinus* species. The leaf and root pastes are used to relieve headache. Leaf sap is taken as an antidote for opium toxicity. The roots are antimalarial and febrifuge. A root paste with black pepper is given to treat leucorrhoea, piles and spermatorrhoea. The root is chewed to cure oral ulcer; root juice is ingested to control diabetes; a root or bark paste is lapped on the forehead to subside boils of children. A paste of the roots and leaves is applied to calm down headache; a root and seed paste is layered to prevent body ache and joint pain. The stem bark is regarded as an abortifacient, antidysenteric, depurative and brain tonic. A stem decoction is drunk against kidney disorders. The bark

juice with *Saurauia napaulensis* bark is given against indigestion; a bark decoction is useful for abortion.[1-3] Its seeds are acrid, bitter, alterative, antidepressant, anti-oxidant, anti-rheumatic, aphrodisiac, appetizer, diaphoretic, digestive, diuretic, emetic, emollient, febrifuge, laxative, cardiac and nervine tonic and stomachic; used to treat abdominal disorders, menorrhea, asthma, leprosy, dysmenorrhea, gout, paralysis and skin diseases. The seed oil is antidiabetic, antituberculosis, hair tonic, rubefacient, sedative and stimulant and is effective to relieve beriberi, body ache, earache, eczema, edema, itching, leukoderma, pneumonia, rheumatism, scabies, skin diseases, stomach ache, ulcers and to improve memory. The seeds are boiled with an edible oil and applied to subside arthritis. The seeds stimulate intellectual powers and sharpen memory.[1-3]

The seeds of *C. paniculatus* contained alkaloids like celapanin, celapanigin, celapagin, celastrine and paniculatine, celastrol, β-amyrin, β-sitosterol, polyhydric alcohol, sesquiterpene ester, malangunin, paniculatadiol, malkanguniol, dipalmitoyl glycerol, acetic, benzoic, formic and linoleic acids and triglycerides.[1,3,4] The seed essential oil was composed mainly of palmitic acid (38.61%), phytol, erucic acid, trans- β-copaene and linalool. [5]

Caesalpinia bonduc (L.) Roxb., syn. *Caesalpinia crista* Thunb., *Caesalpinia cristata* Prowazek, *Caesalpinia bonducella* (L.) Fleming and *Guilandina bonduc* L. (family Caesalpinaceae), known as kantakareja, karanjwaa, grey nicker, yellow nicker, nicker bean, or knicker nut, grows throughout the hotter parts of India, African countries, Brazil and south-eastern Asia. It is a large, straggling, thorny shrub; branches small, yellow, covered with hard, yellow, downy prickles; leaves large, stipules, foliaceous, compound, bipinnate; leaflets 12-16, elongated, upper part thick; flowers yellow, in dense long at the top; fruits inflated pods, covered with wiry prickles; seeds 1-2 per pod, oblong or globular, hard, grey with a smooth shiny surface. [1] The roots are considered as an anthelmintic, astringent and febrifuge; used to treat leucorrhoea, blennorrhagia, fever, malaria, miscarriage and venereal diseases. The root bark is anthelmintic, emmenagogue, expectorant, febrifuge, rubefacient and stomachic; beneficial against asthma, amenorrhea, chest pain, colic, cough, dyspepsia, dysmenorrhoea, fevers, flatulence, headache, intestinal worms, jaundice, skin diseases, sores and tumors.[6-8] The leaves are anthelmintic, anti-asthmatic, astringent, deobstruent, emmenagogue and febrifuge; used to cure amenorrhoea, asthma, body ache, chest pain, colic, cough, diarrhoea, dysmenorrhoea, elephantiasis, fevers, headache, hepatomegaly, hydrocele, indigestion, intestinal worms, menstruation disorders, pharyngodynia, rheumatism, skin infections, smallpox and splenomegaly. A leaf paste is applied locally to reduce inflammation and pain. The leaf oil is a nervine tonic, useful in convulsions. A leaf decoction is utilized as gargles for sore throat.[6-9] The seeds are acrid, anodyne, anthelmintic, anti-inflammatory, antirheumatic, aphrodisiac, astringent, bitter, contraceptive, digestive, depurative, diuretic, emmenagogue, expectorant, febrifuge, flatulence, laxative, liver-tonic, stomachic, styptic, thermogenic and vesicant; used to treat amenorrhea, arthralgia, asthma, body ache, boils, colic, cough, diabetes, dyspepsia, dysentery, flatulence, hydrocele, hepatomegaly, inflammations, intermittent fevers, intestinal worms, leprosy, leukoderma, malaria, miscarriage, piles, skin diseases, splenomegaly and wounds. Seed oil is applied to relieve pimples, rheumatoid arthritis and osteoarthritis; effective to comfort convulsions and paralysis. Burnt seeds with alum and areca nut are useful as a dentifrice to cure spongy gums.[]6-8] The flowers are bitter and effective against ascites. The fruits are acrid, anthelmintic, aphrodisiac, astringent and taken to relieve leucorrhoea, piles, urinary disorders and wounds. Fruit oil is useful for indolent ulcers.[6]

The *C. bonduc* plant contained diterpenes neocaesalpin H and P, cordylane A, caesalpinin B, bonducellpin E, caesalpinolide A and 17-methylvouacapane-8(14),-9(11)-diene,[10] caesanol and 6, 7-dibenzoyloxyvouacapen-5-ol,[11] α- and β-amyrins, lup-20(29)-en-3 β-ol, lup-20(20)-en-3 β yl acetate, β-sitosterol and its 3-O- galactoside,[12] cassane diterpene hemiketals caesalpinolides A - D, caesalpinolide-E and a cassane furanoditerpene and cassane butenolides.[13-18] The seeds contained a fixed oil composed of monoenoic, dienoic, trienoic and one polyenoic fatty acids, [19] cassane diterpenes neocaesalpin A and B,[20-22] furanocassane-type diterpenes caesalpinins C-P and norcaesalpinins A - F, 2-acetoxy-3-deacetoxycaesaldekarin E, caesalmin B, caesaldekarin E, caesalpin F, 14(17)-dehydrocaesalpin F, 2-acetoxycaesaldekarin E, 7-acetoxybonducellpin C, caesalmin B-G, 2-acetoxy-3-deacetoxycaesaldekarin E and 6-acetoxy-3-deacetoxycaesaldekarin E, [23-27] caesalls A–F, norcaesalpinins, caesalpinins D and bonducellpin D,[28,29] *n*-triacontan-7,13α-diol, *n*-pentatetracontan-23β-ol, *n*-hexacos-15-en-1,5-olide, bunducsteroid, stigmasterol, β-sitosterol and β-sitosterol glucoside,[30] caesalbonducins D – F, 6-deacetoxybonducellpin B, 3-acetxoy-α-caesalpin, 2(3)-en-α-caesalpin, 1α-hydroxycaesalpinin J, 1α-hydroxy-6-deacetoxycaesalpinin J, 6α-hydroxycaesall M and 6α-hydroxy-14(17)-dehydrocaesalpin F.[31] The roots yielded cassane diterpenes including caesaldekarins A, I-L, demethylcaesaldekarin C and bonducellpins A – D,[32-34] taepeenin A-I, nortaepeenin A-B.[35] The bark resulted in the isolation of 17-hydroxy-campesta-4,6-dien-3-one, 13,14-seco-stigmasta-5,14-dien-3alpha-ol, 13,14-seco-stigmasta-9(11),14-dien-3alpha-ol, caesaldekarin J and pipataline,[36] homoisoflavonoids caesalpinianone and 6-O-methylcaesalpinianone, hematoxylol, stereochenol A, 6′ -O-acetyl loganic acid, 4 ′-O-acetylloganic acid, and 2-O-β-D-glucosyloxy-4-methoxybenzenepropanoic acid.[37] The root bark yielded caesaldekarin C, F, H and M,

demethylcaesaldekarin C, vouacapen-5, 19-diol, caesalmin D and E-caesalpin.[38,39]

Premna mollissima Roth, syn. *Gumira mollissima* (Roth) Kuntze, *Premna latifolia* Roxb., *P. mucronata* Roxb. and *P. viburnoides* Wall. (family Lamiaceae), commonly known as Jhatela, Bakarcha, Basota, Agimantha, Nelli, Gonderi, Gunara, Munja, Nappa and Dusky fire brand mark, is distributed in southern Asia including Cambodia, China, India, Indonesia, Laos, Malaysia, Myanmar, Nepal, Philippines, Sri Lanka, Thailand and Vietnam. It is a small tree, up to 8 m high, bark greyish-white; leaves simple, opposite, elliptic lanceolate-ovate, margins entire or serrate and ciliate, apex acute to acuminate or mucronate, subcoriaceous or chartaceous, unpleasant smelling, pubescent beneath, glabrous below, petiole slender, pubescent; base rounded, truncate or cordate; flowers bisexual, greenish-white, numerous, odour unpleasant, in terminal corymbose cymes; fruit a drupe, globose, glabrous, black; seeds oblong. The leaves of *P. mollissima* are diuretic, useful in agalactia, allergy, colic, cough, dropsy, dyspepsia, flatulence, neuralgia, piles, rheumatalgia and tumours.[3] The roots are appetizer, astringent and bitter, useful in abscess, asthma, bronchitis, cardiac disorders, cough, diabetes, diarrhoea, inflammations, neuralgia, obesity, rheumatoid arthritis, rhinitis, stomach disorders and as a post-delivery tonic for women.[40] The roots are applied after parturition in Burma.[41] The roots are added in important Ayurvedic formulations.[42] The stem bark is applied to heal wounds, eczema, ring-worms, boils, skin diseases, itches and to reduce fever.[3, 43]

The leaves contained an essential oil composed mainly of 1-octen-3-ol (35.69%), terpendiols I and II, δ-guaiene, 2-undecanone, α-pinene, palmitic, 8,11,14-docosatrienoic, stearic, linoleic, arachidic, behenic and lignoceric acids, eicosane,[44] 3-octanone, ethyl hexanol, linalool, methyl salicylate and (E)-caryophyllene,[45] furanoid premnalatin,[46] premnoside A,[47] apigenin-4'-methoxy-7-O-arabino-rhamnoside and 5-hydroxy-4'-methoxyflavone-7-O-trioside.[48] The root bark yielded hydroxysandaracopimar-15-enes, bisnorditerpene (premnolal) and diterpenes (nellionol, dehydronellionol and anhydronellionol), 5-dehydronellionol, sandaracopimar-15-en-8 β-ol and β-sitosterol, 14α-hydroxyisopimar-7,15-diene, 7α-hydroxysandaracopimar (8, 14), 15-diene, 7α-hydroxyisopimar-8,15-diene and 1α, 8β,11α-triol - sandaracopimar-15-ene. [49-52] The roots afforded fatty acids, stigmanstane esters and *n*-tetracosanol.[53] The stem bark afforded iridoids, 7-deoxyloganic acid and geniposidic acid, icetexane diterpenes latifolionol, dihydrolatifolionol, latiferanol. [54-57] Keeping in view the high reputation and application of *Celastrus paniculatus*, *Caesalpinia bonduc* and *Premna mollissima* in the indigenous medicinal systems, it has been aimed to carry out isolation and characterization of chemical constituents from these plants.

MATERIALS AND METHODS

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 materials

The seeds of *C. paniculatus* and the leaves of *C. bonduc* were purchased from the local market of Khari Baobli, Delhi. The fresh root bark of *P. mollissima* was collected from the Kumaun region, Uttarakhand, India. These plant materials were taxonomically identified by Prof. M. P. Sharma, Taxonomist, Department of Botany, Jamia Hamdard, New Delhi. Voucher specimens of these plants are preserved in the herbarium of the Department of Pharmacognosy and Phytochemistry, Jamia Hamdard, New Delhi.

Extraction and isolation

One kilogramme (1.0 kg) each of the seeds of *C. paniculatus*, leaves of *C. bonduc* and root bark of *P. mollissima* were dried, 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, 112.3 g, 137.2 g and 116.8 g, respectively. Small portion of each extract was analyzed chemically to determine the presence of different chemical constituents. Each dried extract (100 g each) was dissolved in a minimum quantity of methanol and adsorbed on silica gel (60-120 mesh) for the preparation of slurries. Each slurry was dried in air and chromatographed individually over silica gel columns (1.6 m x 16 mm x 2 mm) packed in petroleum ether. Each column was eluted successively in increasing order of polarity in various combinations with 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, v/v) and methanol. 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:

Isolation of a phytoconstituent from the seeds of *Celastrus paniculatus*

Glyceryl-1-linoleio-2-oleo-3-stearate (1)

Elution of the column with petroleum ether furnished a yellow semisolid mass of **1**, yield 316 g, purified by preparative TLC using petroleum ether - chloroform (1:1), 211 g, UV λ_{max} (MeOH): 212 nm (log ε 2.2); IR γ_{max} (KBr): 2925, 2853, 1741, 1739, 1735, 1635, 1461, 1260, 1166, 1098, 722 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 5.35 (4H, m, H-9′, H-10′, H-9″ H-10″), 5.27 (1H, m, H-12′), 5.25 (1H, m, H-13′), 4.37 (1H, m, H-2), 4.28 (2H, m, H_2-1), 4.14 (2H, m, H_2-3), 2.81 (2H, t, J = 7.2 Hz, H_2-2′), 2.32 (2H, t, J = 7.5 Hz, H_2-2″), 2.26 (2H, t, J = 7.1 Hz, H_2-2‴), 2.03 (6H, brs, 3 x CH_2), 1.60 (6H, brs, 3 x CH_2), 1.32 (22H, brs, 11 x CH_2), 1.29 (44H, brs, 22 x CH_2), 0.91 (3H, t, J = 6.1 Hz, Me-18′), 0.89 (3H, t, J = 6.3 Hz, Me-18″), 0.87 (3H, t, J = 6.5 Hz, Me-18‴); ^{13}C NMR ($CDCl_3$): δ 177.70 (C-1′), 174.43 (C-1″), 172.29 (C-1‴), 132.81 (C-9′), 131.03 (C-10′), 130.93 (C-12′), 129.37 (C-13′), 129.06 (C-9″), 128.39 (C-10″), 70.69 (C-2), 63.66 (C-1), 63.45 (C-3), 35.11 (C-2′), 33.23 (C-2″, C-2‴), 32.84 (CH_2), 30.95 (28 x CH_2), 30.78 (CH_2), 30.61 (CH_2), 30.39 (CH_2), 28.30 (CH_2), 26.73 (CH_2), 26.61 (CH_2), 26.34 (CH_2), 25.16 (CH_2), 23.90 (CH_2), 21.67 (CH_2), 22.79 (CH_2), 14.67 (Me-18′), 14.65 (Me-18″, Me-18‴); ESI MS *m/z* (rel. int.): 884 $[M]^+$ ($C_{57}H_{104}O_6$) (1.2), 283 (12.6) 281 (26.8), 279 (15.3), 265 (35.8), 263 (5.1).

Isolation of a phytoconstituent from the leaves of *Caesalpinia bonduc*

***n*-Triacont-5(Z), 19(Z)-dienoic acid (2)**

Elution of the column with petroleum ether - chloroform (2:3) yielded pale yellow crystals of **2**, recrystallized from chloroform – methanol (1 : 1), 121 mg; m. p. 109-110 ^{0}C; IR υ_{max} (KBr): 3409, 2932, 2846, 1701, 1648, 1465, 1379, 1204, 1123, 1032, 731 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 5.27 (2H, m, $w_{1/2}$ = 8.7 Hz, H-5, H-6), 5.13 (2H, m, $w_{1/2}$ = 10.2 Hz, H-19, H-20), 2.53 (2H, t, J = 7.1 Hz, H_2-2), 2.23 (2H, m, H_2-4), 2.11 (2H, m, H_2-7), 2.03 (2H, m, H_2-18), 1.96 (2H, m, H_2-21), 1.53 (4H, m, 2 × CH_2), 1.29 (14 H, brs, 7 × CH_2), 1.25 (20 H, brs, 10 × CH_2), 0.85 (3H, t, J = 6.3 Hz, Me-30); ^{13}C NMR ($CDCl_3$): δ 179.21 (C-1), 129.19 (C-5), 129.01 (C-6), 127.03 (C-19), 126.87 (C-20), 38.04 (C-2), 33.05 (C-4), 30.91 (C-7), 30.51 (C-18), 29.89 (C-21), 29.05 (C-3), 29.01 (C-8), 28.94 (C-9), 28.69 (C-10), 28.66 (C-11), 28.63 (C-12), 28.58 (C-13), 28.57 (C-14), 28.51 (C-15), 28.42 (C-16), 28.35 (C-17), 28.33 (C-22), 28.30 (C-23), 28.23 (C-24), 28.14 (C-25), 26.95 (C-26), 26.16 (C-27), 24.60 (C-28), 21.66 (C-29), 14.36 (C-30); ESI MS *m/z* (rel. int.): 448 $[M]^+$ ($C_{30}H_{56}O_2$) (43.6), 361 (23.8), 335 (14.8), 307 (32.6), 281 (19.3), 167 (22.5), 141 (21.2) .

Isolation of phytoconstituents from the root barks of *Premna mollissima*

Myristyl lignocerate (3)

Elution of the column with chloroform gave a colourless solid mass of **3**, yield 298 mg, m. p. 61 – 62 °C; UV λmax (MeOH): 206 nm (log ε 5.1); IR γmax (KBr): 2929, 2846, 1735, 1637, 1465, 1381, 1248, 1187, 741 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 4.12 (2H, t, J = 6.8 Hz, H_2-1'), 2.31 (2H, t, J = 7.4 Hz, H_2-2), 2.23 – 1.54 (10H, m, 5 x CH_2), 1.32 (12H, brs, 6 x CH_2), 1.29 (28H, brs, 14 x CH_2), 1.23 (16H, brs, 8 x CH_2), 0.87 (3H, t, J = 6.6 Hz, Me-24), 0.84 (3H, t, J = 6.3 Hz, Me-14′); ^{13}C NMR ($CDCl_3$): δ 171.92 (C-1), 63.87 (C-1'), 52.81 (C-2), 38.83 (C-3), 33.97 (C-4), 33.65 (C-5), 32.19 (C-6), 31.02 (C-7), 29.58 (22 x CH_2), 29.46 (C-8), 29.27 (C-2′), 29.06 (C-3′), 28.79 (C-4′), 26.11 (C-23), 22.68 (C-13′), 14.17 (C-24), 14.09 (Me-14′); ESI MS *m/z* (rel. int.): 564 $[M]^+$ ($C_{38}H_{76}O_2$) (3.1), 367 (7.4), 351 (9.8), 213 (16.2).

β-Sitosterol oleate (4)

Elution of the column with chloroform-methanol (19:1) afforded a pale yellow amorphous powder of **4**, recystallized by (chloroform-methanol, 1:1), 531 mg, R_f 0.25 (chloroform-methanol, 9:1); UV λmax (MeOH): 216 nm; m. p. 113 - 114 °C; IR γmax (KBr): 2923, 2843, 1727, 1648, 1451, 1393, 1239, 1057, 726 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 5.35 (1H, m, H-6), 5.13 (1H, m, H-9′), 5.06 (1H, m, H-10′), 4.29 (1H, brm, $w_{1/2}$ = 17.6 Hz, H-3α), 1.03 (3H, brs, Me-19), 0.97 (3H, d, J = 6.3 Hz, Me-21), 0.89 (3H, d, J = 6.5 Hz, Me-26), 0.84 (3H, d, J = 6.2 Hz, Me-27), 0.81 (3H, t, J = 6.3 Hz, Me -18′), 0.78 (3H, t, J = 6.4 Hz, Me-29), 0.67 (3H, brs, Me-18), 2.37-1.17 (57H, m, 25 × CH_2, 7 × CH); ^{13}C NMR ($CDCl_3$): δ 38.42 (C-1), 33.87 (C-2), 73.61 (C-3), 42.38 (C-4), 140.71 (C-5), 121.51 (C-6), 33.89 (C-7), 34.32 (C-8), 51.23 (C-9), 36.69 (C-10), 21.23 (C-11), 39.89 (C-12), 43.127 (C-13), 56.78 (C-14), 24.34 (C-15), 28.28 (C-16), 56.13 (C-17), 11.88 (C-18), 19.07 (C-19), 36.08 (C-20), 18.84 (C-21), 29.31 (C-22), 26.12 (C-23), 45.87 (C-24), 29.23 (C-25), 19.41 (C-26), 19.78 (C-27), 23.11 (C-28), 12.09 (C-29), 171.92 (C-1′), 31.92 (C-2′), 29.76 (C-3′), 29.73 (C-4′), 29.67 (C-5′), 29.64 (C-6′), 29.27 (C-7′), 29.12 (C-8′), 128.89 (C-9′), 128.13 (C-10′), 29.74 (C-11′), 29.56 (C-12′), 29.43 (C-13′), 28.99 (C-14′), 28.79 (C-15′), 25.07 (C-16′), 22.67 (C-17′), 14.21 (C-18′); ESI MS m/z (rel. int.): 678 $[M]^+$ ($C_{47}H_{82}O_2$) (5.6), 413 (15.8), 397 (15.1), 281 (16.8), 265 (15.3).

1-Hexacosanol (5)

Further elution of the column with petroleum ether - chloroform (1:1) gave colourless amorphous powder of **5**, yield 184 mg, m. p. 79 - 81 °C; IR γmax (KBr): 3419, 2923, 2848, 1461, 1252, 1163, 1057, 724 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 3.58 (2H, t, J = 6.9 Hz, H_2-1), 1.65 (2H, m, H_2-2), 1.56 (2H, m, H_2-3), 1.36 (2H, m, H_2-4), 1.32 (4H, m, H_2-5, H_2-6), 1.28 (4H, m, H_2-7, H_2-8), 1.23 (34H, br s, 17 × CH_2), 0.86 (3H, t, J = 6.5 Hz, Me-26); ^{13}C NMR ($CDCl_3$): δ 63.83 (C-1), 32.87 (C-2), 31.98 (C-3), 29.87 (14 × CH_2), 29.71 (C-18), 29.61 (C-19), 29.56 (C-20), 29.50 (C-21), 29.46 (C-22), 29.36 (C-23), 25.81 (C-24),

22.71 (C-25), 14.15 (Me-26); ESI MS *m/z* (rel. int.): 382 $[M]^+$ ($C_{26}H_{54}O$) (32.4).

1-Benzoyloxy- 10-plamityloxy geranilane (6)

Elution of the column with chloroform-methanol (49:1) furnished a yellow solid mass of compound **6**, yield 411 mg, R_f : 0.68 (chloroform – ethyl acetate, (9 : 1)); m. p. 85 - 86°C; IR γmax (KBr): 2932, 2848, 1725,1635, 1549, 1466, 1381, 1279, 1131, 1068, 981, 737 cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.71 (2H, m, H-2', H-6'), 7.52 (2H, m, H-3', H-5'), 7.37 (1H, m, H-4'), 4.23 (2H, t, J = 6.8 Hz, H_2-1), 4.11 (2H, d, J = 7.2 Hz, H_2-10), 2.33 (2H, t, J = 7.4 Hz, H_2-2"), 2.11 (1H, m, H-3), 2.07 (1H, m, H-7), 1.85 (1H, m, H_2-2α), 1.76 (1H, m, H_2-2β), 1.73 (1H, m, H_2-6α), 1.64 (1H, m, H_2-6β), 1.56 (2H, m, H_2-5), 1.43 (1H, m, H_2-4α), 1.37 (1H, m, H_2-4β), 1.33 (2H, m, H_2-3"), 1.29 (6H, brs, H_2-4", H_2-5", H_2-6"), 1.23 (18H, brs, 9 x CH_2), 0.98 (3H, d, J = 6.8 Hz, Me-8), 0.93 (3H, d, J = 7.3 Hz, Me-9), 0.86 (3H, t, J = 6.4 Hz, Me-14"); ^{13}C NMR ($CDCl_3$): δ 66.21 (C-1), 34.12 (C-2), 38.81 (C-3), 31.47 (C-4), 23.83 (C-5), 23.02 (C-6), 34.55 (C-7), 18.98 (C-8), 19.04 (C-9), 71.73 (C-10), 138.42 (C-1'), 132.39 (C-2'), 130.84 (C-3'), 128.92 (C-4'), 130.88 (C-5'), 131.28 (C-6'), 167.89 (C-7'), 172.31 (C-1"), 51.43 (C-2"), 31.92 (C-3"), 30.24 (C-4"), 29.98 (C-5"), 29.71 (C-6"), 29.63 (C-7"), 29.57 (C-8"), 29.18 (C-9"), 29.05 (C-10"), 28.93 (C-11"), 28.89 (C-12"), 27.73 (C-13"), 24.55 (C-14"), 22.71 (C-15"), 14.17 (C-16"); ESI MS *m/z* (rel. int.): 516 $[M]^+$ ($C_{33}H_{56}O_4$) (4.8), 395 (29.3), 255 (41.5), 239 (8.1), 121 (18.7).

1-Benzoyloxy- 10- oleiyl geranilane (7)

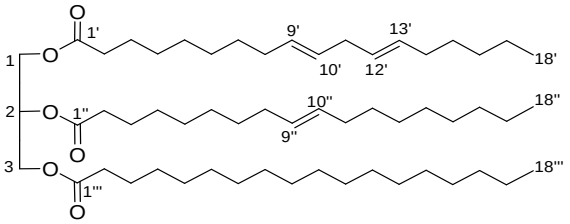
Further elution of the column with chloroform-methanol (49:1) afforded a yellow solid mass of compound **7**, yield 323 mg, R_f : 0.60 (chloroform – ethyl acetate, 9 : 1); m. p. 88 - 89°C; IR γmax (KBr): 2936, 2843, 1727, 1641, 1558, 1461, 1377, 1269, 1144, 1079, 983, 742 cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.75 (2H, m, H-2', H-6'), 7.51 (2H, m, H-3', H-5'), 7.31 (1H, m, H-4'), 5.34 (1H, m, H-9"), 5.30 (1H, m, H-10"), 4.29 (2H, t, J = 6.7 Hz, H_2-1), 4.08 (2H, d, J = 6.8 Hz, H_2-10), 2.36 (2H, t, J = 7.5 Hz, H_2-2"), 2.19 (1H, m, H-3), 2.06 (2H, m, H_2-8"), 2.03 (2H, m, H_2-11"), 1.99 (1H, m, H-7), 1.83 (1H, m, H_2-2α), 1.75 (1H, m, H_2-2β), 1.68 (1H, m, H_2-6α), 1.61 (1H, m, H_2-6β), 1.57 (2H, m, H_2-5), 1.43 (1H, m, H_2-4α), 1.38 (1H, m, H_2-4β), 1.33 (2H, m, H_2-3"), 1.28 (4H, m, H_2-12", H_2-13"), 1.23 (16H, brs, 8 x CH_2), 0.97 (3H, d, J = 6.5 Hz, Me-8), 0.93 (3H, d, J = 6.7 Hz, Me-9), 0.83 (3H, t, J = 6.2 Hz, Me-18"); ^{13}C NMR ($CDCl_3$): δ 68.27 (C-1), 34.13 (C-2), 38.87 (C-3), 31.46 (C-4), 23.82 (C-5), 23.12 (C-6), 34.08 (C-7), 19.12 (C-8), 19.87 (C-9), 71.74 (C-10), 138.23 (C-1'), 132.41 (C-2'), 130.98 (C-3'), 129.12 (C-4'), 130.51 (C-5'), 128.89 (C-6'), 168.17 (C-7'), 172.79 (C-1"), 53.14 (C-2"), 33.91 (C-3"), 29.23 (C-4"), 28.97 (C-5"), 27.69 (C-6"), 26.87 (C-7"), 31.49 (C-8"), 132.51 (C-9"), 127.38 (C-10"), 30.37 (C-11"), 30.23 (C-12"), 29.67 (C-13"), 29.41 (C-14"), 24.92 (C-15"), 24.53 (C-16"), 22.71 (C-17"), 14.19 (C-18"); ESI MS *m/z* (rel. int.): 542 $[M]^+$ ($C_{35}H_{58}O_4$) (2.3), 421 (15.1), 281 (12.7), 265 (28.3), 261 (8.9), 121 (31.6).

1-Benzoyloxy- 10- stearyl geranilane (8)

Elution of the column with chloroform-methanol (19:1) produced a yellow solid mass of compound **8**, yield 412 mg, R_f : 0.51 (chloroform – ethyl acetate, 9 : 1); m. p. 118 - 120 °C; IR γmax (KBr): 2929, 2849, 1733, 1639, 1567, 1461, 1371, 1272, 1125, 1081, 986, 739 cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.73 (2H, m, H-2', H-6'), 7.52 (2H, m, H-3', H-5'), 7.37 (1H, m, H-4'), 4.25 (2H, t, J = 6.7 Hz, H_2-1), 4.11 (2H, d, J = 7.2 Hz, H_2-10), 2.54 (1H, m, H-3), 2.33 (2H, t, J = 7.2 Hz, H_2-2"), 2.05 (2H, m, H_2-3"), 2.02 (2H, m, H_2-4"), 1.96 (1H, m, H-7), 1.89 (1H, m, H_2-2α), 1.78 (1H, m, H_2-2β), 1.76 (1H, m, H_2-6α), 1.63 (1H, m, H_2-6β), 1.57 (2H, m, H_2-5), 1.46 (1H, m, H_2-4α), 1.39 (1H, m, H_2-4β), 1.33 (2H, m, H_2-5"), 1.25 (24H, brs, 12 x CH_2), 0.96 (3H, d, J = 6.5 Hz, Me-10), 0.91 (3H, d, J = 6.9 Hz, Me-9), 0.83 (3H, t, J = 6.7 Hz, Me-18"); ^{13}C NMR ($CDCl_3$): δ 68.31 (C-1), 34.81 (C-2), 36.79 (C-3), 31.39 (C-4), 23.81 (C-5), 23.02 (C-6), 33.92 (C-7), 19.17 (C-8), 19.86 (C-9), 71.83 (C-10), 138.24 (C-1'), 128.32 (C-2'), 130.89 (C-3'), 128.91 (C-4'), 130.94 (C-5'), 128.81 (C-6'), 167.72 (C-7'), 173.41 (C-1"), 51.43 (C-2"), 33.81 (C-3"), 30.22 (C-4"), 29.52 (C-5"), 29.49 (C-6"), 29.47 (C-7"), 29.44 (C-8"), 29.42 (C-9"), 29.39 (C-10"), 29.31 (C-11"), 29.26 (C-12"), 28.96 (C-13"), 27.79 (C-14"), 24.32 (C-15"), (C-16"), 22.71 (C-17"), 14.15 (C-18"); ESI MS *m/z* (rel. int.): 544 $[M]^+$ ($C_{35}H_{60}O_4$) (3.1), 423 (11.8), 283 (14.7), 267 (22.8), 121 (11.2).

RESULTS AND DISCUSSION

Compound **1** was a mixed glyceride characterized as glycerol-1-linoleio-2-oleo-3-stearate (Fig 1).

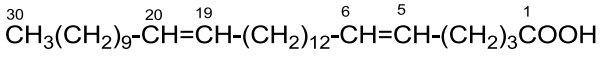


Glycero-1-linoleio-2-oleo-3-stearate **(1)**

Fig 1: Compound 1 isolated from the seeds of *Celastrus paniculata.*

Compound **2** produced effervescences with sodium bicarbonate solution and decolourized bromine water suggesting unsaturated nature of a fatty acid. Its IR spectrum showed characteristic absorption bands for carboxylic group (3409, 1701 cm^{-1}), unsaturation (1648 cm^{-1}) and long aliphatic chain (731 cm^{-1}). Its molecular weight was established at *m/z* 448 on the basis of mass spectrum consistent with a molecular formula of the unsaturated fatty acid, $C_{30}H_{56}O_2$. The ion peaks arising at *m/z* 361 [C_4-C_5 fission, $CH_3(CH_2)_9CH{=}CH$-$(CH_2)_{12}CH{=}CH$, $C_{26}H_{49}]^+$, 335 [C_6-C_7 fission, $CH_3(CH_2)_9CH{=}CH$-$(CH_2)_{12}$, $C_{24}H_{47}]^+$, 167 [C_{18}-C_{19} fission, $CH_3(CH_2)_9CH{=}CH$, $C_{12}H_{23}]^+$, 281 [M – 167]$^+$, 141 [C_{20}-C_{21} fission, $CH_3(CH_2)_9$, $C_{10}H_{21}]^+$ and 307 [M – 141]$^+$ suggested the existence of the vinylic linkages at C-5 and C-19 carbon positions. The ^1H NMR spectrum of **2** showed two two-proton multiplets at δ 5.27 and 5.13 with half-width of 8.7 Hz and 10.2 Hz, respectively,

assigned to cis-oriented vinylic H-5, H-6 and H-19, H-20 protons, respectively. A two-proton triplet at δ 2.53 (J = 7.1 Hz) was ascribed to methylene H_2-2 protons adjacent to the carboxylic function. The other methylene protons resonated as two-proton multiplets at δ 2.23, 2.11, 2.03 and 1.96, as a four-proton multiplet at δ 1.53 and as broad singlets at δ 1.29 (14 H) and 1.25 (20 H). A three-proton triplet at δ 0.85 (J = 6.3 Hz) was accounted to C-30 primary methyl protons. The ^{13}C NMR spectrum of **2** exhibited signals for carboxylic carbon at δ 179.21 (C-1), vinylic carbons at δ 129.19 (C-5), 129.01 (C-6), 127.03 (C-19) and 126.87 (C-20), methylene carbons between δ 38.04 – 21.66 and methyl carbon at δ 14.36 (C-30). On the basis of spectral data analysis and chemical reactions, the structure of **2** has been elucidated as *n*-triacont-5(Z),19(Z)-dienoic acid, a new fatty acid (Fig 2).



n-Triacont-5(Z),19(Z)-dienoic acid (**2**)

Fig 2: Compound 2 isolated from the leaves of *Caesalpinia bonduc*.

Compound **3** was a known fatty acid ester identified as myristyl lignocerate (tetradecyl tetracosanoate) (Fig 3).

Compound **4** was the known steroidal ester and its structure was elucidated as β-sitosteryl oleate (Fig 3).[58,59]

Compound **5** was a long chain aliphatic alcohol characterized as 1-hexacosanol (Fig 3).[60-62]

Compound **6**, named 1-benzoyloxy-10-plamityloxy geranilane, had distinctive IR absorption bands for ester groups (1725 cm^{-1}), aromatic ring (1635, 1549, 1068 cm^{-1}) and aliphatic chain (737 cm^{-1}). On the basis of mass and ^{13}C NMR spectra the molecular ion peak of **6** was determined at *m/z* 516 consistent with a molecular formula of an acyclic monoterpenic diester $C_{33}H_{56}O_4$. The ion peaks arising at *m/z* 121 [C_1 – O fission, $C_6H_5COO]^+$ and 395 [M- 121] $^+$ suggested that benzoyl group was linked to the monoterpenic unit. The ion fragments generated at *m/z* 239 [$C_{1''}$ -O fission, $C_{16}H_{31}O]^+$ and 255 [C_{10} -O fission, $C_{16}H_{31}O_2]^+$ indicated the attachment of the C_{16} palmityl unit to the monoterpenic moiety. The ^{1}H NMR spectra of compound **6** exhibited three multiplets at δ 7.71 (2H), 7.52 (2H) and 7.37 (1H) assigned to aromatic H-2' to H-6' protons. A two-proton triplet at δ 4.23 (J= 6.8 Hz) and a two-proton doublet at δ 4.11 (J= 7.2 Hz) were ascribed to oxymethylene H_2-1 and H_2-10 protons, respectively. A two-proton triplet at δ 2.33 (J= 7.6 Hz) was due to methylene H_2- 2" adjacent to the ester group. Two three-proton doublets at δ 0.98 (J = 6.8 Hz) and 0.93 (J = 7.3 Hz) and a three-proton triplet at δ 0.86 (J = 6.4 Hz) were associated correspondingly with the secondary C-8 and C-9 and primary C-16" methyl protons. The remaining methine and methylene protons appeared as multiplets between δ 2.11 -1.23. The ^{13}C NMR spectra of compound **6** displayed signals for ester carbons at δ 167.89 (C-7') and 172.31 (C-1"), aromatic carbons between δ 138.42 -128.92, methyl carbons at δ 18.98 (C-8), 19.04 (C-9) and 14.17 (C- 16"), oxygenated methylene carbons at δ 66.21 (C-1) and δ 71.73 (C-10), and the remaining methine and methylene carbons from δ 51.43- δ 22.71. The presence of the oxymethylene proton signal of H_2-1 at δ 4.23 in the deshielded region in comparison to H_2-10 signal at δ 4.11 suggested the location of the benzoyloxy group at C-1. On the basis of these evidences the structure of **6** has been determined as 1-benzoyloxy-10-plamityloxy geranilane, a new monoterpenic ester (Fig 3).

Compound **7**, designated as 1-benzoyloxy- 10- oleiyl geranilane, $[M]^+$ at *m/z* 542 ($C_{35}H_{58}O_4$), showed IR absorption bands for ester groups (1727 cm^{-1}), unsaturation (1641 cm^{-1}), aromatic ring (1558, 1073 cm^{-1}) and aliphatic chain (742 cm^{-1}). The mass ion peaks produced at *m/z* 121 [C_1 – O fission, C_6H_5-$COO]^+$ and 421 $[M – 121]^+$ suggested the linkage of benzyloxy group in the molecule. The ion peaks generated at *m/z* 281 [C_{10} – O fission, $CH_3(CH_2)_7CH=CH(CH_2)_7COO]^+$, 265 [$C_{1''}$ - O fission, $CH_3(CH_2)_7CH=CH(CH_2)_7CO]^+$ and 261 $[M – 281]^+$ indicated the attachment of linoleic acid to molecule. The ^{1}H NMR of **7** exhibited two two-proton multiplets at δ 7.75 and 7.51 and a one-proton multiplet a δ 7.31 assigned to aromatic H-2' to H-6' protons, two one-proton multiplets at δ 5.34 and 5.30 ascribed to vinylic H-9" and H-10" protons, respectively, a two-proton triplet at δ 4.29 (J = 6.7) accounted to primary oxymethylene H_2-1, a two-proton doublet at δ 4.08 (J = 6.8 Hz) attributed to secondary oxymethylene H_2-10, two three-proton doublets at δ 0.97 (J = 6.5 Hz) and 0.93 (J = 6.7 Hz) and a three-proton triplet at δ 0.83 (J = 6.2 Hz) associated correspondingly to secondary Me-8 and Me-9 and primary Me-18" methyl protons. The remaining methine and methylene protons resonated between δ 2.36 – 1.23. The ^{13}CNMR spectra of compound **7** displayed signals for ester carbons at δ 168.17 (C-7') and 172.79 (C-1"), aromatic and vinylic carbons in the range of δ 138.23 – 127.38, oxymethylene carbons at δ 68.27 (C-1) and δ 71.74 (C-10) and methyl carbons at δ 19.12 (C-8), 19.87 (C-9) and 14.19 (C-18"). On the basis of the foregoing account the structure of **7** has been established as 1-benzoyloxy-10-(octadec-9"-enoyl) geranilane, a new monoterpenic diester (Fig 3).

Compound **8**, named as 1-benzoyloxy-10-stearyl geranilane, $[M]^+$ at *m/z* 544 ($C_{35}H_{60}O_4$), exhibited IR absorption bands for ester groups (1733 cm^{-1}), aromatic ring (1639, 1567, 1081 cm^{-1}) and aliphatic chain (739 cm^{-1}). The mass ion peaks arose at *m/z* 121 [C_1 – O fission, C_6H_5-$COO]^+$ and 423 $[M – 121]^+$ suggested the linkage of benzyloxy group in the molecule. The ion peaks formed at *m/z* 283 [C_{10} – O fission, $CH_3(CH_2)_{14}$-$COO]^+$, 267 [$C_{1''}$ - O fission, $CH_3(CH_2)_{14}$-$CO]^+$ and 261 $[M – 281]^+$ indicated the attachment of stearic acid to molecule. The ^{1}H NMR of **8** displayed two two-proton multiplets at δ 7.73 and 7.52 and a one-proton multiplet a

δ 7.37 assigned to aromatic H-2' to H-6' protons, a two-proton triplet at δ 4.25 (J = 6.7) accounted to primary oxymethylene H_2-1, a two-proton doublet at δ 4.11 (J = 7.2 Hz), attributed to secondary oxymethylene H_2-10, two three-proton doublets at δ 0.96 (J = 6.5 Hz) and 0.91 (J = 6.9 Hz) and a three-proton triplet at δ 0.83 (J = 6.7 Hz) associated correspondingly to secondary Me-8 and Me-9 and primary Me-18" methyl protons. The remaining methine and methylene protons resonated between δ 2.54 – 1.25. The ^{13}CNMR spectra of compound **8** exhibited signals for ester carbons at δ 167.72 (C-7') and 173.41 (C-1"), aromatic carbons between δ 138.24 – 128.81, oxymethylene carbons at δ 68.31 (C-1) and δ 71.83 (C-10) and methyl carbons at δ 19.17 (C-8), 19.86 (C-9) and 14.15 (C-18"). These evidences led to formulate the structure of **8** as 1-benzoyloxy-10-octadecanyl- geranilane, a new monoterpenic diester (Fig 3).

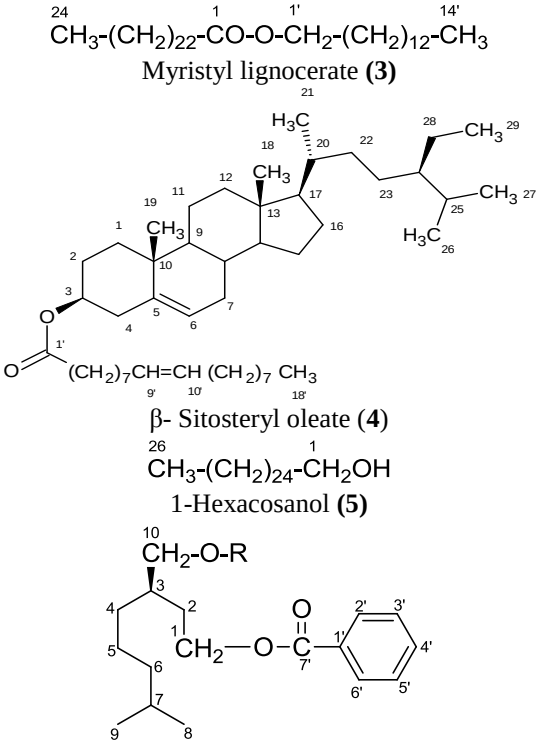


Fig 3: Compound 3 – 8 isolated from the root bark of *Premna mollissima*.

CONCLUSION

Phytochemical investigation of the seeds of *Celustrus paniculata* gave a mixed glyceride identified as glycerol-1-linoleio-2-oleo-3-stearate (**1**). The leaves of *Caesalpinia bonduc* afforded a new fatty acid characterized as *n*-triacont-5(Z),19(Z)-dienoic acid. The root barks of *Premna mollissima* furnished myristyl lignocerate (tetradecyl tetracosanoate, **3**), β-sitosteryl oleate (**4**), 1-hexacosanol (**5**) and new monoterpenic esters 1-benzoyloxy-10-plamityloxy geranilane (**6**), 1-benzoyloxy-10-(octadec-9"-enoyl) geranilane (**7**) and 1-benzoyloxy-10-octadecanyl geranilane (**8**). This work has enhanced understanding about the phytoconstituents of these plants. These compounds may be used as chromatographic markers for standardization of the respective plant parts.

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