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PHYTOCHEMICAL INVESTIGATION OF THE FRUITS OF *COCCINIA INDICA* AND LEAVES OF *TRICHODESMA INDICUM*

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ABSTRACT

Coccinia indica Wight et Arn. (family Cucurbitaceae) is a dioecious, perennial climber with ovoid-oblong, fleshy fruits. Its fruits are edible, used to treat abscesses, asthma, bronchitis, diabetes, fever, hypertension, jaundice, leprosy and tongue sores. *Trichodesma indicum* (L.) Lehm. (family Boraginaceae) is an erect, spreading, branched, annual plant. Its leaves are utilized as a diuretic and to relieve anorexia, arthritis, bleeding, cuts, dysentery, skin diseases, snake bites, stomach disorders, toxicity, urinary diseases and wounds. Our study was planned to isolate chemical constituents from the fruits of *C. indica* and leaves of *T. indicum* and to characterize their structures. Phytochemical investigation of a methanolic extract of the fruits of *C. grandis* afforded two new fatty acid glucosides identified as (Z)-docos-13-enoyl *O*-α-D-glucopyranoside (erucicyl-*O*-α-D-glucoside, **1**) and *n*-docosanoyl *O*-α-D-glucopyranoside (behenyl-*O*-α-D-glucoside, **2**) and two steroidal glucosides, viz., β-sitosterol 3-O-α-D-glucopyranoside (**3**) and β-sitosterol 3-O-α-D-glucopyranosyl-((6'→1")-O-α-D-glucopyranosyl-(6"→1"')-O-α-D-glucopyranoside (β-sitosterol α-D-triglucoside, **4**). The leaf methanolic extract of *T. indicum* furnished three fatty acid esters characterized as *n*-pentadecanyl octadec-9-enoate (*n*-pentadecanyl oleate, **5**), 1-nonacosanolyl (9Z)-octadecenoate (*n*-nonacosanyl oleate, **7**) and *n*-triacontanyl octadec-9-enoate (*n*-triacontanyl oleate, **8**) and a higher aliphatic carboxylic acid, viz., *n*-dotriacont- 5(Z),18(Z)-dienoic acid (**6**). The structures of all these compounds have been established on the basis of spectral data analyses and chemical reactions.

KEYWORDS: *Coccinia indica* fruits, *Trichodesma indicum* leaves, phytoconstituents, isolation, characterization.

INTRODUCTION

Coccinia indica Wight et Arn., syn. *Bryonia grandis* L., *Cephalandra grandis* Kurz, *Coccinia cordifolia* Cogn., *Coccinia grandis* (L.) Voigt, *Coccinia wightiana* M. Roem. and *Momordica bicolor* Blume (family Cucurbitaceae), known as kundru, tendli, ivy gourd and scarlet gourd, is distributed from Africa to Asia including India, the Philippines, Cambodia, China, Indonesia, Malaysia, Myanmar, Saudi Arabia, Thailand, Vietnam, eastern Papua New Guinea, and Australia. This plant is a dioecious, perennial climber with single, simple tendrils; rootstock tuberous; leaves glabrous, 5 lobes, base cordate, margin denticulate, apex acute, upper surface punctate, lower surface glandular; flowers axillary, solitary, campanulate, unisexual, corolla white; fruits ovoid-oblong, fleshy, pulp red; seeds oblong, compressed, many.[1-3] The plant aerial parts are anti-snake venom and detoxicant, taken to relieve chest pain, eyes diseases and headache. A plant decoction is applied on the forehead to cure vertigo.[4,5] The fruits are edible, used to cure abscesses, asthma, bronchitis, diabetes, fever, hypertension, jaundice, leprosy and sore of the tongue.[4-6] The roots are beneficial to manage diabetes, enlarged glands, glycosuria, intestinal disorders, joint pain, osteoarthritis and skin diseases.[6,7] The leaves are edible, recommended to comfort bronchitis, bronchial catarrh, chest colds, diabetes, gonorrhoea, hypertension and urinary tract infection. A leaf paste is applied to relieve earache, headache, rheumatism, scabies, skin eruptions and tonsilitis. A leaf infusion is given to women during child birth. The leaves mixed with ghee are lapped to calm down pain on left side of the abdomen. Leaf juice is messaged on the forehead as a refrigerant and on tumours. Juice from the leaves and roots is hypoglycaemic, antiamoebic, anti-inflammatory, anti-snake venom, effective against bruises, gonorrhoea, jaundice, scorpion and wasp poisoning.[4-8] Stem juice is dropped into the eyes to cure cataract. The flowers are ingested to prevent jaundice. The bark is cathartic, prescribed to overcome gonorrhoea.[6] In veterinary medicine, the leaves are fed to cattle against allergy, dysentery, ephemeral fevers, epistaxis, opacity of cornea, sprains and tympany. The fruits are given to cure dizziness, applied for yolk galls, and with *Gmelina asiatica* to kill lice and insects. The roots with *Helicteres isora* roots and *Jasminum auriculatum* leaves are fed to treat tympany.[6]

The fruits of *C. indica* afforded taraxerone, taraxerol, lupeol, β-amyrin, cucurbitacins, (24R)-24-ethylcholest-5-en-3β-ol glucoside, β-carotene, lycopene, cryptoxanthin, apo-6 ′-lycopenal, β-sitosterol, cucurbitacin B, xyloglucan, stigma-7-en-3-one, amino acids, fatty acids, steroidal saponins, flavonoids, pectin, polyprenol, arabinose, xylose, mannose, galactose and glucose, and coccinosides A - C.[9 – 16] The unripe fruits contained phenolic compounds, carbohydrates, glycosides, steroids, oils and fats.[17] The seeds yielded fat and fixed oil consisting of glycerides of linoleic, oleic and palmitic acids. The stems of *C. grandis* from Tiengiang (Vietnam) yielded 4-hydroxy benzaldehyde, 3,4'-O-dimethyl cedrusin 9'-O-glucopyranoside, (+)-medioresinol, syringaldehyde, vanillic acid and (+)-syringaresinol.[18] The leaves produced ferulic acid, methyl caffeate, ligstroside, trans-p-coumaric acid and kaempferol-3-O-β-D-glucoside, 1-tert-butyl-5,6,7-trimethoxyisoquinolene, rhamnose and other sugars.[19, 20] The leaf essential oil was composed mainly of *n*-tetracosane (39.18%), *n*-eicosane (30.04%), tetratriacotane (2.97%), 7-octadecanal and tricosane.[21] The roots furnished a triterpenoid saponin coccinioside, a flavonoid glycoside ombuin 3-O- arabinofuranoside, β-hydroxylup-20(29)-en-28-oic acid, lupeol, β-amyrin, β-sitosterol and stigmast -7-en-3-one, . resin, alkaloids, starch, fatty acids, carbonic acid, lupeol and taraxerol.[14, 22-24] The whole plant yielded amino acids, polysaccharides, heptacosane, cephalandrol, tritriacontane, β- sitosterol and cephalandrines A and B.[14, 25]

Trichodesma indicum (L.) Lehm., syn. *Borago indica* L., *T. amplexicaule* Roth and *T. hirsutum* Edgew. (family Boraginaceae), known as andhahuli, chota kulpha and Indian borage, is a native to Afghanistan, Bangladesh, Indian subcontinent, Philippines and Thailand; introduced into African countries. It is an erect, spreading, branched, annual plant, up to 50 cm tall; leaves sessile, lanceolate-oblong, apex acute; inflorescences terminal, leafy, flowers axillary; nutlets oblong ovoid, smooth, whitish.[26] The plant is acrid, anodyne, anti-inflammatory, antitoxin, bitter, carminative, depurative, diuretic, emollient, febrifuge, ophthalmic, pectoral and thermogenic, used to treat arthralgia, cough, diarrhoea, dysentery, dysmenorrhoea, dyspepsia, inflammations, influenza, skin diseases, strangury and vitiated states of phlegm and wind.[27] A leaf infusion is depurative. The leaves and roots are used as a diuretic and to treat anorexia, arthritis, bleeding, cuts, dysentery, skin diseases, snake bites, stomach disorders, toxicity, urinary diseases and wounds. A root paste is applied to reduce joint swelling, skin injuries and to relieve anasarca and body ache. The flowers are pectoral and sudorific.[26, 27]

The Indian borage plant contained hexacosane, α-amyrin, lupeol, nonsteroidal compounds, fatty constituents, β-sitosterol, catechin and gallic acid,[28,29] ethyl hexacosanoate, 21,24-hexacosadienoic acid ethyl esters and saponins,[30] ethyl iso-allocholate, catechin, rutin, palmitic, linolenic, gallic, salicylic and benzoic acids,[31] flavonoids, terpenoids and tannins, oleanolic acid, lupeol and linoleic acid.[32,33] The leaves afforded phytol, tetradecamethyl-cyclo-heptasiloxane, hexadecanoic acid methyl ester, cis-10-heptadecenoic acid, cycloheptadecanone, hexacosane, ethyl hexacosanoate and 21, 24-hexacosadienoic acid ethyl ester.[35-37] The seed oil was composed of glycerides of fatty acids - oleic, linoleic, palmitic, stearic, and linolenic acids.[38] The roots yielded *n*-decanyl laurate, *n*-tetradecanyl laurate, *n*-nonacosanyl palmitate, stigmast-5-en-3β-ol-21(24)-olide, *n*-pentacos-9-one, *n*-dotriacont-9-one-13-ene, stigmast-5-en-3β-ol-23-one and lanast-5-en-3β-D-glucopyranosyl-21 (24)-olide.[39]

Keeping in view the various therapeutic values of the plants and the development of ecofriendly, biodegradable and safer herbal preparations, fruits of *Coccinia grandis* and leaves of *Trichodesma indicum* collected from Delhi were extracted with methanol. The concentrated methanolic extracts was used for the isolation of chemical constituents. Structures of the isolated phytoconstituents 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.[40, 41]

General procedures

The melting points were measured on a thermoelectrically operated Perfit apparatus and are uncorrected. UV spectra were recorded on Lambda Bio 20 spectrophotometer (Perkin Elmer, Schwerzenbach, Switzerland) in methanol. The IR spectra were determined on Jasco FT/IR-5000 Spectrometer (FTS 135, Hong Kong) using KBr pellet. The ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR spectra were scanned on Bruker DRX spectrometer (Rheinstetten, 2 Germany) using $CDCl_3$ or DMSO-d_6 as a solvent and TMS (Fluka analytical, Sigma-Aldrich, Netherland) as an internal standard. The coupling constants (J values) are expressed in Hertz (Hz). Mass spectrometric detection was carried out on (Q-TOF-ESI) (Waters Corp., UK) instrument with a +ve ESI Mode 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 (Qualigens, Mumbai, India) with 60-120 mesh particle size and petroleum ether, chloroform, methanol and other solvents used were purchased from Merck Specialties (E. Merck Pvt. Ltd., New Delhi, India). 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 vapours or under UV radiations (254 and 366 nm) and spraying with ceric sulphate solution.

Collection of plant materials

The fresh fruits of *C. grandis* and leaves of *T. indicum* were procured from Delhi. These plant materials were identified by Prof. M. P. Sharma, Taxonomist, Department of Botany, Faculty of Science, Jamia Hamdard. The voucher specimens of these drugs are preserved in the Department of Pharmacognosy and Phytochemistry, School of Pharmaceutical Education and Research, Jamia Hamdard, New Delhi.

Extraction and isolation

The pulverized materials (2.0 kg each) were extracted exhaustively in a Soxhlet apparatus with methanol. The combined extracts of each drug were dried under reduced pressure separately to secure a viscous dark brown residues (278 g and 416 g, respectively). A small portion of the each extract was analyzed chemically to determine the presence of different types of chemical constituents. The dried residues (200 g each) were dissolved in minimum amount of methanol and adsorbed on silica gel column grade (60-120 mesh) to obtain slurries. The slurries were air-dried and chromatographed individually over a silica gel column loaded in petroleum ether (b. p. 60 - 80°C). 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, *v/v*). Various fractions were collected 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 chemical constituents from the fruits of *Coccinia grandis*

Erucicyl O-α-D-glucoside (1)

Elution of the column with chloroform-methanol (19:1) yielded a yellow powder of **1**, recrystallized from acetone - methanol (1:1), 221 mg, m. p. 118 – 119 ^{o} C, UV λmax (MeOH): 213 nm, IR υ_{max} (KBr): 3410, 3366, 3281, 2919, 2849, 1739, 1627, 1467, 1376, 1261, 1169, 1081, 1038, 720 cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 5.37 (1H, m, $w_{1/2}$ = 5.3 Hz, H-13), 5.23 (1H, m, $w_{1/2}$ = 3.2 Hz, H-14), 2.52 (2H, t, J = 7.1 Hz, H_2-2), 1.93 (2H, m, H_2-12), 1.91 (2H, m, H_2-15), 1.51 (2H, m, H_2-3), 1.33 (2H, m, H_2-4), 1.30 (4H, m, H_2-11, H_2-16), 1.27 (4H, m, H_2-10, H_2-17), 1.23 (18H, brs, 9 x CH_2), 0.84 (3H, t, J = 6.5 Hz, Me-22), 5.57 (1H, d, J = 5.13 Hz, H-1'α), 4.52 (1H, m, H-5'), 4.13 (1H, dd, J = 5.1, 6.5 Hz, H-2'), 3.85 (1H, m, H-3'), 3.67 (1H, m, H-4'), 3.10 (2H, d, J = 8.5 Hz, H_2-6'); ^{13}C NMR (DMSO-d_6): δ 171.89 (C-1), 48.63 (C-2), 31.30 (C-3), 30.16 (C-4), 29.61 (C-5), 29.12 (C-6), 29.02 (C-7), 28.72 (C-8), 28.67 (C-9), 31.32 (C-10), 29.12 (C-11), 32.41 (C-12), 129.61 (C-13), 129.63 (C-14), 29.08 (C-15), 29.03 (C-16), 29.01 (C-17), 26.24 (C-18), 24.78 (C-19), 24.17 (C-20), 22.11 (C-21), 13.88 (C-22), 103.47 (C-1'), 73.31 (C-2'), 72.83 (C-3'), 70.91 (C-4'), 76.73 (C-5'), 61.48 (C-6'); ESI-MS *m/z* (rel.int.): 500 $[M]^+$ ($C_{28}H_{52}O_7$) (1.8), 337 (100), 321 (8.2), 179 (3.5), 163 (11.7).

Behenyl O-α-D-glucoside (**2**)

Further elution of the column with chloroform-methanol (19:1) furnished a buff-colored powder of **2**, recrystallized from chloroform-methanol (1:1), 270 g, R_f 0.35 (chloroform-methanol, 9:1), m. p. 123 – 124 ^{0}C; UV λmax (MeOH): 210 nm; IR υ_{max} (KBr): 3311, 3250, 2918, 2849, 1733, 1468, 1392, 1178, 1104, 1047, 991, 944, 721 cm^{-1}; ^{1}H NMR (DMSO-d_6) : δ 2.27 (2H, t, J = 7.6 Hz, H_2-2), 1.53 (2H, m, H_2-3), 1.31 (2H, m, H_2-4), 1.27 (4H, m, H_2-5, H_2-6), 1.24 (30 H, brs, 15 x CH_2), 0.86 (3 H, t , J = 6.8 Hz, Me-22), 4.97 (1H, d, J = 4.8 Hz, H-1'), 4.45 (1H, m, H-5'), 4.02 (1H, dd, J = 4.4, 4.8 Hz, H-2'), 3.91 (1H, m, H-3'), 3.65 (1H, m, H-4'), 3.19 (2H, m, H_2-6'); ^{13}C NMR (DMSO-d_6): δ 171.62 (C-1), 33.69 (C-2), 32.92 (C-3), 31.30 (C-4), 30.71 (C-5), 29.7 2 (C-6), 29.06 (C-7), 29.03 (C-8 to C-16), 29.01 (C-17), 28.52 (C-18), 25.11 (C-19), 24.56 (C-20), 22.10 (C-21), 14.43 (C-22), 99.49 (C-1'), 69.74 (C-2'), 66.12 (C-3'), 63.07 (C-4'), 78.91 (C-5'), 60.41 (C-6'); ESI-MS *m/z* (rel.int.): 502 $[M]^+$ ($C_{28}H_{54}O_7$) (2.8), 339 (100), 163 (9.2).

β-Sitosterol α-D-glucoside (3)

Elution of the column with chloroform – methanol (9:1) afforded an amorphous powder of **3**, recrystallized from chloroform-methanol (1:3), yield 186 mg, R_f 0.4 (chloroform-methanol, 5:2), m. p. 275-277 °C; UV λmax (MeOH): 207 nm (log ε 3.1); IR υ_{max} (KBr): 3410, 3311, 2919, 2849, 1639, 1465, 1378, 1254, 1196, 1166, 1073, 1023, 824 cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 5.32 (1H, d, J = 7.8 Hz, H-6), 3.49 (1H, brs, $w_{1/2}$ = 18.5 Hz, H-3), 1.01 (3H, brs, Me-19), 0.91 (3H, d, J = 6.5 Hz, Me-21), 0.87 (3H, d, J = 6.6 Hz, Me-26), 0.84 (3H, d, J = 6.3 Hz, Me-27), 0.81 (3H, t, J = 6.5 Hz, Me-29), 0.66 (3H, brs, Me-18), 2.55 - 1.03 (29H, m, 11 × CH_2, 7 × CH), 4.90 (1H, d, J = 4.2 Hz, H-1′), 4.85 (1H, m, H-5′), 4.39 (1H, dd, J = 4.2, 5.8 Hz, H-2′), 4.24 (1H, m, H-3′), 3.66 (1H, m, H-4′), 3.08 (2H, d, J = 7.9 Hz, H_2- 6′); ^{13}C NMR (DMSO-d_6): δ 38.90 (C-1), 31.37 (C-2), 73.37 (C-3), 41.81 (C-4), 141.06 (C-5), 121.15 (C-6), 39.31 (C-7), 35.47 (C-8), 49.56 (C-9), 36.18 (C-10), 22.54 (C-11), 39.52 (C-12), 39.73 (C-13), 56.14 (C-14), 28.59 (C-15), 29.03 (C-16), 55.36 (C-17), 11.72 (C-18), 19.05 (C-19), 34.26 (C-20), 18.82 (C-21), 33.97 (C-22), 26.18 (C-23), 45.11 (C-24), 29.23 (C-25), 18.52 (C-26), 19.64 (C-27), 20.55 (C-28), 11.60 (C-29), 100.78 (C-1'), 76.71 (C-2'), 71.68 (C-3'), 69.98 (C-4'), 76.97 (C-5'), 61.27 (C- 6'); ESI-MS *m/z* (rel. int.): 576 $[M]^+$ ($C_{35}H_{60}O_6$) (1.8), 413 (21.2), 397 (10.8), 179 (9.6), 163 (32.5).

β-Sitosterol α-D-triglucoside (4)

Elution of the column with chloroform – methanol (17:3) furnished a light brown powder of **4**, recrystallized from chloroform-methanol (1:1), yield 252 mg, R_f 0.53 (benzene-chloroform-methanol, 5:4:1), m. p. 270 - 272 °C; UV λmax (MeOH): 212 nm (log ε 4.2); IR υ_{max} (KBr): 3515, 3411, 3360, 3241, 2918, 2851, 1636, 1439, 1365, 1246, 1121, 1024 cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 5.34 (1H, m, H-6), 3.59 (1H, brs, $w_{1/2}$ = 18.2 Hz, H-3α), 0.97 (3H, brs, Me-19), 0.91 (3H, d, J = 6.1 Hz, Me-21), 0.86 (3H, d, J = 6.3 Hz, Me-26), 0.83 (3H, d, J = 6.5 Hz,

Me-27), 0.81 (3H, t, J = 6.2 Hz, Me-29), 0.68 (3H, brs, Me-18), 2.51 - 1.02 (29H, m, 11 × CH_2, 7 × CH), 5.11 (1H, d, J = 4.8 Hz, H-1′), 4.30 (1H, m, H-5′), 3.86 (1H, m, H-2′), 3.52 (1H, m, H-3′), 3.46 (1H, m, H-4′), 3.36 (2H, d, J = 8.8 Hz, H_2- 6′), 4.89 (1H, d, J = 3.8 Hz, H-1″), 4.28 (1H, m, H-5″), 3.83 (1H, m, H-2″), 3.49 (1H, m, H-3″), 3.44 (1H, m, H-4″), 3.18 (2H, d, J = 8.5 Hz, H_2- 6″), 4.82 (1H, d, J = 4.5 Hz, H-1‴), 4.23 (1H, m, H-5‴), 3.80 (1H, m, H-2‴), 3.47 (1H, m, H-3‴), 3.41 (1H, m, H-4‴), 3.10 (2H, d, J = 9.1 Hz, H_2- 6‴); ^{13}C NMR (DMSO-d_6): δ 37.28 (C-1), 31.17 (C-2), 72.29 (C-3), 42.31 (C-4), 140.89 (C-5), 121.72 (C-6), 29.71 (C-7), 31.87 (C-8), 50.04 (C-9), 36.67 (C-10), 22.56 (C-11), 38.73 (C-12), 39.86 (C-13), 56.62 (C-14), 28.27 (C-15), 29.12 (C-16), 55.85 (C-17), 12.24 (C-18), 19.38 (C-19), 35.94 (C-20), 19.07 (C-21), 33.77 (C-22), 25.82 (C-23), 45.56 (C-24), 29.97 (C-25), 21.05 (C-26), 19.57 (C-27), 24.33 (C-28), 12.14 (C-29), 104.28 (C-1'), 75.68 (C-2'), 73.09 (C-3'), 68.16 (C-4'), 82.31 (C-5'), 63.85 (C- 6'), 102.19 (C-1"), 73.91 (C-2"), 72.93 (C-3"), 71.17 (C-4"), 77.34 (C-5"), 63.42 (C- 6"), 101.18 (C-1'"), 73.19 (C-2'"), 72.85 (C-3'"), 70.29 (C-4'"), 77.18 (C-5'"), 61.52 (C- 6'"); ESI-MS *m/z* (rel. int.): 900 $[M]^+$ ($C_{47}H_{80}O_{16}$) (1.3), 487 (8.1), 413 (7.3), 397 (10.8), 271 (21.5), 341 (12.7), 212 (10.6), 179 (5.8), 163 (6.2).

Isolation of phytoconstituents from the leaves of *Trichodesma indicum*

***n*-Pentadecanyl oleate (5)**

Elution of the column with petroleum ether – chloroform (1:1) gave a pale yellow semisolid mass of **5**, yield 191 mg, UV λmax (MeOH): 207 nm (log ε 4.3); IR υ_{max} (KBr): 2925, 2852, 1739, 1635, 1461, 1373, 1170, 1116, 1018, 723 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 5.35 (1H, m, H-9), 5.33 (1H, m, H-10), 4.15 (2H, t, J = 7.3 Hz, H_2 -1′), 2.77 (2H, t, J = 6.8 Hz, H_2 -2), 2.30 (2H, m, H_2 -8), 2.11 (2H, m, H_2 -11), 1.63 (2H, m, H_2-7), 1.57 (2H, m, H_2-3), 1.35 (4H, m, H_2 -12, H_2 -2′), 1.28 (40H, brs, 20 x CH_2), 0.89 (3H, t, J = 6.4 Hz, Me-18), 0.85 (3H, t, J = 6.5 Hz, Me-15′); ^{13}C NMR ($CDCl_3$): δ 173.16 (C-1), 51.46 (C-2), 29.74 (C-3), 29.43 (C-4), 29.34 (C-5), 29.69 (C-6), 29.67 (C-7), 34.30 (C-8), 129.95 (C-9), 127.82 (C-10), 31.80 (C-11), 29.70 (C-12), 29.41 (C-13), 29.23 (C-14), 28.86 (C-15), 27.13 (C-16), 24.86 (C-17), 14.07 (C-18), 63.26 (C-1′), 29.73 (C-2′), 29.71 (C-3′), 29.65 (C-4′), 29.64 (C-5′), 29.57 (C-6′), 29.50 (C-7′), 29.30 (C-8′), 29.28 (C-9′), 29.12 (C-10′), 29.08 (C-11′), 28.27 (C-12′), 27.16 (C-13′), 22.66 (C-14′), 12.99 (C-15′); ESI MS *m/*z (rel.int.): 492 $[M]^+$ ($C_{33}H_{64}O_2$) (3.8), 281 (8.5), 265 (14.8).

***n*-Dotriacont-15(Z),18(Z)-dienoic acid (6)**

Elution of the column with petroleum ether - chloroform (1:3) afforded pale yellow crystals of **6**, recrystallized from chloroform – methanol (1 : 1), 132 mg; m. p. 113-114 ^{0}C; UV λmax (MeOH): 212 nm (log ε 4.6); IR υmax (KBr): 3421, 2918, 2856, 1705, 1635, 1415, 1309, 1244, 1172, 1024, 952, 721 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 5.36 (1H, m, $w_{1/2}$ = 8.9 Hz, H-15), 5.34 (1H, m, $w_{1/2}$ = 9.1 Hz, H-16), 5.31 (1H, m, $w_{1/2}$ = 10.1 Hz, H-18), 5.26 (1H, m, $w_{1/2}$ = 10.6 Hz, H-19), 2.76 (2H, m, H_2-17), 2.31 (2H, t, J = 7.2 Hz, H_2-2), 2.05 (2H, m, H_2-14), 2.01 (2H, m, H_2-20), 1.62 (2H, m, H_2-3), 1.59 (2H, m, H_2-4), 1.56 (2H, m, H_2-4), 1.30 (18 H, brs, 9 × CH_2), 1.25 (20 H, brs, 10 × CH_2), 0.89 (3H, t, J = 6.8 Hz, Me-32); ^{13}C NMR ($CDCl_3$): δ 179.92 (C-1), 31.56 (C-2), 29.70 (C-3), 29.63 (C-4), 29.63 (C-5), 29.57 (C-6), 29.30 (C-7), 29.36 (C-8), 29.36 (C-9), 29.41 (C-10), 29.45 (C-11), 29.50 (C-12), 29.65 (C-13), 29.80 (C-14), 130.20 (C-15), 130.01 (C-16), 33.97 (C-17), 129.73 (C-18), 127.93 (C-19), 29.77 (C-20), 29.57 (C-21), 29.30 (C-22), 29.21 (C-23), 29.14 (C-24), 29.03 (C-25), 27.75 (C-26), 27.23 (C-27), 25.66 (C-28), 24.90 (C-29), 24.78 (C-30), 22.73 (C-31), 14.13 (C-32); ESI MS *m/z* (rel. int.): 476 $[M]^+$ ($C_{32}H_{60}O_2$) (100), 361 (23.8), 253 (13.3), 249 (12.6), 209 (9.3), 183 (12.5).

***n*-Nonacosanyl oleate (7)**

Elution of the column with petroleum ether – chloroform (1:3) afforded a pale yellow semisolid mass of **7**, yield 179 mg, UV λmax (MeOH): 207 nm (log ε 4.3); IR υ_{max} (KBr): 2933, 2850, 1734, 1636, 1452, 1377, 1246, 1168, 1099, 725 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 5.36 (1H, m, H-9), 5.32 (1H, m, H-10), 4.12 (2H, t, J = 6.1 Hz, H_2 -1′), 2.31 (2H, t, J = 7.6 Hz, H_2 -2), 2.03 (2H, m, H_2 -8), 2.01 (2H, m, H_2 -11), 1.61 (2H, m, H_2-3), 1.59 (2H, m, H_2-7), 1.33 (2H, m, H_2 -12), 1.30 (8H, brs, 4 x CH_2), 1.28 (56H, brs, 28 x CH_2), 0.89 (3H, t, J = 6.5 Hz, Me-18), 0.86 (3H, t, J = 7.2 Hz, Me-29′); ^{13}C NMR ($CDCl_3$): δ 173.30 (C-1), 51.24 (C-2), 31.44 (C-3), 30.39 (C-4), 29.77 (C-5), 29.71 (C-6), 29.71 (C-7), 34.11 (C-8), 129.75 (C-9), 128.05 (C-10), 31.93 (C-11), 29.71 (C-12), 29.69 (C-13), 29.67 (C-14), 29.63 (C-15), 27.20 (C-16), 24.87 (C-17), 14.12 (C-18), 63.67 (C-1′), 29.71 (C-2′ to C-14′), 29.48 (C-15′), 29.37 (C-16′), 29.33 (C-17′), 29.28 (C-18′), 29.20 (C-19′), 29.18 (C-20′), 29.16 (C-21′), 29.12 (C-22′), 29.09 (C-23′), 29.05 (C-24′), 28.89 (C-25′), 27.97 (C-26′), 25.83 (C-27′), 22.69 (C-28′), 14.08 (C-29′); ESI MS *m/*z (rel.int.): 688 $[M]^+$ ($C_{47}H_{92}O_2$) (52.5), 423 (16.1), 281 (12.3), 265 (10.4).

***n*-Triacontanyl oleate (8)**

Further elution of the column with petroleum ether – chloroform (1:3) furnished colourless crystals of **8**, yield 127 mg, m. p. 107- 109 ^{o}C, UV λmax (MeOH): 207 nm (log ε 4.8); IR υ_{max} (KBr): 2924, 2852, 1732, 1635, 1463, 1374, 1243, 1176, 1062, 974, 722 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 5.33 (1H, m, H-9), 5.31 (1H, m, H-10), 4.15 (2H, t, J = 7.2 Hz, H_2 -1′), 2.32 (2H, t, J = 7.6 Hz, H_2 -2), 2.01 (2H, m, H_2 -8), 1.98 (2H, m, H_2 -11), 1.62 (2H, m, H_2-3), 1.58 (2H, m, H_2-7), 1.31 (18H, brs, 9 x CH_2), 1.25 (56H, brs, 28 x CH_2), 0.89 (3H, t, J = 6.4 Hz, Me-18), 0.86 (3H, t, J = 6.8 Hz, Me-30′); ^{13}C NMR ($CDCl_3$): δ 173.95 (C-1), 51.29 (C-2), 31.68 (C-3), 29.69 (C-4), 29.64 (C-5), 29.63 (C-6), 29.61 (C-7), 31.72 (C-8), 129.91 (C-9), 128.01 (C-10), 31.86 (C-11), 29.61 (C-12), 29.61 (C-13), 29.61 (C-14), 28.91 (C-15), 25.57 (C-16), 24.69 (C-17), 14.02 (C-18), 64.87 (C-1′), 29.61 (C-2′ to C-17′), 29.53 (C-18′), 29.55 (C-19′), 29.45 (C-20′), 29.40 (C-21′), 29.28 (C-22′), 29.25 (C-23′), 29.21 (C-24′), 29.10 (C-25′), 29.05 (C-26′), 28.77 (C-27′), 27.15 (C-

28′), 22.62 (C-29′), 14.04 (C-30′); ESI MS *m/z* (rel.int.): 702 $[M]^+$ ($C_{48}H_{94}O_2$) (100), 437 (8.6), 281 (11.2), 265 (13.4).

RESULTS AND DISCUSSION

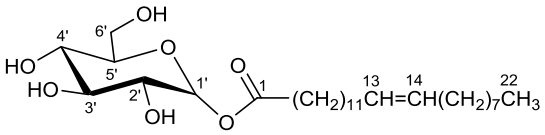
Compound **1**, designated as erucicyl O-α-D-glucoside, responded positive tests for glycosides and displayed characteristic IR absorption bands for hydroxyl groups (3410, 3366, 3281 cm^{-1}), ester function (1739 cm^{-1}), unsaturation (1627 cm^{-1}) and long chain aliphatic hydrocarbon (720 cm^{-1}). Its molecular ion peak was determined at *m/z* 500 on the basis of mass and ^{13}C NMR spectral data consistent with a molecular formula of a fatty acid glycoside $C_{28}H_{52}O_7$. The mass ion fragments generated at *m/z* 321 [C_1 – O fission, CH_3-$(CH_2)_7CH$=CH-$(CH)_{11}$-CO]$^+$, 179 [M – 321, $C_6H_{11}O_6]^+$, 337 [$C_{1'}$ – O fission, CH_3-$(CH_2)_7CH$=CH-$(CH)_{11}$-COO]$^+$ and 163 [M - 336, $C_6H_{11}O_5]^+$ indicated that a hexose unit was linked with a C-22 unsaturated erucic fatty acid. The ^1H NMR spectrum of **1** exhibited two one-proton multiplets at δ 5.37 and 5.23 with half- widths of 5.3 and 3.1 Hz assigned to cis-oriented vinylic H-13 and H-14 protons of erucic acid, respectively, methylene protons between δ 2.52 – 1.23 and a three-proton triplet at δ 0.84 (J = 6.5 Hz) ascribed to primary C-22 methyl protons. A one-proton doublet at δ 5.57 (J = 5.1 Hz) was attributed to α-oriented anomeric H-1′ proton, other sugar protons as one-protons multiplets due to oxymethine protons at δ 4.52 (H-5'), 3.85 (H-3') and 3.67 (H-4'), as a one-proton double doublet at δ 4.13 (J = 5.1, 6.5 Hz, H-2') and as a two-proton doublet at δ 3.10 (J = 8.5 Hz) accounted to hydroxymethylene H_2-6' protons. The ^{13}C NMR spectrum of **1** displayed signals for the ester carbon at δ 171.89 (C-1), vinylic carbons at δ 129.61 (C-13) and 129.63 (C-14), anomeric carbon at δ 103.47 (C-1′), other sugar carbons in the range from δ 76.73 to 61.48, methylene carbons between δ 48.63 - 22.118 and methy1 carbon at δ 13.88 (C- 22). Acid hydrolysis of **1** yielded erucic acid, m. p. 31-33 ^{o} C, and D-glucose, R_f 0.26 (*n*-butanol- acetic acid – water, 4 : 1 : 5). On the basis of these spectral data analysis and chemical reactions, the structure of **1** has been established as (Z)-docos-13-enoyl *O*-α-D-glucopyranoside, a new acyl glucoside (Fig. 1).

Compound **2**, designated as behenyl O-α-D-glucoside, gave glycosidic tests positively and exhibited distinctive IR absorption bands for hydroxyl groups (3311, 3250 cm^{-1}), ester function (1733 cm^{-1}) and long chain aliphatic hydrocarbon (721 cm^{-1}). On the basis of mass and ^{13}C NMR spectral data the molecular ion peak of **2** was established at *m/z* 502 consistent with a molecular formula of a fatty acid glycoside $C_{28}H_{54}O_7$. The mass ion fragments generated at *m/z* 163 [$C_{1'}$ - O fission, $C_6H_{11}O_5]^+$ and 339 [M – 163, CH_3-$(CH_2)_{20}$-COO]$^+$ suggested that a hexose unit was linked with a C-22 saturated fatty acid (behenic acid). The ^1H NMR spectrum of **2** exhibited a two-proton triplet at δ 2.27 (J = 7.6 Hz) assigned to methylene H_2-2 protons adjacent to the ester group, other methylene protons as multiplets at δ 1.53 (H_2-3), 1.31 (H_2-4) and 1.27 (H_2-5, H_2-6) and as a singlet at δ 1.24 (15 x CH_2) and a three-proton triplet at δ 0.86 (J = 6.8 Hz) ascribed to primary C-22 methyl protons. A one-proton doublet at δ 4.97 (J = 4.8 Hz) was attributed to α-oriented anomeric H-1′ proton. The remaining sugar protons resonated as one-protons multiplets from δ 4.45 to 3.65 accounted to carbinol H-2′ to H-4′ protons and as a two-proton multiplet at δ 3.19 associated with hydroxymethylene H_2-6′ protons. The ^{13}C NMR spectrum of **2** displayed signals for the ester carbon at δ 171.62 (C-1), anomeric carbon at δ 99.49 (C-1′), other sugar carbons in the range from δ 78.91 to 60.41, methylene carbons between δ 33.69 - 22.10 and methy1 carbon at δ 14.43 (C- 22). Acid hydrolysis of **2** yielded behenic acid, m. p. 78 – 80 ^{0}C, R_f 0.47 (chloroform-methanol, 1:1), and D-glucose, R_f 0.26 (*n*-butanol- acetic acid – water, 4 : 1 : 5). On the basis of these evidences the structure of **2** has been elucidated as *n*-docosanoyl *O*-α-D-glucopyranoside, a new acyl glucoside (Fig. 1).

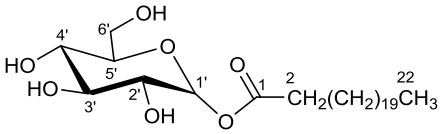
Compound **3**, $[M]^+$ at *m/z* 576 ($C_{35}H_{60}O_6$), gave positive tests of steroidal glycosides and showed IR absorption bands for hydroxyl groups (3410, 3311 cm^{-1}) and unsaturation (1639 cm^{-1}). The mass ion peaks generated at *m/z* 413 [M – $C_6H_{11}O_5$, 163]$^+$, 397 [413 - Me]$^+$ and 180 $[C_6H_{12}O_6]^+$ suggested that β-sitosterol was linked with a hexoside unit. The ^1H NMR spectrum of **3** displayed two one-proton doublets at δ 5.32 (J = 7.8 Hz) and 4.90 (J = 4.2 Hz) assigned to vinylic H-6 and α-oriented anomeric H-1′ protons, respectively. The other sugar proton appeared as one-proton multiplets at δ 4.85, 4.24 and 3.66, as a one-proton double doublet at δ 4.39 (J = 4.2, 5.8 Hz) correspondingly due to oxymethine H-5′, H-3′ , H-4′ and H-2′ protons and as a two-proton doublet at δ 3.08 (J = 7.9 Hz) accounted to hydroxymethylene H_2-6′ protons. A one-proton broad multiplet at δ 3.49 with half width of 18.5 Hz was attributed to *α*-oriented oxymethine H-3 proton. Two three-proton broad singlets at δ 0.66 and 1.01 were assigned to tertiary C-18 and C-19 methyl protons, respectively. Three doublets at δ 0.91 (J = 6.5 Hz), 0.87 (J = 6.6 Hz) and 0.84 (J = 6.3 Hz) and a triplet at δ 0.81 (J = 6.5 Hz), all integrating for three protons each, were accounted to secondary C-21, C-26 and C-27 methyl and primary C-29 methyl protons, respectively, all attached to the saturated carbons. The remaining methylene and methine protons resonated between δ 2.55 - 1.03. The ^{13}C NMR spectrum of **3** showed signals for 35 carbons including vinylic carbons at δ 141.06 (C-5) and 121.15 (C-6), oxymethine carbon at δ 73.37 (C-3), anomeric carbon at δ 100.78 (C-1′), other sugar carbons from δ 76.97 to 61.10 and the remaining methyl, methylene and methine carbons between δ 56.14 -11.60. The ^1H-^1H COSY spectrum exhibited interactions of H_2-4 with H_2-2, H-3 and H-6; H-8 with H-6, H_2-7, H-9 and H-14; H-17 with H_2-16, H_3-18, H-20, H_3-21 and H_2-22; H-25 with H_2-23, H-24, H_3-26 and H_3-27; and H-1′ with H-2′, H-3′, H-5′ and H-3. The ^1H NMR and ^{13}C NMR spectral data of the steroidal nucleus of **3** were compared with other stigmasterol-type molecules.[42,43] Acid hydrolysis of **3** yielded β-sitosterol,

R_f 0.35 (chloroform – methanol, 9:1); m. p. 137-138 ^{o} C; and D-glucose, R_f 0.26 (*n*-butanol- acetic acid – water, 4 : 1 : 5). On the basis of spectral data analysis and chemical reactions, the structure of **3** has been formulated as β-sitosterol 3-O-α-D-glucopyranoside, a rare steroidal glucoside (Fig. 1).

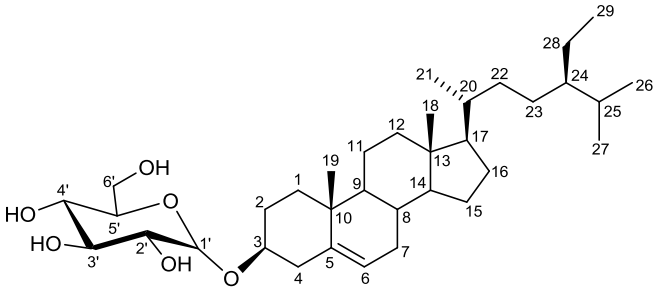
Compound **4**, $[M]^+$ at *m/z* 900 ($C_{47}H_{80}O_{16}$), responded positive tests of steroidal glycosides and displayed characteristic IR absorption bands for hydroxyl groups (3515, 3411, 3360, 3241 cm^{-1}) and unsaturation (1636 cm^{-1}). The mass ion peaks produced at *m/z* 413 [$C_{1'}$ – O fission, $C_{29}H_{49}O]^+$, 397 [C_3 – O fission, $C_{29}H_{49}]^+$, 271 [413 – side chain]$^+$ and 486 [M – 413, $C_{18}H_{31}O_{15}]^+$ indicated that a sterol was linked with a trihexoside unit. The ion fragments generated at *m/z* 163 [$C_{1'''}$ – O fission, $C_6H_{11}O_5]^+$, 179 [$C_{6''}$ – O fission, $C_6H_{11}O_5]^+$ and 341 [$C_{6'}$ – O fission, $C_{12}H_{21}O_{11}]^+$ also supported the existence of the trihexoside unit. The ^{1}H NMR spectrum of **4** showed a one-proton multiplet at δ 5.34 assigned to vinylic H-6 proton. A one-proton broad multiplet at δ 3.59 with half width of 18.2 Hz was attributed to *α*-oriented oxymethine H-3 proton. Two three-proton broad singlets at δ 0.68 and 0.97 were assigned to tertiary C-18 and C-19 methyl protons, respectively. Three doublets at δ 0.91 (J = 6.1 Hz), 0.86 (J = 6.3 Hz) and 0.83 (J = 6.5 Hz) and a triplet at δ 0.81 (J = 6.2 Hz), all integrating for three protons each, were accounted to secondary C-21, C-26 and C-27 methyl and primary C-29 methyl protons, respectively, all attached to the saturated carbons. The remaining methylene and methine protons resonated between δ 2.51 - 1.02. Three one-proton doublets at δ 5.11 (J = 4.8 Hz), 4.89 (J = 3.8 Hz) and 4.82 (J = 4.5 Hz) were ascribed to α-oriented anomeric H-1′, H-1″ and H-1‴ protons, respectively. The other sugar oxymethine protons appeared as one-proton multiplets between δ 4.30 – 3.41. Three two-proton doublets at δ 3.36 (J = 8.8 Hz), 3.18 (J = 8.5 Hz) and 3.10 (J = 9.1 Hz) were attributed correspondingly to sugar oxymethylene H_2- 6′, H_2- 6″ and H_2- 6‴ protons. The ^{13}C NMR spectrum of **4** showed signals for 47 carbons including vinylic carbons at δ 140.89 (C-5) and 121.72 (C-6), oxymethine carbon at δ 72.29 (C-3), methyl carbons in the range of δ 21.05 – 12.14, anomeric carbons at δ 104.28 (C-1′), 102.19 (C-1") and 101.18 (C-1‴) and other sugar carbons from δ 75.68 to 61.52. The DEPT spectrum of **4** showed the presence of six methyl, fourteen methylene and twenty three methine carbons. The presence of oxymethylene signals in the deshielded region as two-proton doublets at δ 3.36 (J = 8.8 Hz, H_2- 6′) and 3.18 (J = 8.5 Hz, H_2- 6″) and their respective carbon signals at δ 63.85 (C- 6') and 63.42 (C- 6") suggested attachment of the sugar units through (6'→1") and (6"→1‴) linkages, respectively. The ^{1}H-^{1}H COSY spectrum of **4** displayed interactions of H_2-4 with H_2-2, H-3 and H-6; H-8 with H-6, H_2-7, H-9 and H-14; H-17 with H_2-16, H_3-18, H-20, H_3-21 and H_2-22; H-25 with H_2-23, H-24, H_3-26 and H-27; H-1′ with H-2′, H-3′, H-5′ and H-3; H-1" with H-2", H-5" and H_2-6' ; and H-1‴ with H-2‴, H-5‴ and H_2-1″. The HMBC spectrum of **4** indicated that H-3, H_2-4, H-6 and H_2-7 interacted with C-5; H_2-15, H_2-16, H_3-18, H-20, H_3-21 and H_2-22 interacted with C-17; H-3, H-2′, H-3′ and H-5′ interacted with C- 1'; H_2- 6′, H-2″, H-3″ and H-5″ interacted with C-1″ ; and H_2- 6″, H-2‴, H-3‴, and H-5‴ interacted with C-1‴. The ^{1}H NMR and ^{13}C NMR spectral data of the steroidal nucleus of **4** were compared with other stigmasterol-type molecules.[42,43] Acid hydrolysis of **4** yielded β-sitosterol, R_f 0.35 (chloroform – methanol, 9: 1); m. p. 137-138 ^{o} C; and D-glucose, R_f 0.26 (*n*-butanol- acetic acid – water, 4 : 1 : 5). On the basis of spectral data analysis and chemical reactions, the structure of **4** has been determined as β-sitosterol 3-O-α-D-glucopyranosyl – ((6'→1")- O-α-D-glucopyranosyl-(6"→1‴)- O-α-D-glucopyranoside, a new steroidal glucoside (Fig. 1).



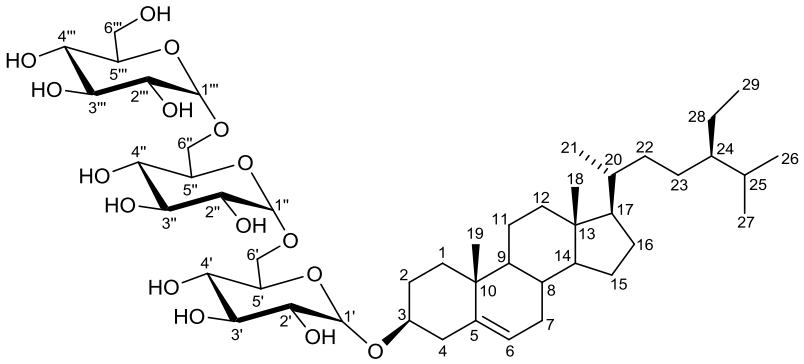
Erucicyl-*O*-α-D-glucoside **(1)**



Behenyl-*O*-α-D-glucoside **(2)**



β-Sitosterol-α-D-glucoside **(3)**



β-Sitosterol-α-D-triglucoside **(4)**

Fig. 1. Structural formulae of the phytoconstituents 1 - 4 isolated from the fruits of *Coccinia grandis*.

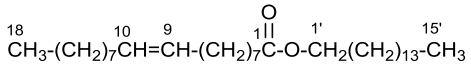
Compound **5** was a known fatty acid ester characterized as *n*-pentadecanyl octadec-9-enoate (*n*-pentadecanyl oleate) (Fig. 2).[44]

Compound **6** 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 a carboxylic group (3421, 1705 cm^{-1}), unsaturation (1635 cm^{-1}) and long aliphatic chain (721 cm^{-1}). Its molecular weight was established at *m/z* 476 on the basis of mass spectrum consistent with a molecular formula of the unsaturated fatty acid, $C_{32}H_{60}O_2$. The ion peaks arising at *m/z* 249 [C_{14}-C_{15} fission, $CH_3(CH_2)_{12}CH{=}CH\text{-}CH_2\text{-}CH{=}CH$, $C_{18}H_{33}]^+$ and 253 [C_{16}-C_{17} fission, $CH{=}CH\text{-}(CH_2)_{13}\text{-}COOH$, $C_{16}H_{29}O_2]$+ suggested the presence of one of the vinylic linkage at C_{15} carbon position and carboxylic group at one of the terminal carbon. The ion fragments produced at *m/z* 209 [C_{17}-C_{18} fission, $CH_3(CH_2)_{12}CH{=}CH$, $C_{15}H_{29}]^+$ and 183 [C_{19}-C_{20} fission, $CH_3(CH_2)_{12}$, $C_{13}H_{27}]^+$ indicated the existence of the another vinylic linkage at C-18 carbon position. The ^{1}H NMR spectrum of **6** showed four one-proton multiplets at δ 5.36, 5.34, 5.31 and 5.26 with half-width between 10.6 – 8.9 Hz assigned to cis-oriented vinylic H-15, H-16, H-18 and H-19 protons, respectively. A two-proton multiplet in the deshielded region at δ 2.76 was ascribed to methylene H_2-17 present between two vinylic carbons. A two-proton triplet at δ 2.31 (J = 7.2 Hz) was attributed to C_2 methylene protons adjacent to the carboxylic function. The other methylene protons resonated as two-proton multiplets at δ 2.05, 2.01, 1.62, 1.59 and 1.56, and as broad singlets at δ 1.30 (18 H) and 1.25 (20 H). A three-proton triplet at δ 0.89 (J = 6.8 Hz) was accounted to C- 32 primary methyl protons. The ^{13}C NMR spectrum of **6** exhibited signals for carboxylic carbon at δ 179.92 (C-1), vinylic carbons at δ 130.20 (C-15), 130.01 (C-16), 129.73 (C-18) and 127.93 (C-19), methylene carbons between δ 33.97 – 22.73 and methyl carbon at δ 14.13 (C-32). On the basis of spectral data analysis and chemical reactions, the structure of **6** has been elucidated as *n*-dotriacont- 15(Z),18(Z)-dienoic acid, a new fatty acid (Fig. 2).

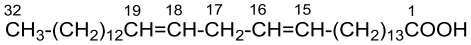
Compound **7** showed IR absorption bands for an ester group (1734 cm^{-1}) and a long aliphatic chain (725 cm^{-1}). Its mass spectrum displayed a molecular ion peak at *m/z* 688 corresponding to a molecular formula of a fatty acid ester, $C_{47}H_{92}O_2$. The ion peaks generating at *m/z* 281 [C1′ – O fission, $CH_3(CH_2)_7\text{-}CH{=}CH\text{-}(CH_2)_7COO]^+$, 265 [$C_1$ – O fission, $CH_3(CH_2)_7\text{-}CH{=}CH\text{-}(CH_2)_7CO]^+$ and 423 [M – 265, $CH_3\text{-}(CH_2)_{27}CH_2O]^+$ suggested the esterification of oleic acid with nonacosan-1-ol. The ^{1}H NMR spectrum of **7** exhibited two one-proton multiplets at δ 5.36 and 5.32 assigned to vinylic H-9 and H-10 protons, two two-proton triplets at δ 4.12 (J = 6.1 Hz) and 2.31 (J = 7.6 Hz) ascribed correspondingly to oxygenated methylene H_2 -1′ and methylene H_2 -2 adjacent to the ester function. The other methylene protons resonated as two-proton multiplets in the range of 2.03 – 1.33 and as broad singlets at δ 1.30 (8H) and 1.28 (56H). Two three-proton triplets at δ 0.89 (J = 6.5 Hz) and 0.86 (J = 7.2 Hz) were accounted to terminal C-18 and C-29′ primary methyl protons, respectively. The ^{13}C NMR spectrum of **7** showed signals for the ester carbon at δ 173.30 (C-1), oxymethylene carbon at δ 63.67 (C-1′), methylene carbons between δ 51.24 - 22.69 and methyl carbons at δ 14.12 (C-18) and 14.08 (C-29′).

On the basis of foregoing spectral data analysis, the structure of **7** has been established as 1-nonacosanolyl (9Z)-octadecenoate, a new fatty acid ester (Fig. 2).

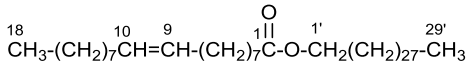
Compound **8,** designated as *n*-triacontanyl oleate, responded positive tests of unsaturation. Its IR spectrum exhibited important absorption bands for ester group (1732 cm^{-1}), unsaturation (1635 cm^{-1}) and aliphatic moiety (722 cm^{-1}). On the basis of mass and ^{13}C NMR spectra, its molecular mass was determined at *m/z* 702 consistent with the molecular formula $C_{48}H_{94}O_2$ of a fatty acid ester. The ion peaks produced at *m/z* 281 [$C_{1'}$ – O fission, $CH_3(CH_2)_7$-CH=CH-$(CH_2)_7COO]^+$, 265 [C_1 – O fission, $CH_3(CH_2)_7$-CH=CH-$(CH_2)_7CO]^+$ and 437 [M – 265, CH_3-$(CH_2)_{28}CH_2O]^+$ supported the esterification of oleic acid with triacontan-1-ol. The ^1H NMR of **8** exhibited two downfield one-proton multiplets at δ 5.33 and 5.31 ascribed to vinylic H-9 and H-10 protons, respectively. Two two-proton multiplets at δ 2.01 and 1.98 were attributed correspondingly to methylene H_2-8 and H_2-11 protons adjacent to the vinylic linkage. Two two-proton triplets at δ 4.15 (J = 7.2 Hz) and 2.32 (J = 7.6 Hz) were attributed to oxygenated methylene H_2- 1′ protons and H_2-2 methylene protons adjacent to ester linkage. The remaining methylene protons resonated from δ 1.64 to 1.25. Two three-proton triplets at δ 0.89 (J = 6.4 Hz) and 0.86 (J = 6.8 Hz) were associated with primary methyl protons Me-18 and Me-30′, respectively. The ^{13}C NMR spectrum of compound **8** displayed important signals for ester carbon at δ 173.95 (C-1), vinylic carbons at δ 129.91 (C-9) and 128.01 (C-10), oxymethylene carbon at δ 64.86 (C-1′) and methyl carbons at δ 14.02 (C-18) and 14.04 (C-30′). The remaining methylene carbons appeared from δ 51.29 to 22.62. On the basis of above discussion the structure of compound **8** was formulated as *n*-triacontanyl octadec-9-enoate, a new fatty acid ester (Fig. 2).



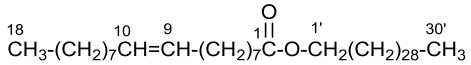
n-Pentadecanyl oleate **(5)**



n-Dotriacont- 15(Z),18(Z)-dienoic acid **(6)**



n-Nonacosanyl oleate **(7)**



n-Triacontanyl oleate **(8)**

Fig. 2. Structural formulae of the phytoconstituents 5 – 8 isolated from the leaves of *Trichodesma indicum*.

CONCLUSION

Phytochemical investigation of the methanolic extract of the fruits of *Coccinia grandis* afforded two fatty acid glucosides identified as (Z)-docos-13-enoyl O-α-D-glucopyranoside (erucicyl-*O*-α-D-glucoside, **1**) and *n*-docosanoyl O-α-D-glucopyranoside (behenyl-*O*-α-D-glucoside, **2**) and two steroidal glucosides, viz., β-sitosterol 3-O-α-D-glucopyranoside (**3**) and β-sitosterol 3-O-α-D-glucopyranosyl- ((6'→1")-O-α-D-glucopyranosyl-(6"→1'")- O-α-D-glucopyranoside (β-sitosterol α-D-triglucoside, **4**). The leaf methanolic extract of *T. indicum* furnished three fatty acid esters characterized as *n*-pentadecanyl octadec-9-enoate (*n*-pentadecanyl oleate, **5**), 1-nonacosanolyl (9Z)-octadecenoate (*n*-nonacosanyl oleate, **7**) and *n*-triacontanyl octadec-9-enoate (*n*-triacontanyl oleate, **8**) and a higher carboxylic acid, viz., *n*-dotriacont- 5(Z), 18(Z)-dienoic acid (**6**). 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.

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