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Chemical constituents from the leaves of *Adenanthera pavonina* and *Erythrina variegata* and roots of *Heliotropium eichwaldii*

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ABSTRACT

Background: *Adenanthera pavonina* L. (Leguminosae) is a fast-growing, attractive, perennial tree found in southern China, India, Florida and South America. Our study was planned to isolate chemical constituents from the leaves of *A. pavonina* and *E. variegata* and roots of *H. eichwaldii* and to characterize their structures.

Material and Methods: The air-dried powders of the leaves of *A. pavonina* and *E. Variegata* and roots of *H. eichwaldii* were exhaustively extracted with methanol individually and the concentrated each extract was adsorbed on silica gel (60-120 mesh) separately for the preparation of slurries. Each dried slurry was chromatographed over silica gel column packed in petroleum ether. The columns were eluted with petroleum ether, chloroform, and methanol, successively, in order of increasing polarity to isolate the compounds.

Results: Phytochemical investigation of a methanolic extract of the leaves of *A. pavonina* afforded 1-tricosanol (**1**) and α-D-glucopyranosyl-(6→1′)-α-D-glucopyranosyl-(6′→1′′)-α-D-glucopyranosyl-(6′′→1′′′)-α-D-glucopyranoside (α-6-O-D-tetraglucoside, (**2**) Column chromatography of a methanolic extract of the leaves of *E. variegata* gave 1-hexatetracontanol, (**3**) and 1-octanyloxy- O-β-D-glucopyranosyl-(6′→1′′) -O-β-D-glucopyranoside, (**4**) A methanolic extract of the roots of *H. eichwaldii* on subjection to silica gel column furnished *n*-nonanyl oleate, (**5**) *n*-dodecanyl stearate, (**6**) triarachidin, (**7**) stigmast-5,23–dien-3β –ol (23-epistigmasterol, (**8**) *n*-undecanyl *n*-docosanoate (*n*-undecanyl behenate, (**9**) and (17Z)-*n*-tetracont -17-enoic acid (**10**). The structures of all the isolated phytoconstituents have been established on the basis of spectral data analysis and chemical reactions.

Conclusion: The leaves of *A. pavonina* possessed an aliphatic alcohol and a tetraglucoside. A long chain alcohol and an alkyloxy diglucoside were isolated from the *E. variegata* leaves. The *H. eichwaldii* roots afforded fatty acid esters, a phytosterol, and a long chain unsaturated fatty acid.

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INTRODUCTION

Adenanthera pavonina L., syn. *A. gersenii* Scheff, *Corallaria parvifolia* Rumph. (Leguminosae), commonly known as rakt Chandan, red lucky seed, acacia coral and red sandalwood, is a fast-growing, attractive, perennial tree found in southern China, India, Florida and South America (Anonymous, 1986). Its leaves and bark are used as an astringent, anthelmintic, aphrodisiac and to treat diarrhea, gout, rheumatism, leprosy, haematuria, and haematemesis. The bark extract is utilized to wash hair. The wood is effective as an antiseptic, tonic and to relieve migraines and headaches. The seeds are prescribed to alleviate boils, inflammations, cholera, general paralysis and pulmonary affections. The roots are regarded as an emetic (WHO, 1998; Kirtikar and

Basu, 2006; Warrier, 2003). The plant contained galactitol, 2,4-dihydroxybenzoic acid, O-acetylethanolamine and 1H-imidazole (Devi et al., 2007; Gennaro and Nasini, 1972). The seeds possessed HCN-glucoside, lignoceric acid, dulcitol, stigmasterol, its glucoside, polysaccharide, amino acids, a fat consisting mainly of saturated fatty acids and flavones (Yadava and Vishwakarma, 2013; Chourasia and Rao, 2005, 2006; Zarnowski et al., 2004). The leaves afforded alkanes, squalene, palmitate ester, β-sitosterol, its 3β-D- glucoside and flavones (Nigam et al., 1973; Ahmad et al., 2002; Enuo et al., 2007; Mohammeda et al., 2014; George et al., 2017;). The roots furnished oleanolic and echinocystic acid sapogenins and pavonin (Ali et al., 2005). The bark yielded glucose, protein, and fatty acids (Yadav et al., 1976; Ara et al., 2012).

Erythrina variegata L., syn. *E. indica* Lam., *E. orientalis* (L.) Merr. (Papilionoideae), known as pangri, dadak, Indian coral tree , and sunshine tree, is distributed as a medium-sized, spiny, deciduous tree from India to Malaysia, China, Africa and Polynesia. The plant parts are used as an antiabortion, aphrodisiac, antibilious, astringent, expectorant, febrifuge, lactagogue and to treat stomach disorders, malaria, liver problems, rheumatism, amenorrhea, anorexia, cough, cholesterol imbalance, convulsions, cuts, cystitis, dysuria, dysmenorrhea, earache, erectile dysfunction, fever, fissures, joint pain, inflammation, insomnia, intestinal worms, menstrual disorders, obesity, oedema, toothache, wounds and parasitic infections (Nadkarni et al., 1991; Kiritikar and Basu, 1991; Warrier et al., 1994; Anonymous, 2002; Kumar et al, 2010). The plant contained spiroamine alkaloids, scoulerine, (+)-coreximine, reticuline, erybidine, erysovine, stachydrine, hypaphorine, its methyl ester, N, N-dimethyltryptophan, isoquinoline (erythritol) and isococcolinine alkaloids; flavonoids erythrinins A, B, and C, osajin, alpinum isoflavone, oxyresveratrol and dihydroxyresveratrol, robustone, 4-O-methylalpinum isoflavone, erycricstagallin, orientanol B, erystagallin A, stigmasterol, campesterol, stigmoidins A, B, and C, phaseollin, erythrabyssin II, dihydrofolinin, octacosanyl ferulate, wax alcohol, wax acids, alkyl ferulates and alkyl phenolates. The seeds possessed protein, pentosan, gum, isolecitins, trypsin inhibitors and chymotrypsin inhibitor (Chawla et al, 1988; 1993; Tanaka et al., 2000, 2002; 2003; 2004; Ahmad et al., 2002; Sato et al. 2003; Xiaoli et al., 2006; Rahman et al., 2007, 2010; Samanta and Laskar, 2008). A plant extract revealed the presence of 3-eicosyne, acyclic diterpenol, butanoic ester, phytol, 1,2-benzenedicarboxylic acid, diundecyl ester, 2-butyl-1-octanol, squalene and 2H-pyran, 2-(7-heptadecynyloxy) tetrahydro-derivative (Muthukrishnan et al., 2016).

Heliotropium eichwaldii Steud., syn. *H. macrocarpum* Guss (Boraginaceae), known as atwin, bithua, shahdevi, nilkattai, and chiraghas , is an erect, hairy, annual herb up to 30 cm in height found in north-western India including Punjab, Rajasthan, and Kashmir. Its leaves are used to treat earache, erysipelas, headache, itching, pimples, ulcers and warts. The plant contained pyrrolizidine alkaloids heliotrine, 7-angeloylheliotrine, and lasiocarpine (Suri et al., 1975; El-Shazly and Wink, 2014). The pyrrolizidine alkaloids are hepatotoxic (Datta et al., 1978). The roots showed antioxidant activity and protective effect against nephrotoxicity induced by cisplatin (Sharma and Goyal, 2012a, 2012b). Keeping in view the various therapeutic values of the plants and the development of ecofriendly, biodegradable and safer herbal preparations the leaves of *A. pavonina* and E. *variegata* and the roots of *H. eichwaldii* were screened for the isolation and characterization of their chemical constituents.

MATERIALS AND METHODS

General procedures

Melting points were determined on a Perfit apparatus without correction. UV spectra were scanned in methanol on a Lambda Bio 20 spectrophotometer (Shimadzu-U, Singapore). IR spectra were recorded in KBr pellets on a Win IR FTS 135 instrument (Biorad, Philadelphia, PA, USA). The ^{1}H Nuclear Magnetic Resonance (NMR) (300 MHz) and ^{13}C NMR (75 MHz) spectra were recorded on a Brucker spectrometer (Brucker, Billerica, MA, USA) in a deuterated solvent with tetramethyl silane (TMS) as an internal standard. The mass spectroscopy spectra were taken on a JEOL-Accu TOF machine equipped with a Direct Analysis in Real Time (DART) ion source and helium was used as a gas of collision. Silica gel (60–120 mesh, Qualigens, Mumbai, India) was used for column chromatography. Precoated silica gel plates (Merck, Silica gel 60 F_{254}) were used for analytical thin layer chromatography and the spots were visualized by exposure to UV radiations and iodine vapors and spraying with ceric sulfate solution.

Plant materials

The leaves of *Adenanthera pavonina* and *Erythrina variegata* were procured from Cochin, Kerala. The roots of *Heliotropium eichwaldii* were collected from Chhatisgarh. The plant materials were authenticated by Dr. M. P. Sharma, Department of Botany, Jamia Hamdard, New Delhi. The voucher specimens of these drugs were deposited in the herbarium of the Phytochemistry Research Laboratory, Jamia Hamdard, New Delhi, India.

Preparation of extracts

Each 1 kg of the leaves of A. *pavonina* and E. *variegata* and roots of H. *eichwaldii* were coarsely powdered and extracted exhaustively separately with methanol in a Soxhlet apparatus. The extracts were concentrated under reduced pressure to get dark brown masses, 105.3 g, 112.6 g and 118.2 g, respectively. A small portion of the each extract was analyzed chemically to determine the presence of different chemical constituents.

Isolation of phytoconstituents

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 individually. Each column was eluted with petroleum ether, petroleum ether - chloroform mixtures, chloroform, and chloroform - methanol mixtures in order of increasing polarity. Various fractions were collected separately and matched by TLC to check homogeneity. Similar fractions having the same R_f values were combined and crystallized to obtain the compounds.

Isolation of phytoconstituents from the leaves of *Adenanthera parviflora*

1-Tricosanol (1)

Elution of the column with chloroform gave colourless mass of **1**, yield 137 mg, R_f 0.46 (chloroform – methanol, 9:1), m. p. 74 - 76 °C; IR γ_{max} (KBr): 3401, 2925, 2841, 1647, 1425, 1216, 1096, 727 cm^{-1}; ^1H NMR ($CDCl_3$): δ 3.34 (2H, t, J = 6.3 Hz, H_2-1), 2.04 – 1.34 (8H, m, 4 x CH_2), 1.29 (8H, brs, 4 × CH_2), 1.23 (24H, brs, 12 × CH_2), 0.87 (3H, t, J = 6.6 Hz, Me-23); ^{13}C NMR ($CDCl_3$): δ 62.89 (C-1), 33.04 (CH_2), 31.84 (CH_2), 29.90 (16 × CH_2), 27.57 (CH_2), 25.37 (CH_2), 22.90 (CH_2), 14.32 (C-23); ESI MS *m/z* (rel. int.): 340 $[M]^+$ ($C_{23}H_{48}O$) (47.9).

α-6-O-D-Tetraglucoside (2)

Elution of the column with chloroform - methanol (9:1) afforded colourless crystals of **2**, yield 105 mg, R_f 0.49 (chloroform – methanol, 9:1); m. p. 130 - 132 °C; IR γ_{max} (KBr): 3412, 3341, 3212, 3038, 2951, 2842, 1647, 1429, 1386, 1219, 1095 cm^{-1}; ^1H NMR (MeOD): δ 5.17 (1H, d, J = 3.9 Hz, H-1α), 3.95 (1H, m, H-5), 3.79 (1H, m, H-2), 3.69 (1H, m, H-3), 3.59 (2H, m, H-4, H-4′), 3.31 (2H, d, J = 8.3 Hz, H_2-6), 4.49 (1H, d, J = 6.1 Hz, H-1′), 3.89 (1H, m, H-5′), 3.77 (1H, m, H-2′), 3.67 (1H, m, H-3′), 3.25 (2H, d, J = 9.8 Hz, H_2-6′), 4.06 (1H, d, J=5.1 Hz, H-1′′), 3.84 (1H, m, H-5′′), 3.75 (1H, m, H-2′′), 3.65 (1H, m, H-3′′), 3.56 (1H, m, H-4′′), 3.21 (2H, d, J = 5.1 Hz, H_2-6′′), 4.01 (1H, d, J = 3.1 Hz, H-1′′′), 3.82 (1H, m, H-5′′′), 3.73 (1H, m, H-2′′′), 3.61 (1H, m, H-3′′′), 3.54 (1H, m, H-4′′′), 3.11 (2H, d, J = 4.8 Hz, H_2-6′′′); ^{13}C NMR ($CDCl_3$): δ 103.31 (C-1), 77.69 (C-2), 73.11 (C-3), 71.87 (C-4), 84.98 (C-5), 64.65 (C-6), 99.29 (C-1′), 76.92 (C-2′), 72.68 (C-3′), 71.35 (C-4′), 83.38 (C-5′), 62.87 (C-6′2), 98.32 (C-1′′), 76.44 (C-2′′), 72.13 (C-3′′), 69.55 (C-4′′), 78.19 (C-5′′), 62.95 (C-6′′), 94.08 (C-1′′′), 76.42 (C-2′′′), 71.95 (C-3′′′), 66.08 (C-4′′′), 78.11 (C-5′′′), 60.89 (C-6′′′); ESI MS *m/z* (rel. int.): 666 $[M]^+$ ($C_{24}H_{42}O_{21}$) (20.8), 503 (12.8) 342 (78.2), 324 (10.6), 179 (7.1), 163 (9.8).

Isolation of phytoconstituents from the leaves of *Erythrina indica*

1-Hexatetracontanol (3)

Elution of the column with chloroform - methanol (19:1) furnished colorless crystals of **3**, yield 212 mg, R_f 0.41 (chloroform – methanol, 4:1), m. p. 173 – 174 °C; UV λmax (MeOH): 207 nm; IR γmax (KBr): 3413, 2969, 2843, 1612, 1471, 1213, 1043, 928, 872, 735 cm^{-1}; ^1H NMR (MeOD): δ 3.79 (2H, t, J = 9.0 Hz, H_2-1), 2.07 (2H, m, H_2-2), 1.59 (2H, m, CH_2), 1.46 (2H, m, CH_2), 1.38 (6H, brs, 3 × CH_2), 1.29 (56H, brs, 28 × CH_2), 1.25 (20H, brs, 10 × CH_2), 0.89 (3H, t, J = 6.5 Hz, Me-46); ^{13}C NMR ($CDCl_3$): δ 62.52 (C-1), 34.28 (CH_2), 32.09 (CH_2), 29.35 (18 × CH_2), 29.11 (10 x CH_2), 29.37 (11 x CH_2), 27.48 (CH_2), 25.41 (CH2), 22.69 (CH_2), 14.06 (C-46); ESI MS *m/z* (rel. int.): 662 $[M]^+$ ($C_{46}H_{94}O$) (100).

1-Octanol O-β-D-diglucoside (4)

Elution of the column with chloroform - methanol (9:1) yielded colourless crystals of **4**, yield 161 mg; m. p. 113 – 115 °C; IR γmax (KBr): 3401, 3343, 3019, 2975, 2842, 1645, 1425, 1384, 1216, 1095, 758 cm^{-1}; ^1H NMR (MeOD): δ 3.34 (2H, t, J = 9.0 Hz, H_2-1), 2.30 (2H, m, H_2-2), 1.54 (2H, m, CH_2), 1.28 (8H, brs, 4 × CH_2), 0.89 (3H, t, J = 6.6 Hz, Me-8), 5.17 (1H, d, J = 7.8 Hz, H-1′), 4.34 (1H, m, H-5′), 3.89 (1H, m, H-2′), 3.73 (1H, m, H-3′), 3.55 (1H, m, H-4′), 3.28 (2H, d, J = 7.5 Hz, H_2-6′), 5.07 (1H, d, J = 7.3 Hz, H-1′′), 4.04 (1H, m, H-5′′), 3.82 (1H, m, H-2′′), 3.68 (1H, m, H-3′′), 3.48 (1H, m, H-4′′), 3.12 (2H, d, J = 9.3 Hz, H_2-6′′); ^{13}C NMR ($CDCl_3$): δ 66.05 (C-1), 30.93 (C-2), 30.08 (C-3), 29.91 (C-4, C-5), 25.83 (C-6), 22.69 (C-7), 14.16 (C-8), 100.32 (C-1′), 73.58 (C-2′), 72.15 (C-3′), 71.37 (C-4′), 75.43 (C-5′), 64.68 (C-6′), 91.03 (C-1′′), 72.69 (C-2′′), 71.98 (C-3′′), 69.57 (C-4′′), 74.88 (C-5′′), 60.92 (C-6′′); ESI MS *m/z* (rel. int.): 454 $[M]^+$ ($C_{20}H_{38}O_{11}$) (6.1), 325 (11.2), 179 (6.3), 129 (10.6).

Isolation of phytoconstituents from the roots of *Heliotropium eichwaldii*

n-Nonanyl oleate (5)

Elution of the column with petroleum ether gave a pale yellow gummy mass of **5**, yield 165 mg, UV max (MeOH): 211 nm (log ε 2.6); IR umax (KBr): 2919, 2850, 1736, 1462, 1378, 1260, 1170, 1098, 914, 875, 749 cm^{-1}; ^1H NMR ($CDCl_3$): δ 5.41 (1H, m, H-9), 5.36 (1H, m, H-10), 4.17 (2H, t, J = 8.9 Hz, H_2-1′), 2.28 (2H, t, J = 7.2 Hz, H_2-2), 2.04 (2H, m, H_2-8), 2.01 (2H, m, H_2-11), 1.56 (2H, m, CH_2), 1.34 (2H, m, CH_2), 1.29 (18H, m, 9 x CH_2), 1.27 (16H, brs, 8 x CH_2), 0.87 (3H, t, J = 6.5 Hz, Me-18), 0.84 (3H, t, J = 6.1 Hz, Me-9′); ^{13}C NMR ($CDCl_3$): δ 171.42 (C-1), 61.29 (C-1′), 34.61 (C-2), 32.05 (CH_2), 29.17 (15 x CH_2), 27.68 (CH_2), 25.88 (CH_2), 23.31 (CH_2), 22.69 (CH_2), 14.23 (Me-22), 14.08 (Me-9′); ESI MS *m/z* (rel. int.): 408 $[M]^+$ ($C_{27}H_{52}O_2$) (100), 281 (3.1), 265 (4.3).

n-Dodecanyl stearate (6)

Elution of the column with petroleum ether – chloroform (3 :1) produced a colourless semisolid mass of **6**, yield 227 mg, UV max (MeOH): 205 nm (log ε 3.5); IR umax (KBr): 2926, 2845, 1729, 1614, 1459, 1384, 1281, 1121, 827, 746 cm^{-1}; ^1H NMR ($CDCl_3$): δ 4.04 (2H, t, J = 8.2 Hz, H_2-1′), 2.27 (2H, t, J = 7.2 Hz, H_2-2), 1.68 (2H, m, CH_2), 1.58 (2H, m, CH_2), 1.32 (2H, m, CH_2), 1.29 (20H, m, 10 x CH_2), 1.25 (24H, m, 12 x CH_2), 0.88 (3H, t, J = 6.5 Hz, Me-18), 0.85 (3H, t, J = 6.2 Hz, Me-12′); ESI *m/z* (rel. int.): 452 $[M]^+$ ($C_{30}H_{60}O_2$) (89.3), 283 (10.1), 267 (19.3).

Triarachidin (7)

Elution of the column with petroleum ether – chloroform (3:1) furnished colourless amorphous powder of **7**, yield 205 mg, R_f 0.48 (petroleum ether – chloroform, 3:1), m. p. 63 -65 °C, UV λmax (methanol) : 207 nm (log ε 3.9), IR γmax (KBr) : 2924, 2854, 1733, 1635, 1458, 1394, 1274, 1084, 725 cm^{-1}; ^1H NMR ($CDCl_3$): δ 4.27 (1H, m, H -2), 4.12 (2H, d, J = 6. 7 Hz, H_2 -1), 4.02 (2H, d, J = 7. 1 Hz, H_2 -3), 2.33 (2H, t, J = 7.2 Hz, H_2 -2′), 2.29 (2H, t, J = 7.6 Hz, H_2 -2′′), 2.25 (2H, t, J = 7.6 Hz, H_2 -2′′′), 1.58 (4H, m, 2 x CH_2), 1.25 (98H, brs, 49 x CH_2), 0.86 (3H, t, J = 6.3 Hz, Me-20′), 0.83 (3H, t, J = 6.1 Hz, Me-20′′), 0.81 (3H, t, J = 6.0 Hz, Me-20′′′); ^{13}C NMR ($CDCl_3$): 174.65 (C-1′), 173.96 (C-1′′), 171.69 (C-1′′′), 68.24 (C-2), 64.97 (C-1), 63.26 (C-3), 34.77 (CH_2), 34.11 (CH_2), 31.93 (CH_2), 29.47 (48 x CH_2), 27.14 (CH_2), 24.89 (CH2), 22.69 (CH_2), 14.11 (Me-20′), 14.09 (Me-20′′, Me-20′′′); ESI MS *m/z* (rel. int.): 974 $[M]^+$ ($C_{63}H_{122}O_6$) (11.6), 283 (81.5).

23-Epistigmasterol (8)

Elution of the column with petroleum ether – chloroform (1:1) afforded colourless amorphous powder of **8**, yield 199 mg, R_f 0.69 (petroleum ether–chloroform, 2:3), m. p. 119-120 °C, UV λmax (methanol) : 211 nm (log ε 4.3); IR γmax (KBr): 3398, 2932, 2825, 1645, 1461, 1374, 1225, 1057, 956, 837 cm^{-1}; ^1H NMR ($CDCl_3$): δ 5.41 (1H, m, H-6), 5.36 (1H, m, H-23), 3.79 (1H, brm, $w_{1/2}$ = 16.5 Hz, H-3α), 2.01 to 1.23 (25 H, m, 9 x CH_2. 7 x CH), 1.01 (3H, brs, Me-19), 0.93 (3H, d, J = 6.4 Hz, Me-21), 0.86 (3H, d, J = 6.0 Hz, Me-26), 0.84 (3H, d, J = 6.1 Hz, Me-27), 0.82 (3H, t, J = 6.3 Hz, Me-29), 0.66 (3H, brs, Me-18); ^{13}C NMR ($CDCl_3$): δ 37.59 (C-1), 31.80 (C-2), 73.01 (C-3), 42.32 (C-4), 144.71 (C-5), 123.83 (C-6), 31.89 (C-7), 38.81 (C-8), 49.94 (C-9), 36.76 (C-10), 21.05 (C-11), 39.56 (C-12), 39.68 (C-13), 56.67 (C-14), 24.27 (C-15), 28.26 (C-16), 56.16 (C-17), 11.86 (C-18), 19.22 (C-19), 36.50 (C-20), 18.73 (C-21), 36.19 (C-22), 123.82 (C-23), 138.50 (C-24), 28.03 (C-25), 26.83 (C-26), 23.84 (C-27), 27.38 (C-28), 22.58 (C-29); EIS MS *m/z* (rel. int.): 412 $[M]^+$ ($C_{29}H_{48}O$) (8.4), 394 (100), 397 (8.9).

n-Undecanyl behenate (9)

Elution of the column with chloroform offered a pale yellow powder of **9**, yield 1.21 g, R_f : 0.44 (chloroform - methanol, 9:1), m. p. : 78 -79 °C; UV max (MeOH): 209 nm (log ε 3.5); IR γmax (KBr): 2927, 2841, 1723, 1641, 1465, 1278, 1179, 1056, 813, 722 cm^{-1}; ^1H NMR ($CDCl_3$): δ 4.06 (2H, t, J = 9.6 Hz, H_2-1′), 2.30 (2H, t, J = 7.2 Hz, H_2-2), 1.99 (2H, m, CH_2), 1.52 (2H, m, CH_2), 1.32 (2H, m, CH_2), 1.29 (2H, m, CH_2), 1.25 (46H, brs, 23 x CH_2), 0.85 (3H, t, J = 6.5 Hz, Me-22), 0.82 (3H, t, J = 6.1 Hz, Me-11′); ^{13}C NMR ($CDCl_3$): δ 172.89 (C-1), 60.22 (C-1′), 34.41 (C-2), 31.95 (CH_2), 29.731 (29 x CH_2), 29.41 (CH_2), 29.30 (CH_2), 29.21 (CH_2), 27.87 (CH_2), 28.77 (CH_2), 25.94 (CH_2), 23.15 (CH_2), 22.71 (CH_2), 14.30 (Me-22), 14.13 (Me-11′); ESI MS *m/z* (rel. int.): 494 $[M]^+$ ($C_{33}H_{66}O_2$) (92.8), 339 (11.2), 323 (71.6).

n-Tetracont-17-enoic acid (10)

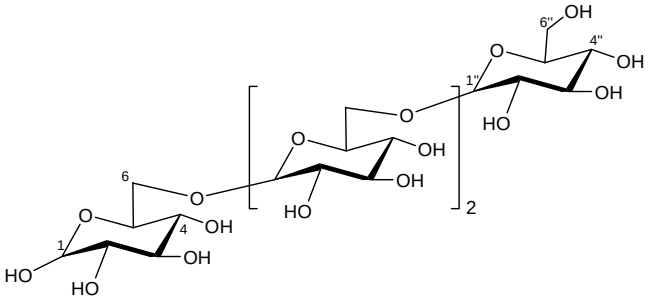
Elution of the column with chloroform-methanol (19:1) produced a pale yellow powder of **10**, recrystallized from chloroform-methanol (1:1), yield 152 mg, R_f 0.52 (chloroform-methanol, 19:1), m. p. 83 -84 °C, UV λmax (MeOH), 213 nm (log ε 3.1); IR γmax (KBr): 3134, 2928, 2854, 1702, 1643, 1461, 1376, 1280, 939, 723 cm^{-1}; ^1H NMR ($CDCl_3$): δ 5.35 (1H, m, $w_{1/2}$ = 8.5 Hz, H-17), 5.30 (1H, m, $w_{1/2}$ = 8.8 Hz, H-18), 2.76 (2H, t, J = 7.2 Hz, H_2-2), 2.33 (2H, m, H_2- 19), 2.30 (2H, m, H_2-16), 2.24 (2H, m, CH_2), 2.01 (2H, m, CH_2), 1.92 (2H, m, CH_2), 1.64 (4H, m, 2 x CH_2), 1.55 (2H, m, CH_2), 1.28 (16H, brs, 8 x CH_2), 1.25 (38H, brs, 19 x CH_2), 0.85 (3H, t, J = 6.1 Hz, Me-40); ^{13}C NMR ($CDCl_3$): δ 180.37 (C-1), 130.22 (C-17),

127.95 (C-18), 34.15 (CH_2), 31.97 (CH_2), 31.58 (CH_2), 29.73 (15 x CH_2), 29.41 (6 x CH_2), 29.32 (2 x CH_2), 29.12 (6 x CH_2), 27.24 (CH_2), 25.57 (CH_2), 24.71 (CH_2), 22.72 (CH_2), 14.12 (Me-40); ESI MS *m/z* (rel. Int.): 590 $[M]^+$ ($C_{40}H_{78}O_2$) (6.1), 335 (9.7), 309 (14.4), 255 (10.3).

RESULTS AND DISCUSSION

$CH_3(CH_2)_{21}CH_2OH$

1-Tricosanol (**1**)



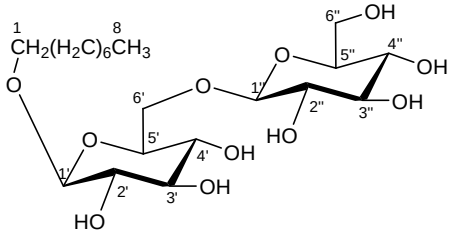
α-6-O- L-Tetraglucoside (**2**)

Compound **1** was a known aliphatic alcohol characterized as 1-tricosanol (Khan et al., 2002).

Compound **2**, named as α-6-O-D-tetraglucoside, showed IR diagnostic absorption bands for hydroxyl groups (3412, 3341, 3212, 3038 cm^{-1}) and responded positively to the general test for glycosides. On the basis of mass and ^{13}C NMR spectral data, the molecular ion peak of **2** was determined at *m/z* 666 consistent with a molecular formula of a tetraglycoside, $C_{24}H_{42}O_{21}$. The ion peaks generating at *m/z* 163 $[C_6H_{11}O_5]^+$, 179 $[C_6H_{11}O_6]^+$, 342 $[M - C_6H_{11}O_6 - C_6H_{11}O_5]^+$ and 503 $[M - 163]^+$ suggested that the compound 2 was a tetraglycoside containing hexose units. The ^1H NMR spectrum of **2** showed four one-proton doublets at δ 5.17 (J = 3.9 Hz), 4.49 (J = 6.2 Hz), 4.06 (J = 6.1 Hz) and 4.01 (J = 3.1 Hz) ascribed as α-oriented anomeric H-1, H-1′, H-1′′ and H-1′′′ protons, respectively. The other sugar protons appeared as multiplets from δ 3.95 to 3.54 and as two-proton doublets at δ 3.31 (J = 8.3 Hz, H_2-6), 3.25 (J = 9.8 Hz, H_2-6′), 3.21 (J = 5.1 Hz, H_2-6′′) and 3.11 (J = 4.8 Hz, H_2-6′′′). The ^{13}C NMR of **2** exhibited important signals for anomeric carbons at δ 103.31 (C-1), 99.29 (C-1′), 98.32 (C-1′′) and 94.08 (C-1′′′) and other sugar carbons between δ 84.98 and 60.89. The existence of oxymethylene protons in the ^1H NMR spectrum in the downfield region at δ 3.31 (H_2-6), 3.25 (H_2-6′) and 3.21 (H_2-6′′) and carbon signals at δ 64.65 (C-6), 62.87 (C-6′) and 62.95 (C-6′′) suggested (6→1) linkages of the sugar units. Acid hydrolysis of **2** yielded D-glucose characterized on the basis of co-TLC comparison and specific rotation. On the basis of above discussion the structure of **2** was elucidated as α-D-glucopyranosyl-(6→1′)-α-D-glucopyranosyl-(6′→1′′)-α-D-glucopyranosyl-(6′′→1′′′)-α-D-glucopyranoside.

$CH_3(CH_2)_{44}CH_2OH$

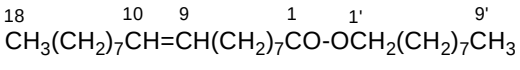
1-Hexatetracontanol (**3**)



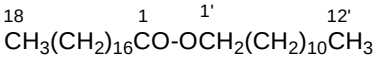
1-Octanol-O-β-D-diglucoside (**4**)

Compound **3** was a known aliphatic alcohol characterized as 1-hexatetracontanol (Ndom et al., 2001).

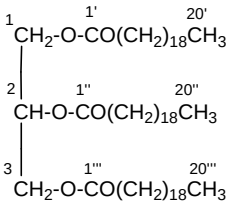
Compound **4**, named 1-octanol O-β-D-diglucoside, gave positive tests for glycosides and exhibited IR absorption bands for hydroxyl groups (3401, 3343, 3019 cm^{-1}) and aliphatic chain (758 cm^{-1}). On the basis of its mass and ^{13}C NMR spectra, its molecular weight was established at *m/z* 454 consistent with a molecular formula of a fatty acid diglycoside, $C_{30}H_{52}O_{12}$. The ion peaks generated at *m/z* 179 $[C_6H_{11}O_6]^+$, 129 $[C_8H_{17}O]+$ and 325 $[M - 129]^+$ suggested the presence of a dihexose unit linked to an octanol moiety. The ^1H NMR spectrum of **4** displayed a two-proton triplet at δ 3.34 (J = 9.0 Hz) assigned to oxymethylene H_2-1 protons, two one-proton doublets at δ 5.17 (J = 7.8 Hz) and 5.07 (J = 7.3 Hz) accounted to anomeric H-1′ and H-1′′ protons, respectively, other sugar protons as one–proton multiplets between δ 4.34- 3.48 and as two-proton doublets at δ 3.28 (J = 7.5 Hz, H_2-6′) and 3.12 (J = 9.3 Hz, H_2-6′′), methylene protons as two-proton multiplets at δ 2.30 and 1.54, as a singlet at δ 1.28 (8H) and as a three-proton triplet at δ 0.89 (J = 6.6 Hz) ascribed to the terminal C-8 primary methyl protons. The ^{13}C NMR spectrum of **4** showed important signals for oxymethylene carbon at δ 66.05 (C-1), anomeric carbons at δ 100.32 (C-1′) and 91.03 (C-1′′), other sugar carbons between δ 75.43 - 60.92, methylene carbons from δ 30.08 to 22.69 and methyl carbon at δ 14.16 (C-8). The presence of the oxymethylene protons signal in the deshielded region at δ 3.28 (H_2-6′) and the respective carbon signal at δ 64.68 (C-6′) suggested (6′→1′′) linkage of the sugar units. Acid hydrolysis of **4** yielded D-glucose, co-TLC comparable. On the basis of these findings, the structure of **4** has been established as 1-octanyloxy O-β-D-glucopyranosyl-(6′→1′′)-O-β-D-glucopyranoside, a new aliphatic alcoholic diglucoside.



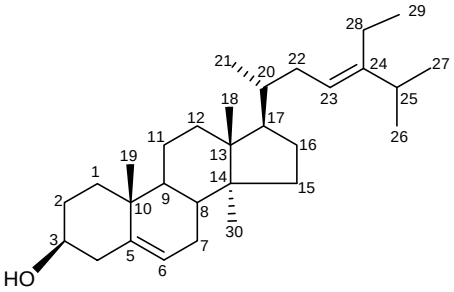
n-Nonyl oleate **(5)**



n-Dodecanyl stearate **(6)**



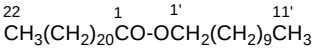
Triarachidin **(7)**



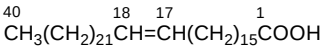
23-Epistigmasterol **(8)**

Compounds **5** and **6** were the fatty esters characterized as *n*-nonanyl oleate and *n*-dodecanyl stearate, respectively. Compound **7** was a lipid component identified as triarachidin.

Compound **8**, named 23-epistigmasterol, responded positively to steroidal tests and showed characteristic IR absorption bands for hydroxyl group (3398 cm^{-1}) and unsaturation (1645 cm^{-1}). On the basis of mass and ^{13}C NMR spectra, its molecular ion peak was established at *m/z* 412 consistent with a molecular formula of a sterol, $C_{29}H_{48}O$. The prominent ion peaks generating at *m/z* 394 $[M - H_2O]^+$ and 397 $[M - Me]^+$ suggested that it was a stigmasterol-type sterol. The ^1H NMR spectrum of **8** displayed two one-proton multiplets at δ 5.41 and 5.36 assigned to vinylic H-6 and H-23 protons, respectively. A one-proton broad multiplet at δ 3.79 with half width of 16.5 Hz was attributed to α-oriented carbinol H-3 proton. Two three - proton broad singlets at δ 0.66 and 1.01 were ascribed to tertiary C-18 and C-19 methyl protons, respectively. Three doublets at δ 0.93 (J = 6.4 Hz), 0.86 (J = 6.0 Hz), 0.84 (J = 6.1 Hz) and a triplet at δ 0.82 (J = 6.3 Hz), all integrating for three protons each, were accounted to secondary C-21, C-26 and C-27 and primary C-29 methyl protons, respectively, all attached to the saturated carbons. The remaining methylene and methine protons resonated between δ 2.01-1.23. The ^{13}C NMR spectrum of **8** showed important signals for vinylic carbons at δ 144.71 (C-5), 123.83 (C-6), 123.82 (C-23) and 138.50 (C-24), carbinol carbon at δ 73.80 (C-3) and the remaining methyl, methylene and methine carbons between δ 56.67 – 11.86. The ^1H NMR and ^{13}C NMR spectral data of the steroidal skeleton of **8** were compared with other stigmastene-type molecules (Jung et al., 2012; Mustafa and Ali, 2011). On the basis of spectral data analysis the structure of **8** has been established as stigmast-5, 23–dien-3β –ol.



n-Undecanyl behenate **(9)**



n-Tetracont-17-enoic acid **(10)**

Compound **9**, named *n*-undecanyl behenate, was a fatty ester and its structure was established as *n*-undecanyl *n*-docosanoate.

Compound **10** gave effervescences with sodium bicarbonate indicating carboxylic nature of the compound. Its IR spectrum showed characteristic absorption bands for the carboxylic group (3134, 1702 cm^{-1}), unsaturation (1643 cm^{-1}) and long aliphatic chain (723 cm^{-1}). On the basis of mass and ^{13}C NMR spectra, the molecular ion peak of **10** was established at m/z 590 corresponding to a molecular formula of a long chain unsaturated fatty acid, $C_{40}H_{78}O_2$. The ion peaks arising at *m/z* 255 $[(CH_2)_{15}COOH]^+$, 309 $[CH_3(CH_2)_{21}]^+$ and *m/z* 335 $[CH_3(CH_2)_{21}CH{=}CH]^+$ indicated the existence of the vinylic linkage at C-17 carbon. The ^1H NMR spectrum of **10** displayed two one-proton multiplets at δ 5.35 ($w_{1/2}$ = 8.5 Hz) and 5.30 ($w_{1/2}$ = 8.8 Hz) assigned to cis-oriented vinylic H-17 and H-18 protons, respectively. A two-proton triplet at δ 2.76 (J = 7.2 Hz) was ascribed to methylene H_2-2 protons adjacent to the carboxylic group. The other methylene protons appeared between δ 2.33 –1.25. A three-proton triplet at δ 0.85 (J = 6.1 Hz) was accounted to the primary C-40 methyl protons. The ^{13}C NMR spectrum of **10** exhibited important signals for carboxylic carbon at δ 180.37 (C-1), vinylic carbons at δ 130.22 (C-17) and 127.95 (C-18), methyl carbon at δ 14.12 (C-40) and methylene carbons between δ 34.15 – 22.72. On the basis of the spectral data analysis, the structure of **10** has been elucidated as (17 Z)-*n*-tetracont-17-enoic acid, a new fatty acid.

CONCLUSION

Phytochemical investigation of a methanolic extract of the leaves of *Adenanthera pavonina* afforded 1-tricosanol and an α-6-O-D-tetraglucoside. The leaves of

Erythrina variegata gave 1-hexatetracontanol and 1-octanyloxy-O-β-D-diglucoside. The root extract of *Heliotropium eichwaldii* on subjection to column chromatography furnished fatty esters, triarachidin, 23-epistigmasterol and *n*-tetracont-17-enoic acid. 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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CONFLICTS OF INTERESTS

The authors declare that they have no conflict of interest.

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