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Identification of new compounds from *Fumaria parviflora* Lam.

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ABSTRACT

Fumaria parviflora Lam. (Fumariaceae) is a traditional medicinal herb chiefly used as a blood, skin and liver related disorder along with several human ailments like abdominal cramps, diarrhea, fever, antidyspeptic, cholagogue, diaphoretic, diuretic, laxative, sedative, tonic and syphilis. Phytochemical investigation of a methanolic extract of the plant of the plant led isolation of six new compounds characterized as 2-hexadecanoxybenzyl alcohol (**2**), 2-*O*-β-*D*-arabinopyranosyloxy-2'-*O*-β-*D*-arabinopyranosyl-(8'''-geranilanyl) benzoate (**3**), *n*-tetracosanoyl- *O*-β-*D*-arabinopyranoside (**4**), *n*-tetracosanoyl O-β-D-arabinopyranosyl-(2′→1″)-O-β-D- arabinopyranoside (**5**), 2,6,7- trihydroxynaphthyl-2-*O*-β-*D*-glucopyranosyl-(6a→1b)-*O*-β-*D*-glucopyranosyl-(6b→1c)-*O*-β-*D*-glucuranopyranosyl-(2c→1d)-*O*-β-*D*-glucuranopyranosyl-2d-*n*-octadecanoate (**6**) and 2-hydroxy benzoic acid-2-*O*-β-*D*-arabinopyranosyl-(2a→1b)-*O*-β-*D*-arabinopyranosyl-(2b→1c)-*O*-β-*D*-arabinopyranosyl-(2c→1d)-*O*-β-*D*-glucuranopyranosyl-2d-(2',6',10',14'-tetramethyl)-*n*-hexadecan-1'-oate (**7**) along with a known fatty acid *n*- triacontanoic acid (**1**). The structural elucidations of the compounds were established on the basis of spectral data analysis and chemical means.

INTRODUCTION

Fumaria parviflora Lam., (Fumariaceae) is widely used herb in folkloric as well as traditional system of medicine with several synonyms like earth smoke, beggary, wax dolls in English (Orhan *et al.,* 2010), Pitpapra in Hindi, Shahtrah in Urdu (Chopra *et al.,* 2002) and Homaira in Arabic (Mossa *et al.,* 1987). It is small, annually and wildly growing weedy herb in agricultural fields, plains and lower hills (Suau *et al.,* 2002). It is 10-40 cm in height (Kirtikar and Basu, 1999) and consists of 18 genera and 450 species globally and mainly distributed in North America, Europe, Asia and Africa (Mabberley, 2008). It is imported from Persia as a chief ingredients of blood purifying agent and used for skin diseases in Unani system of medicine (Khare, 2004). It is considered useful to treat abdominal cramps (Duke *et al.,* 2002), diarrhea, fever (Haq and Hussain, 1993), cholagogue, diaphoretic, diuretic, laxative, sedative, tonic, jaundice and leprosy (Nadkarni, 1976), antidyspeptic (Anonymous, 2006) and tuberculosis (Anonymous, 2005) due to presence of several vital secondary metabolites including flavonoids, tannins, saponins, phenolic compounds (Naz *et al.,* 2013), alkaloids (Popova *et al.,* 1982; Rahman *et al.,* 1992; Suau *et al.,* 2002), glycosides (Hussain *et al.,* 1980) citric, coumaric, ferulic, fumaric, malic, 3-hydroxybenzoic, protocatechuic and caffeic acids (Sousek *et al.,* 1999). Traditional medicines have undeniably result oriented, diversifying, inventive and successful approach for discovering novel crucial unmatched lead molecules that might be transform in practice to accept the future challenges (Fakim, 2006). Therefore an integrated approach towards the isolation of new chemical entities with ethnomedicinal implications for promotion and development of traditional medicine by column chromatographic methods has been made.

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Previously isolation of n-octacosan-7β-ol (Jameel *et al.*, 2014A), n-propyl-3,4-dioxymethylene benzene 5β,6,7,8,9,10β-hexahydrocoumarin and 2,6-dimethyl dodecan-10-oyl-12,15-olide (Jameel *et al*., 2014B), (5αH,11αH)-8-oxo-homoiridolide, *n*-docosanyl-2-*O*-β-*D*-glucopyranosyl salicylate, 2-methyl-6-hydroxymethylenedodecan-10-oyl-12, 15-olide14-*O*-β-*D*-xylopyranoside, 4-oxo-stigmast-5-en-3β-ol-*D*-glucopyranoside and salicylic acid-*O*-β-*D*-xylopyranoside (Jameel *et al*., 2014C) have been done from this plant grown in Delhi region.

MATERIAL AND METHODS

General

UV spectra were measured with a Lambda Bio 20 Spectrophotometer (Perkin Elmer, Rotkreuz, Switzerland) in methanol. The ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR spectra were recorded on a Bruker ARX-Spectrometer (Rheinstetten, Baden-Wurttemberg, Germany), using $CDCl_3$ and DMSO-d_6 as solvents and TMS (Fluka analytical, Sigma-Aldrich, Netherland) as an internal standard. Melting points were determined by a thermoelectrically heated Perfit melting point apparatus (Ambala, India) without correction. Infra Red (IR) spectra were recorded using KBr pellets with a Jasco FT-IR-5000 Spectrometer (FTS 135, Kawloon, Hong Kong). Mass-spectrometric detection was carried out on ESI MS (Q-TOF-ESI) (Waters Corp., Manchester, UK), an electrospray-ionisation (ESI) technique with positive ionization mode. Column chromatography was performed on silica gel (Qualigens, Mumbai, India), 60-120 mesh and solvents taken were purchased from Merck Specialties (E. Merck, Pvt. Ltd. New Delhi, India). Pre-coated Aluminum TLC plates of silica gel 60 F_{254} (Merck, Darmstadt, Germany) were used to run and spots were visualized by exposure to iodine vapors, and UV radiations and spraying with anisaldehyde- sulphuric acid solution.

Methods

Plant material

The *F. parviflora* whole plant was collected from the herbal garden of Jamia Hamdard, New Delhi and identified by Prof. Javed Ahmad, In-charge of the herbal garden. A specimen voucher of the drug was deposited in the herbarium of the Faculty of Pharmacy with a reference number PRL-JH/2011/05.

Preparation of extract and isolation

The dried *F. parviflora* whole plant (2.5 kg) was coarsely powdered and extracted with methanol for 48 h using a Soxhlet extractor in hot and cold cycle of interval of 5-6 hrs. The extract was dried under reduced pressure to obtain a dark brown residue (380 g). The extract was partioned with hexane (750 ml X 3) and chloroform (500 ml X 3) and excluded. The remaining portion of extract (80 g) was dissolved in minimum amount of methanol and adsorbed mechanically by heating on water bath with column grade silica gel (60-120 mesh) to obtain a slurry that was chromatographed over silica gel column loaded in chloroform and the eluants of each fraction were examined by precoated Aluminum TLC plate. The column was eluted with gradient mixtures of chloroform-methanol (99:1, 97:3, 19:1, 93:7, 9:1, 3:1, *v/v*) to isolate the compounds **1**-**7**.

Further a spot of TLC chromatogram of methanolic extract was scraped out, sonicated to 2-3 min at 30 ºC in MS grade methanol and filtered first whatman's filter paper and then 0.2 sized µ filter for Mass spectrometer. Its molecular weight and m/z fragments were analyzed and compared with the same R_f of eluants of column which is showed in Fig.1.

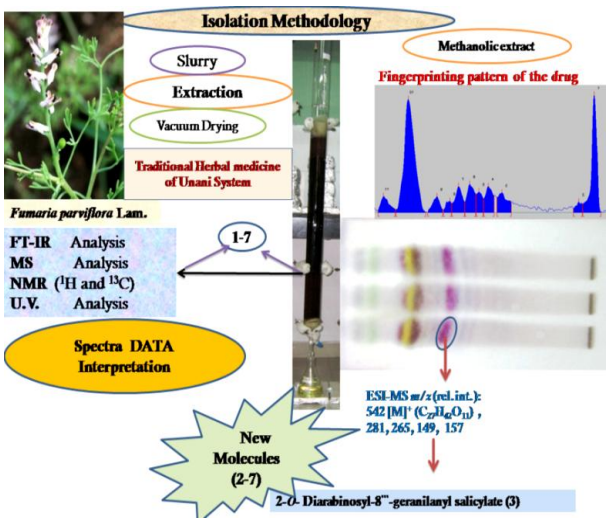


Fig. 1: Layout of Isolation.

RESULTS

Melissic acid (1)

Elution of the column with chloroform-methanol (99:1, *v/v*) gave colorless amorphous mass of **1**, purified from chloroform-methanol (1:1, *v/v*), 652 mg (0.052 % yield), m.p. 92-93 ºC; R_f 0.7 (chloroform), UV λ_{max} (MeOH): 206, 223 nm (log ε 3.7, 1.8). IR ν_{max} (KBr): 3485, 2924, 2854, 1704, 1458, 1255, 1178, 974, 725 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 2.28 (2H, t, *J*=7.2 Hz, H_2-2), 1.99 (2H, m, CH_2), 1.57 (4H, brs, 2 x CH_2), 1.21 (48 H, brs, 24 x CH_2), 0.83 (3H, t, *J*=6.8 Hz, Me-1). ^{13}C NMR ($CDCl_3$): δ 179.18 (C-1), 39.30 (C-2), 31.74 (CH_2), 29.68 (10 x CH_2), 29.64 (5 x CH_2), 29.59 (CH_2), 29.43 (CH_2), 29.34 (CH_2), 29.24 (CH_2), 29.07 (CH_2), 27.96 (CH_2), 27.71 (CH_2), 27.42 (CH_2), 27.19 (CH_2), 24.47 (CH_2), 22.56 (CH_2), 14.28 (Me-30). ESI MS *m/z* (rel.int.): 452 $[M]^+$ ($C_{30}H_{60}O_2$) (68.3).

2-Palmityloxybenzyl alcohol (2)

Elution of the column with chloroform-methanol (97:3, *v/v*) yielded greenish semisolid mass of **2**, purified by TLC using chloroform-methanol (3:1, *v/v*), 997 mg (0.08 % yield), R_f 0.6 ($CHCl_3$), UV λ_{max} (MeOH): 204, 224, 287 nm (log ε 4.6, 2.1, 1.8), IR λmax (KBr): 3321, 2920, 2852, 1724, 1622, 1525, 1463, 1375, 1247, 1037, 719 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 7.20 (1H, d, *J*=7.6 Hz, H- 3), 6.24 (1H, dd, *J*= 8.4, 2.3 Hz, H-6), 5.94 (1H, m, H-5), 5.43 (1H, m, H-4), 3.56 (2H, brs, H_2-7), 2.82 (2H, t, *J*=6.5 Hz, H_2–2'), 2.38 (2H, m, CH_2), 2.13 (2H, m, CH_2), 2.03 (2H, m, CH_2), 1.68 (2H,

m, CH_2), 1.49 (2H, m, CH_2), 1.32 (18H, brs, 9 x CH_2), 0.96 (3H, t, *J*= 6.0 Hz, Me–16'); ^{13}C NMR ($CDCl_3$): δ 135.03 (C-1), 142.58 (C-2), 131.93 (C-3), 130.22 (C-4), 128.24 (C-5), 127.11 (C-6), 71.85 (C-7), 173.12 (C-1'), 37.46 (C-2'), 37.29 (C-3'), 33.98 (C-4'), 33.09 (C-5'), 31.92 (C-6'), 29.69 (C-7'), 29.65 (C-8'), 29.50 (C-9'), 29.35 (C-10'), 29.19 (C-11'), 29.10 (C-12'), 25.58 (C-13'), 24.79 (C-14'), 22.65 (C-15'), 14.11 (C-16'). ESI MS *m/z* (rel. int.): 362 $[M]^+$ ($C_{23}H_{38}O_3$) (21.9), 255 (19.1), 239 (15.6), 123 (7.3).

2-*O*- Diarabinosyl-8‴-geranilanyl salicylate (3)

Elution of the column with chloroform-methanol (19:1, *v/v*) afforded a green semisolid mass of **3,** purified by TLC using acetone-methanol (3:1, *v/v*), 3 g (0.24 % yield), R_f 0.5 (chloroform), UV λ_{max} (MeOH): 205, 227, 281 nm (log ε 4.1, 1.8, 1.7). IR λ_{max} (KBr): 3482, 3390, 3292, 2926, 2854, 1721, 1635, 1525, 1445, 1381, 1273, 1197, 1085, 979 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 7.63 (1H, dd, *J*=3.0, 8.8 Hz, H-3), 7.45 (1H, dd, *J*= 3.0, 9.2 Hz H-6), 7.31 (1H, m, H–5), 7.27 (1H, m, H–4), 5.31 (1H, d, *J*=7.3 Hz, H-1′), 4.98 (1H, d, *J*=7.1 Hz, H-1″), 4.38 (1H, m, H–2′), 4.25 (1H, m, H–2″), 4.18 (1H, m, H–3′), 4.05 (1H, m, H–3″), 4.01 (2H, d, *J*=6.8 Hz, H_2- 8‴), 3.85 (1H, m, H–4′), 3.78 (1H, m, H–4‴), 3.67 (2H, d, *J*=9.1 Hz, H_2 -5′), 3.56 (2H, d, *J*= 9.6 Hz, H $_2$-5″), 2.24 (2H, m, H_2–6‴), 2.11 (1H, m, H-7‴), 1.97 (2H, m, H_2-4‴), 1.74 (1H, m, H_2-3‴), 1.50 (2H, m, H_2-5‴), 1.19 (2H, m, H_2-2‴), 1.16 (3H, d, J=6.8 Hz, Me–6‴), 0.90 (3H, d, J=6.7 Hz, Me–10‴), 0.78 (3H, t, *J*=6.0 Hz, Me–1‴), ^{13}C NMR ($CDCl_3$): δ 132.63 (C-1), 149.11 (C-2), 128.42 (C-3), 122.56 (C-4), 118.73 (C-5), 119.36 (C-6), 170.38 (C-7), 102.53 (C-1′), 82.49 (C-2′), 71.82 (C-3′), 68.13 (C-4′), 62.41 (C-5′), 93.62 (C-1″), 76.19 (C-2″), 71.80 (C-3″), 67.95 (C-4″), 62.24 (C-5″), 14.25 (C-1‴), 27.71 (C-2‴), 47.26 (C-3‴), 29.26 (C-4‴), 29.70 (C-5‴), 31.08 (C-6‴), 42.44 (C-7‴), 66.34 (C-8‴), 22.69 (C-9‴), 19.16 (C-10‴). ESI-MS *m/z* (rel.int.): 542 $[M]^+$ ($C_{27}H_{42}O_{11}$) (8.6), 281 (10.3), 265 (6.3), 149 (29.1), 157 (5.8).

Lignoceryl *O*-β-*D*- arabinoside (4)

Elution of the column with chloroform-methanol (93:7, *v/v*) gave greenish sticky mass of **4**, purified by TLC using chloroform- methanol (3:1, *v/v*), 5 g (0.4 % yield), R_f 0.4 (chloroform) UV λ_{max} (MeOH): 209 nm (log ε 3.7), IR λmax (KBr): 3435, 3338, 2927, 2854, 1722, 1636, 1440, 1379, 1261, 1207, 975, 722 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 5.31 (1H, d, *J*=7.3 Hz, H-1'), 4.42 (1H, m, H-2'), 4.24 (1H, m, H-3'), 4.05 (1H, m, H-4'), 3.84 (2H, d, *J*=6.8 Hz, H-5'), 2.58 (2H, t, *J*=9.6 Hz, H_2-2), 2.27 (1H, m, CH_2), 2.17 (2H, m, CH_2), 1.60 (2H, m, CH_2), 1.57 (2H, m, CH_2), 1.29 (6H, brs, 3 × CH_2), 1.24 (8H, brs, 4 × CH_2), 1.21 (10H, brs, 10 × CH_2), 0.84 (3H, t, *J*=7.6 Hz, Me–24); ^{13}C NMR ($CDCl_3$) δ 175.25 (C–1), 55.94 (C–2), 51.95 (C–3), 34.02 (C–4), 31.92 (C–5), 29.70 (C–6), 29.16 (C–7), 29.36 (C–8), 29.28 (C–9), 29.11 (C–10), 28.89 (C–11), 31.44 (C–12), 29.36 (C–13), 29.36 (C–14), 29.28 (C–15), 29.21 (C–16), 29.15 (C–17), 29.11 (C–18), 28.89 (C–19), 28.80 (C–20), 27.97(C–21), 24.63 (C–22), 22.70 (C–23), 14.15 (C–24), 103.65 (C–1'), 71.18 (C– 2'), 68.47 (C–3'), 66.21 (C–4'), 63.76 (C–5'); ESI-MS *m/z* (rel int.): 500 $[M]^+$ ($C_{29}H_{56}O_6$) (28.3), 367 (16.1), 351 (33.8), 149 (31.8), 133 (85).

Lignoceryl diarabinoside (5)

Elution of the column with chloroform-methanol (9:1, *v/v*) gave dark brown sticky mass of **5**, purified by TLC using chloroform-methanol (9:1, *v/v*), 5 g (0.4 % yield), R_f 0.3 (chloroform); UV λ_{max} (MeOH): 207 nm (log ε 3.1), IR λmax (KBr): 3455, 3390, 3290, 2926, 2854, 1721, 1440, 1635, 1458, 1259, 1039, 722 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 5.31 (1H, d, *J*=7.5 Hz, H-1'), 5.28 (1H, d, *J*=7.1 H-1"), 4.30 (1H, m, H-2'), 4.08 (1H, m, H-2"), 3.90 (1H, m, H-3'), 3.84 (1H, m, H-3"), 3.79 (1H, m, H-4'), 3.76 (1H, m, H-4"), 3.63 (2H, d, *J*=5.6 Hz, H_2-5'), 3.57 (2H, d, *J*=6.6 Hz, H_2-5"), 2.79 (2H, t, *J*=7.3 Hz, H_2-2), 2.57 (2H, m, CH_2), 2.26 (2H, m, CH_2), 2.23 (2H, m, CH_2), 2.16 (2H, m, CH_2), 2.01 (2H, m, CH_2), 1.60 (2H, m, CH_2), 1.53 (2H, m, CH_2), 1.29 (2H, m, CH_2), 1.24 (6H, brs, 3 × CH_2), 1.21 (20H, brs, 10 × CH_2), 0.83 (3H, t, *J*=7.2 Hz, Me- 24). ^{13}C NMR ($CDCl_3$): δ 171.56 (C–1), 42.91 (C–2), 34.01 (C–3), 31.91 (C–4), 31.43 (C–5), 30.18 (C-6), 29.69 (C–7), 29.69 (C–8), 29.69 (C–9), 29.69 (C–10), 29.69 (C–11), 29.51 (C–12), 29.48 (C–13), 29.35 (C–14), 29.14 (C–15), 29.47 (C–16), 29.85 (C–17), 29.96 (C–18), 27.73 (C–19), 27.15 (C–20), 25.32(C-21), 24.77 (C–22), 22.68 (C–23), 14.10 (C–24), 101.93 (C–1'), 80.21 (C–2'), 74.55 (C–3'), 65.61 (C–4'), 63.16 (C–5'), 102.48 (C-1"), 72.34 (C-2"), 68.42 (C-3"), 64.35 (C-4"), 62.27 (C-5"). ESI-MS *m/z* (rel.int.): 632 $[M]^+$ ($C_{34}H_{64}O_{10}$) (38.4), 367 (10.8), 351 (53.1), 281 (32.3), 265 (11.2), 149 (2.7).

Trihydroxynaphthyl tetraglycosidic stearate (6)

Elution of the column with chloroform-methanol (3:1, *v/v*) furnished red semisolid mass of **6**, purified by TLC using methanol, 7.91 g (0.64 % yield); R_f 0.2 (chloroform-methanol 3:1); UV λmax (MeOH): 207, 285 nm. (log ε 4.1, 1.3). IR λmax (KBr): 3490, 3392, 3281, 2927, 2858, 1721, 1664, 1630, 1525, 1458, 1381, 1263, 1070, 722 cm^{-1}. ^{1}H NMR (DMSO-d_6): δ 7.03 (1H, d, *J*=2.2 Hz, H-1), 6.75 (1H, dd, *J*=2.2, 8.0 Hz, H-3), 6.41 (1H, d, *J*=8.0 Hz, H-4), 5.93 (1H, s, H-5), 5.91 (1H, s, H-8), 5.40 (1H, d, *J*=7.3 Hz, H-1a), 3.97 (1H, m, H-5a), 3.73, (1H, m, H-2a), 3.58 (1H, m, H-3a), 3.50 (1H, m, H-4a), 3.29 (2H, d, *J*=4.8 Hz, H_2-6), 5.28 (1H, d, *J*=7.1 Hz, H-1b), 3.95 (1H, m, H-5b), 3.67(1H, m, H-2b), 3.56 (1H, m, H-3b), 3.48 (1H, m, H-4b), 3.24 (2H, d, *J*=5.6 Hz, H_2-6b), 5.20 (1H, d, *J*=7.2 Hz, H-1c), 4.02 (1H, m, H-2c), 3.91(1H, m, H-5c), 3.54 (1H, m, H-3c), 3.45 (1H, m, H-4c), 5.10 (1H, d, *J*=7.0 Hz, H-1d), 4.33 (1H, m, H-2d), 3.90 (1H, m, H-5d), 3.51 (1H, m, H-3d), 3.38 (1H, m, H-4d), 2.59 (2H, t, *J*=7.2 Hz, H_2-2'), 2.18 (2H, m, CH_2), 2.08 (2H, m, CH_2), 1.88 (2H, m, CH_2), 1.40 (2H, m, CH_2), 1.15 (4H, brs, 2 x CH_2), 1.14 (4H, brs, 2 x CH_2), 1.12 (14H, brs, 7 x CH_2), 0.72 (3H, t, *J*=6.2 Hz, Me-18'); ^{13}C NMR (DMSO-d_6): δ 146.16 (C-1), 151.03 (C-2), 128.24 (C-3), 122.32 (C-4), 142.31 (C-5), 148.56 (C-6), 147.14 (C-7), 140.06 (C-8), 131.83 (C-9), 144.41 (C-10), 108.89 (C-1a), 74.08 (C-2a), 74.39 (C-3a), 70.20 (C-4a), 77.13 (C-5a), 63.60 (C-6a), 108.51 (C-1b), 73.31 (C-2b), 74.39 (C-3b), 69.64 (C-4b), 77.13 (C-5b), 63.60 (C-6b), 106.50 (C-1c), 85.01 (C-2c), 72.13 (C-3c), 69.53 (C-4c), 76.28 (C-5c), 176.18 (C-6c), 101.98 (C-1d), 82.67 (C-2d), 74.39 (C-3d), 69.39 (C-4d), 75.03 (C-5d), 177.48 (C-6d), 174.02 (C-1'), 55.40 (C-2'), 42.57 (C-3'), 34.12 (C-4'), 31.79 (C-5'), 30.79 (C-6'),

29.60 (C-7'), 29.60 (C-8'), 29.60 (C-9'), 29.41 (C-10'), 29.37 (C-11'), 29.19 (C-12'), 29.11 (C-13'), 27.13 (C-14'), 25.62 (C-15'), 24.87 (C-16'), 22.69 (C-17'), 14.33 (C-18'). ESI MS *m/z* (rel.int.): 1118 $[M]^+$ ($C_{52}H_{78}O_{26}$) (4.5), 675 (12.8), 619 (13.1), 499 (12.2), 443 (31.7), 267 (17.5), 175 (13.2).

Salicylic acid 2-*O*-β-tetra-arabinosyl tetra methyl hexadecanoate (7)

Elution of the column with chloroform-methanol (3:1, *v/v*) furnished brown colour semisolid mass of **7**, purified by TLC using chloroform-methanol (1:1, *v/v*), 10 g (0.8 % yield); R_f 0.2 (chloroform), UV λmax (MeOH): 207, 257, 275 nm; (log ε 4.6, 2.8, 2.1). IR ν_{max} (KBr): 3505, 3431, 3255, 2933, 2870, 1723, 1690, 1635, 1597, 1440, 1394, 1263, 1066 cm^{-1}. ^1H NMR (DMSO-d_6): δ 7.66 (1H, dd, *J*=2.8, 8.5 Hz, H-3), 7.45 (1H, dd, *J*=2.3, 8.5 Hz, H-6), 7.35 (1H, m, H-5), 7.30 (1H, m, H-4), 5.21 (1H, d, *J*=7.2 Hz, H-1a), 5.10 (1H, d, *J*=7.1 Hz, H-1b), 5.01 (2H, d, J=7.3 Hz, H-1c, H-1d), 4.20 (1H, m, H-2d), 4.03 (1H, m, H-2a), 3.97 (1H, m, H-2b), 3.92 (1H, m,H-2c), 3.74 (1H, m, H-3a), 3.70 (1H, m, H-3b), 3.68 (1H, m, H-3c), 3.65 (1H, m, H-3d), 3.62 (2H, m, H-4a, H-4b), 3.60 (2H, m, H-4c, H-4d), 3.58 (2H, d, *J*=6.0 Hz, H_2-5a), 3.55 (2H, d, *J*=11.2 Hz, H_2-5b), 3.52 (2H, d, *J*=7.2 Hz, H_2-5c), 3.49 (2H, d, *J*=6.0 Hz, H_2-5d), 2.28 (1H, m, H-2'), 2.20 (1H, m, H-6'), 1.91 (1H, m, H-10'), 1.88 (1H, m, H-14'), 1.49 (2H, m, H_2-3'), 1.47 (2H, m, H_2-4'), 1.20 (2H, m, H_2-5', H_2-7'), 1.16 (12H, brs, 6 x CH_2), 0.94 (3H, d, *J*=6.0 Hz, Me-17'), 0.90 (3H, d, *J*=6.0 Hz, Me-19'), 0.87 (3H, d, *J*=7.2 Hz, Me-18'), 0.84 (3H, d, *J*=6.8 Hz, Me-20'), 0.79 (3H, d, *J*=6.8 Hz, Me-16'); ^{13}C NMR (DMSO-d_6); δ 141.98 (C-1), 153.21 (C-2), 131.45 (C-3), 127.67 (C-4), 127.88 (C-5), 128.70 (C-6), 180.36 (C-7), 103.65 (C-1a), 76.73 (C-2a), 73.70 (C-3a), 70.09 (C-4a), 62.67 (C-5a), 103.49 (C-1b), 75.22 (C-2b), 72.81 (C-3b), 70.29 (C-4b), 62.91 (C-5b), 101.74 (C-1c), 74.77 (C-2c), 71.91 (C-3c), 69.66 (C-4c), 63.15 (C-5c), 98.04 (C-1d), 83.15 (C-2d), 71.73 (C-3d), 69.02 (C-4d), 63.23 (C-5d), 171.63 (C-1'), 56.59 (C-2'), 31.60 (C-3'), 29.39 (C-4'), 29.13 (C-5'), 39.99 (C-6'), 24.61 (C-7'), 24.25 (C-8'), 24.38 (C-9'), 36.46 (C-10'), 22.31 (C-11'), 21.88 (C-12'), 21.55 (C-13'), 33.83 (C-14'), 20.91 (C-15'), 10.99 (C-16'), 17.71 (C-17'), 16.82 (C-18'), 14.23 (C-19'), 13.22 (C-20'). ESI MS *m/z* (rel.int.): 960 $[M]^+$ ($C_{47}H_{76}O_{20}$) (2.1), 665 (6.8), 427 (14.5), 401 (12.3), 295 (18.2), 137 (11.8).

RESULTS AND DISCUSSION

Compound **1** was a known fatty acid identified as *n*-triacontanoic acid (melissic acid) (Chibnall et al., 1933).

Compound **2**, designated as 2-palmityloxybenzyl alcohol, showed characteristic IR absorption bands for hydroxyl group (3321 cm^{-1}), ester function (1724 cm^{-1}), aromatic ring (1622, 1525 cm^{-1}) and long aliphatic chain (719 cm^{-1}). On the basis of mass and ^{13}C NMR spectra the molecular ion peak of **2** was determined at *m/z* 362 consisting to the molecular formula of a phenyl alcoholic ester $C_{23}H_{38}O_3$. The ion fragments arising at *m/z* 239 $[CH_3(CH_2)_{14}CO]^+$, *m/z* 255 $[CH_3(CH_2)_{14}COO]^+$ and 123 [M-239; $C_6H_4OCH_2OH$] indicated that palmitic acid was esterified with a hydroxyl benzyl alcohol. The ^1H NMR spectrum of **2** showed a one-proton doublet at δ 7.20 (*J*=7.6 Hz), a one-proton double doublet at 6.24 (*J*=2.3, 8.4 Hz), and two one-proton multiplets at δ 5.94 and 5.43 assigned to aromatic H-3, H-6, H-5 and H-4 protons, respectively. A two-proton broad singlet at δ 3.56 was ascribed to hydroxylmethylene H_2-7 protons. A two-proton triplet at δ 2.82 (*J*= 6.5 Hz), four two- proton multiplets between δ 2.38-1.49 and a broad singlet at δ 1.32 (18H) were associated with the methylene protons of the acyl chain. A three-proton triplet at δ 0.96 (*J*=6.0 Hz) was accounted to C-16' primary methyl protons. The ^{13}C NMR spectrum of **2** showed signals for aromatic carbons between δ 135.03-127.11, benzyl methylene carbon at δ 71.85 (C-7), ester carbon at δ 173.12 (C-1') and remaining carbon signals are resonated between δ 37.46-14.11. Acid hydrolysis of **2** yielded pamitic acid, co-TLC comparable. On the basis of above discussion the structure of compound **2** was formulated as 2-hexadecanoxybenzyl alcohol. This is a new aromatic alcohol ester.

Compound **3**, named 2-*O*-diarabinosyl-8'''-geranilanyl salicylate, was obtained as a green amorphous mass from chloroform-methanol (19:1 *v/v*) eluants. It gave positive tests for carbohydrates and displayed characteristic IR absorption bands for hydroxyl groups (3482, 3390, 3292 cm^{-1}), ester function (1721 cm^{-1}) and aromatic ring (1635, 1525, 1085 cm^{-1}). The molecular ion peak of **3** was determined at *m/z* 542 on the basis of mass and ^{13}C NMR spectra corresponding to a molecular formula of a phenolic acid glycosidic ester $C_{27}H_{42}O_{11}$. The ion peaks arising at *m/z* 149 $[C_5H_9O_5]^+$, 265 $[C_5H_9O_5\text{-}C_5H_8O_3]^+$ and 281 $[C_5H_9O_5\text{-}C_5H_8O_4]^+$ suggested that two C-5 sugar units were linked to phenyl group. An ion peak formed at *m/z* 157 $[C_{10}H_{21}O]^+$ suggested that a C-10 alcohol was esterified with the aromatic acid. The ^1H NMR spectrum of **3** exhibited two one-proton double doublets at δ 7.63 (*J*=3.0, 8.8 Hz) and 7.45 (*J*=3.0, 9.0 Hz) and two one-proton multiplets at δ 7.31 and 7.27 assigned to aromatic H-3, H-6, H-5 and H-4 protons, respectively. Two one-proton doublets at δ 5.31 (*J*=7.3 Hz) and 4.98 (*J*=7.1 Hz) were ascribed to anomeric H-1' and H-1'' protons, respectively. The other sugar protons appeared between δ 4.38–3.56. A two-proton doublet at δ 4.01 (*J*= 6.8 Hz) was accounted to oxygenated methylene H_2-8''' protons and its presence in the deshielded region indicated the location of the ester group at C-8'''. Two three-proton doublets at δ 1.16 (*J* =6.8 Hz) and 0.90 (*J*=6.7 Hz) and a three-proton triplet at δ 0.78 (*J*=6.0 Hz) were associated correspondingly with the secondary C-9''' and C-10''' and primary C-1''' methyl protons of monoterpenic unit. The remaining methylene and methine proton resonated from δ 2.24 to 1.19. The ^{13}C NMR spectrum of **3** showed signals for aromatic carbons between δ 149.11 -119.36, ester group at δ 170.38 (C-7), anomeric carbons at δ 102.53 (C-1') and δ 93.62 (C-1''), while remaining sugar protons appeared between δ 82.49-62.24. Downfield signal of terpenic carbon at δ 66.34 (C-8''') due to adjacent to ester group, remaining methine and methylene carbon are appeared between δ 27.71-31.08 and methyl carbons at δ 14.25 (C-1''') and δ 19.16 (C-10'''). On the basis of aforementioned evidences the structure of **3** was elucidated as 2-*O*-β-*D*-arabinopyranosyloxy-2'-*O*-β-*D*-arabinopyranosyl-(8'''-geranilanyl) benzoate. This is a new phenolic glycoside. Compound **4**,

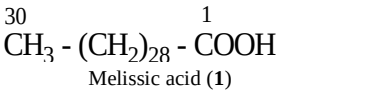
designated as lignoceryl *O*-β-*D*- arabinoside, gave positive tests for glycosides and displayed characteristic IR absorption bands for hydroxyl groups (3435 and 3338 cm^{-1}), ester function (1722 cm^{-1}), and long chain aliphatic hydrocarbon (722 cm^{-1}). On the basis of mass and ^{13}C NMR spectra the molecular ion peak of **4** was established at *m/z* 500 corresponding to a molecular formula of an acyl glycoside, $C_{29}H_{56}O_6$. The ion peaks arising at *m/z* 133 $[C_5H_9O_4]^+$, 149 $[C_5H_9O_5]^+$, 367 [M-133, $CH_3(CH_2)_{22}COO]^+$ and 351 [M-149, $CH_3(CH_2)_{22}CO]^+$ suggested that a C_5 sugar unit was esterified with a C_{24} fatty acid. The ^1H NMR spectrum of **4** exhibited a one-proton doublet at δ 5.31 (*J*= 7.3 Hz) assigned to anomeric H-1′ protons. Three one-protons multiplets between δ 4.42-4.05 were ascribed to sugar carbinol protons, a two-protons doublets at δ 3.84 (*J*=8.5 Hz) was accounted to oxygenated methylene H_2-5′ protons. A two-proton triplets at δ 2.58 (*J*=9.6 Hz) was due to methylene H_2-2 proton adjacent to the ester group, and remaining methylene protons appeared between δ 2.27-1.21. A three-proton triplet at δ 0.84 (*J*= 7.6 Hz) was associated with the terminal methyl H_3-24 protons. The ^{13}C NMR spectrum of **4** exhibited signals for the ester carbon at δ 175.25 (C-1), anomeric carbon at δ 103.65 (C-1′), other sugar carbons in the range from δ 71.18 to 63.76, methylene carbons between δ 55.94- 22.70 and methy1 carbon at 14.15 (C-24). Acid hydrolysis of **4** yielded lignoceric acid and D-arabinose, co-TLC comparable. On the basis of the above mentioned discussion the structure of **4** has been elucidated as *n*-tetracosanoyl- *O*-β-*D*-arabinopyranoside. This is new acyl glycoside.

Compound **5**, designated as lignoceryl diarabinoside, was a dark brown semisolid mass. It gave positive tests for glycosides and displayed characteristic IR absorption bands for hydroxyl groups (3455, 3390, 3290 cm^{-1}), ester function (1721 cm^{-1}), and long chain aliphatic hydrocarbon (722 cm^{-1}). The molecular ion peak arising at *m/z* 632 consistent to molecular formula of acyl diglycoside, $C_{34}H_{64}O_{10}$. The ion fragments at *m/z* 149 $[C_5H_9O_5]^+$, 265 $[C_5H_9O_5\text{-}C_5H_8O_3]^+$ and 281 $[C_5H_9O_5\text{-}C_5H_8O_4]^+$, 367 [M-265, $CH_3(CH_2)_{22}COO]^+$ and 351 [M-281, $CH_3(CH_2)_{22}CO]^+$ suggested that behenic acid was esterified with a diarabinose unit. The ^1H NMR spectrum of **5** exhibited two one-proton doublets at δ 5.31 (*J*=7.5 Hz) and δ 5.28 (*J*=7.1 Hz) assigned to anomeric H-1′ and H-1″ protons, respectively. Six one-protons multiplets appeared between δ 4.30- 3.76 each assigned for carbinol protons, two two-proton doublets at δ 3.63 (*J*=5.6 Hz) and δ 3.57 (*J*=6.6 Hz) were accounted to oxygenated methylene H_2-5′ and H_2-5″ protons, respectively. A two-proton triplet at δ 2.79 (*J*=7.3 Hz) was ascribed to methylene H_2-2 proton nearby to the ester function, and the remaining methylene protons appeared between δ 2.57-1.21. A three-proton triplet at δ 0.83 (*J*=7.2 Hz) was due to terminal methyl H_3-24 protons. The ^{13}C NMR spectrum of **5** exhibited signals for the ester carbon at δ 171.56 (C-1), anomeric carbons at δ 101.93 (C-1′) and δ 102.48 (C-1″), other sugar carbons in the range from δ 80.21 to 62.27, methylene carbons between δ 42.91-22.68 and methy1 carbon at 14.06 (C-24). The presence of ^1H NMR signal for H-2′ in the deshielded region at δ 4.30 and ^{13}C NMR signals for C-2′ at δ 80.21 suggested the attachment of another sugar at (2′→1″) linkage. Acid hydrolysis of **5** yielded lignoceric acid and *D*-arabinose, co-TLC comparable. On the basis of these evidences the structure of **5** has been elucidated as *n*-tetracosanoyl-*O*-β-*D*- arabinopyranosyl-(2′→1″)-*O*-β-*D*-arabinopyranoside. This is a new fatty ester diarabinoside.

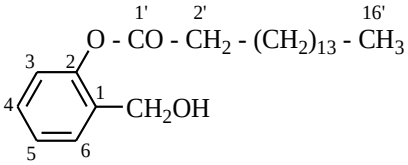
Compound **6**, named as trihydroxynaphthyl tetraglycosidic stearate, was obtained as a red sticky semisolid mass. It responded glycosidic tests positively and showed IR absorption bands for hydroxyl groups (3490, 3392, 3281 cm^{-1}), ester group (1721 cm^{-1}), aromatic ring (1525, 1458, 1070 cm^{-1}) and long chain aliphatic hydrocarbon (722 cm^{-1}). On the basis of mass and ^{13}C NMR spectral data, the molecular ion peak of **6** was established at *m/z* 1118 corresponding to the molecular formula of a trihydroxynaphthyl tetraglycosidic ester ($C_{52}H_{78}O_{26}$). An ion peak generating at *m/z* 175 [O-C_{1a} fission, $C_{10}H_7(O)_3]^+$ suggested the existence of a trihydroxynaphthyl unit in the molecule. The ion fragments arising at *m/z* 267 $[CH_3(CH_2)_{16}CO]^+$ indicated the attachment of a C_{18} acyl unit in the compound. The ion peaks produced at *m/z* 443 $[CH_3(CH_2)_{16}CO\text{-}C_5H_8O_4COOH]^+$, 619 $[CH_3(CH_2)_{16}CO\text{-}(C_5H_8O_4COOH)_2]^+$, 675 [M-443]$^+$ and 499 [M-619]$^+$ supported the presence of stearyl substituted glycosidic acid units at the terminal position linked to the remaining glycosidic chain. The ^1H NMR spectra of **6** exhibited two one- proton doublets at δ 7.03 (*J*=2.2 Hz) and 6.41 (*J*=8.0 Hz), a one-proton double doublet at δ 6.75 (*J*=2.2, 8.0 Hz), and two one-proton singlet at δ 5.93 and 5.91 assigned, respectively, to aromatic meta-coupled H-1 adjacent to the glycoside linked phenolic group, ortho-coupled H-4, meta, ortho-coupled H-3 and para-coupled H-5 and H-8 protons. Four one-proton doublets at δ 5.40 (*J*=7.3 Hz), 5.28 (*J*=7.1Hz), 5.20 (*J*=7.2 Hz) and 5.10 (*J*=7.0 Hz) were assigned to sugar anomeric H-1a, 1b, 1c and 1d protons, respectively. The other sugar protons resonated from δ 4.33 to 3.18. A two-proton triplet at δ 2.59 (*J*=7.2 Hz) was ascribed to methylene H_2-2' protons adjacent to the ester group. A three-proton triplet at δ 0.72 (*J*=6.2 Hz) was due to terminal C-18' primary methyl protons. The remaining methylene protons appeared between δ 2.18-1.12. The ^{13}C NMR spectrum of **6** displayed signals for naphthalenic carbons between δ 151.03 to 122.32 , ester carbon at δ 174.02 (C-1'), anomeric carbons at δ 108.89 (C-1a), 108.51 (C-1b), 106.50 (C-1c) and 101.98 (C-1d), carboxylic carbons at δ 176.18 (C-6c) and δ 177.48 (C-6d), remaining sugar carbons resonated between δ 82.67 to 63.60, methylene carbons from δ 55.40 to 22.69, and terminal methyl carbon at δ 14.33 (C-18'). The presence of downfield signals of two oxygenated methylene protons as doublets at δ 3.29 (*J*=4.8 Hz) for H_2-6a and at δ 3.24 (*J*=5.6 Hz) for H_2-6b in the ^1H NMR spectrum and carbon signals δ 63.60 (C-6a, C-6b) in the ^{13}C NMR spectrum suggested the (6a→1b) and (6b→1c) linkages between two sugar units, and the existence of two downfield signals as multiplets at δ 4.02 (H-2c) and δ 4.33 (H-2d) and the carbon signals at δ 85.01 (C-2c) and δ 82.67 (C-2d) suggested that (2c→1d) and (2d→1') linkages of two sugar units and aliphatic chain. On the basis of above discussion the compound **6** is structurally elucidated as 2,6,7- trihydroxynaphthyl-2-*O*-β-*D*-

glucopyranosyl-(6a→1b)-*O*-β-*D*-glucopyranosyl-(6b→1c)-*O*-β-*D*-glucuranopyranosyl-(2c→1d)-*O*-β-*D*-glucuranopyranosyl-2d-*n*-octadecanoate. This is new naphthol tetraglycosidic ester.

Compound **7**, named salicylic acid 2-*O*-β-tetra-arabinosyl tetra methyl hexadecanoate, responded glycosidic tests positively and showed IR absorption bands for hydroxyl groups (3505, 3431, cm^{-1}), carboxylic group (3255, 1690 cm^{-1}), ester group (1723 cm^{-1}), and aromatic ring (1597, 1066 cm^{-1}). On the basis of mass and ^{13}C NMR spectral data, the molecular ion peak of **7** was established at *m/z* 960 consistent with the molecular formula of a salicyl tetraglycosidic ester ($C_{47}H_{76}O_{20}$). An ion peak generating at *m/z* 137 [O-1a fission, $C_6H_4(O)COOH]^+$ indicated the location of salicylic unit at one of the terminal of the molecule. The ion fragments produced at *m/z* 295 [O-C_1 fission, $C_{20}H_{39}O]^+$, 427 [O-1d fission, $C_{20}H_{39}O\text{-}C_5H_8O_4]^+$, and 401 [O-1c fission, $C_6H_5(COOH)\text{-}C_5H_8O_5\text{-}C_5H_8O_4]^+$, suggested the diterpenic unit at another terminal and presence of four C_5 sugar units in the molecule. The ^1H NMR spectrum of **7** displayed two one-proton double doublets at δ 7.66 (*J*=2.8, 8.5 Hz) and 7.45 (*J*=2.3, 8.5 Hz), and two one-proton multiplets at δ 7.35 and 7.30 assigned to aromatic H-3 adjacent to glycosidic oxygen, H-6, H-5, and H-4 protons, respectively. Two one-proton doublets at δ 5.21 (*J*=7.2 Hz) and 5.10 (*J*=7.1 Hz) and a two-proton doublets at δ 5.01 (*J*=7.3 Hz) assigned to sugar anomeric H-1a, H-1b, H-1c, and H-1d protons, respectively. The other sugar protons resonated from δ 4.20 to 3.49. Four three-proton doublets at δ 0.94 (*J*=6.8 Hz), 0.90 (*J*=6.0 Hz), 0.87 (*J*=7.2 Hz), and 0.84 (*J*=6.8 Hz), and a three-proton triplet at δ 0.79 (*J*=6.8 Hz) were accounted to secondary C-17', C-19', C-18' and C-20' and primary C-16' methyl protons, respectively. The remaining methine and methylene protons appeared between δ 2.28-1.16. The ^{13}C NMR spectrum of **7** displayed signals for aromatic carbons between δ 153.21 to 127.67, carboxylic carbon at δ 180.36 (C-7), ester carbon at δ 171.63 (C-1'), anomeric carbons at δ 103.65 (C-1a), 103.49 (C-1b), 101.74 (C-1c) and 98.08 (C-1d) and the remaining sugar carbons between δ 83.15 to 62.67. Other methylene and methine carbons are resonated between δ 56.59-20.91, primary and secondary methyl carbon appeared between δ 17.71-10.99. The presence ^1H NMR signals in the downfield region as multiplets at δ 4.03 (H-2a), δ 3.97 (H-2b), δ 3.92 (H-2c), and δ 4.20 (H-2d) and their respective carbon signals at δ 76.73 (C-2a), 75.22 (C-2b), 74.77 (C-2c) and 85.15 (C-2d) suggested the (2a→1b), (2b→1c) and (2c→1d) linkages between four sugar units and aliphatic chain at C-2d. On the basis of above discussion the compound **7** is structurally elucidated as 2-hydroxy benzoic acid-2-*O*-β-*D*-arabinopyranosyl-(2a→1b)-*O*-β-*D*-arabinopyranosyl-(2b→1c)-*O*-β-*D*-arabino- pyranosyl-(2c→1d)-*O*-β-*D*-glucuranopyranosyl-2d-(2',6',10',14'-tetramethyl)-*n*-hexadecan-1'-oate. This is a new salicylic acid tetraglyosidic diterpenoid.



Melissic acid (**1**)



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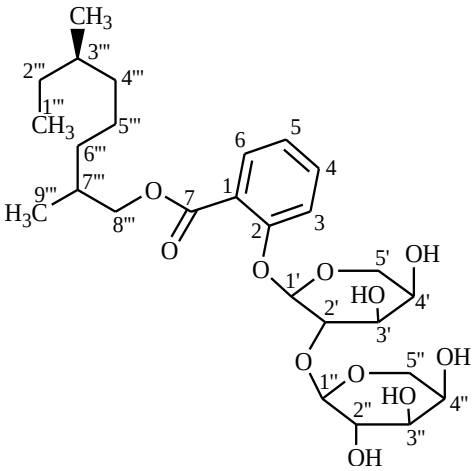
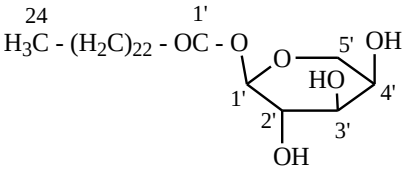
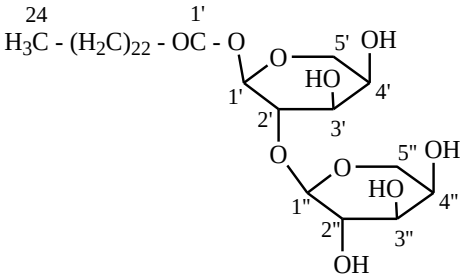


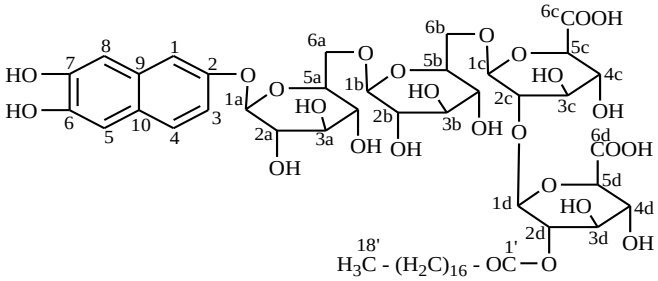
2-*O*-Diarabinosyl-8‴-geranilanyl salicylate (**3**)



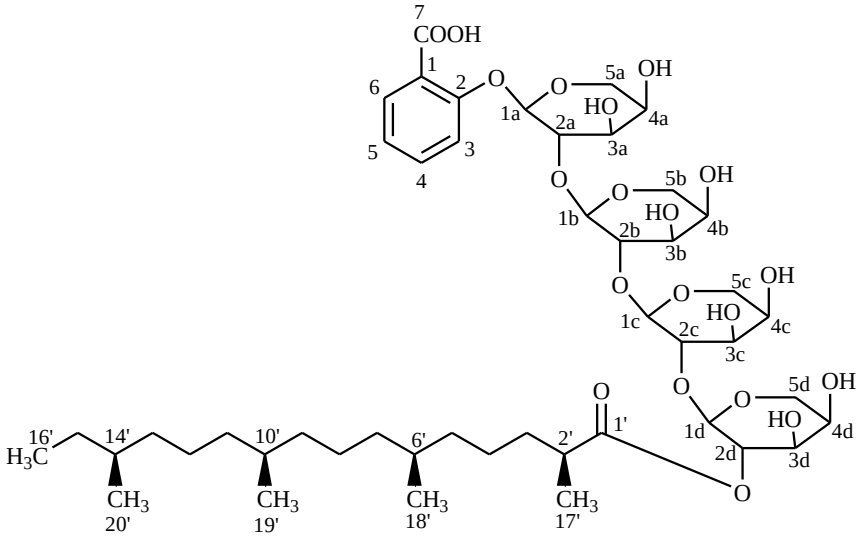
Lignoceryl *O-β-D*-arabinoside (**4**)



Lignoceryl diarabinoside (**5**)



Trihydroxynaphthyl tetraglycosidic stearate (**6**)



Salicylic acid-2-*O*-*β*-tetra-arabinosyl tetramethylhexadecanoate (**7**)

CONCLUSION

Plants have been used as medicine for humankind since ancient times in the indigenous systems and many tribal communities. They produced diverse range of novel bioactive secondary metabolites making them a prosperous therapeutic application. Isolation of six new crucially unmatched complex and potentially active secondary metabolites from this traditional medicinal herb has proven their ancient and folkloric utilization. Further chemical pattern of this plant could be used as reference compounds and establish a platform for authentication and quality control purposes in future.

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