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Fatty acid Glycosides from the Roots of *Ricinus Communis* L.

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ABSTRACT

Phytochemical investigation of the roots of *Ricinus communis* L. (Euphorbiaceae), used to cure lumbago and toothache, led to the isolation of acyl glycosides characterized as *n*-octadec-9-enoyl β -D-glucopyranoside (**4**), *n*-octadecanoyl- β -D-glucopyranoside (**5**), *n*-docosanyl- β -D-glucopyranoside (**6**), *n*-hexadecanoyl β -D-glucuranylopyranoside (**7**) and *n*-octadecanoyl- β -arabinopyranosyl – (2 \rightarrow 1'') – β -arabinopyranoside (**9**), a trisaccharide β -D-glucuranylopyranosyl-(2 \rightarrow 1)- β -D-arabinopyranosyl-(2 \rightarrow 1) – β -D-arabinopyranoside (**8**), a tetraglycoside β -D-glucopyranosyl-(2 \rightarrow 1)- β -D-arabinopyranosyl-(2 \rightarrow 1)- β -D-arabinopyranosyl-(2 \rightarrow 1)- β -D-arabinopyranoside (**10**), a triterpenic glucoside α -amyrin β -D-glucopyranoside (**1**) and two steroidal glycosides viz., stigmasterol β -L-arabinopyranoside(**2**) and β -sitosterol β -D-glucopyranoside(**3**). The structures of all these glycosides, isolated from the roots for the first time, have been characterized on the basis of spectral data analysis and chemical reactions.

Keywords: *Ricinus communis*, Euphorbiaceae, Roots, acyl glycosides, steroidal glycosides.

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INTRODUCTION

Ricinus communis L. (Euphorbiaceae), known as castor plant, is an annual or perennial soft wooded small tree up to 6 m in height (Figure. 1). It is widespread throughout tropics and warm temperate regions of the world and cultivated in India and other countries up to 2,000 m altitude^{1,2}. It is an important oil seed crop which produces an oil rich in ricinoleic acid conferring unique properties of the oil^{3,4,5}. The castor oil is cathartic and is official in some pharmacopoeias. It is used to prepare some liquid disinfectants like phenyls, hair oils, fixers, aromatic perfumes, lipsticks, hair lotions and tonics². An emulsion of the oil and soap is effective against some crop pests.



Figure. 1. *Ricinus communis* plant

The oil is a source of N-isobutyl undecylate amide, a valuable synergist which can be used with pyrethrum. It is applied as a preservative to food grains and pulses. The plant leaves are prescribed to treat boils, sores, swellings, stomachache, jaundice, teeth carries, flatulence in children, guinea worm sores and as a lactagogue. A decoction of the root is administered to relieve lumbago, and a root paste is applied to alleviate toothache. The efficacy of the seeds as a contraceptive drug has been studied with several traditional applications^{6,7}. They have been used with arguable success in the treatment of warts, cold, tumours and indurations of the mammary glands, corns and moles^{8,9,10}. Its extracts were found to cause proportional increase in mean wheal diameter in skin tests in castor bean allergic workers¹¹. The anti-inflammatory and the free radical scavenging activity were well demonstrated¹². Nowadays, there is increasing interest in the use of naturally occurring substances for the preservation of food. Plant essential oils and their components have been known to exhibit biological activities. The root bark is a powerful purgative^{2,13}, anti-inflammatory, antioxidant¹², antifertility, contraceptive¹⁴, antibacterial¹⁵, and yields 3-O-benzoyl-stigmast-5,22-dien-3 β , 21-diol and 3 α -hydroxy-pentatriacont-14-en-26-one¹⁶. The leaves afforded ricinine, n-demethylricinine and flavonoid glycosides^{17,18}. The seeds

possessed gallotannins, lupeol and 30-nor lupan-3 β -ol-20-one^{2,19,20}. An essential oil of the aerial parts is consisted of α -pinene, 1,8-cineole, α -thujone, camphor and camphene²¹. The present paper describes the isolation and characterization of fatty acid glycosides along with steroidal and triterpenic glycosides from the roots of *R. communis* of arid region.

MATERIALS AND METHOD

General experimental Procedures

The chemicals and solvents (S.D. Fine, Mumbai) were purchased from Chopra Chemicals, New Delhi. Melting points were measured on a thermoelectrically operated Perfit apparatus without correction. UV spectra were determined on Shimadzu-120 double beam spectrophotometer with methanol as a solvent. IR spectra were recorded in KBr pellet on Shimadzu FTIR-8400 spectrophotometer. ¹H NMR (400 MHz) and ¹³C NMR (100 MHz) spectra were scanned by Bruker spectropin NMR instrument, using TMS as internal standard. FAB mass spectra were recorded on a Jeol D-300 spectrometer. Column chromatography separations were carried out on silica gel (Merck, 60-120 mesh). Precoated silica gel plates (Merck, Silica gel 60 F₂₅₄) were used for analytical thin layer chromatography. The spots were visualized by exposure of the TLC plates to iodine vapours, UV radiations and by spraying with ceric sulphate solution. The percentage yields of the isolated compounds were calculated on the basis of dried plant material taken for extraction.

Plant material

The fresh roots of *R. communis* were collected from the arid waste land of Jaipur (Rajasthan). The plant material was identified on the basis of exomorphic characters and reviews of literature by Prof. M. P. Sharma, taxonomist, Department of Botany, Jamia Hamdard, New Delhi. A voucher specimen No. PRL/ JH / 09 / 12 is deposited in the herbarium of the Department of Pharmacognosy and Phytochemistry, Jamia Hamdard, New Delhi.

Extraction and isolation

The air-dried roots (2 kg) of *R. communis* were coarsely powdered and extracted exhaustively in a Soxhlet apparatus with methanol for 72 hr. The methanolic extract was concentrated under reduced pressure to obtain a dark brown viscous mass (234 g). Small portion of the extract was analyzed chemically to determine the presence of different chemical constituents. The viscous brown mass was dissolved in minimum amount of methanol and adsorbed on silica gel (60-120 mesh) for preparation of slurry. The slurry (200 g) was air dried and chromatographed over silica gel column packed in petroleum ether. The column was eluted successively with petroleum

ether, mixture of petroleum ether and chloroform (9:1, 3:1, 1:1, 1:3, v/v), pure chloroform and finally the mixture of chloroform and methanol (99:1, 97:3, 95:5, 92:8, 9:1, 3:1, 1:1, 1:3, v/v). Various fractions were collected separately and matched by TLC to check homogeneity. Similar fractions having same R_f values were combined and crystallized. The isolated compounds were recrystallized to get the following pure compounds:

α -Amyrin glucopyranoside (1)

Elution of the column with chloroform- methanol (19:1) afforded colourless crystals of **1**, recrystallized from methanol, 35 mg (0.033% yield); R_f : 0.38 (chloroform- methanol 24:1); m.p : 228-230 °C; +ve ion FAB MS m/z (rel. int.) : 588 $[M]^+$, ($C_{36}H_{60}O_6$) (25.1).

Stigmasterol- β - L- arabinoside (2)

Further elution of the column with chloroform-methanol (19:1) yielded colorless amorphous powder of **2**; recrystallized from methanol, 45 mg (0.042 % yield.); R_f : 0.72 (toluene : ethylformate : formic acid ; 5:5: 1.5); m.p. : 208-210 °C; +ve FAB MS m/z (rel. int.) : 544 $[M]^+$ ($C_{34}H_{56}O_5$) (22.1).

β -Sitosterol glucoside (3)

Elution of the column with chloroform-methanol (93:7) mixture gave colorless amorphous powder of **3**, recrystallized from methanol, 53 mg (0.050 % yield); R_f : 0.53 (benzene : chloroform : methanol; 5:4:1); m.p. : 270-272 °C ; +ve FAB MS m/z (rel. int.) : 576 $[M]^+$ ($C_{35}H_{60}O_6$) (10.2), 413 $[M-sugar]^+$ ($C_{29}H_{50}O$) (4.3), 398 (15.5), 396 (20.5), 381 (15.3), 273 (4.3), 255 (11.7), 240 (7.6), 213 (8.2).

Oleiyglucoside (4)

Elution of the column with chloroform-methanol (9:1) furnished colourless crystals of **4**, recrystallized from methanol, 55 mg (0.052% yield); R_f : 0.89 (chloroform – methanol ; 22:3); m.p. : 113-114 °C; UV λ_{max} (MeOH) : 207, 222 nm (log ϵ 3-8, 3.9); IR ν_{max} (KBr) : 3477, 3265, 2927, 1741, 1617, 1470, 1373, 1279, 1090, 793, 718 cm^{-1} ; 1H NMR (DMSO- d_6) : δ 5.28 (2H, m, H-9, H-10), 4.96 (1H, d, $J=7.1$ Hz, H-1') 4.45 (1H, m, H-5'), 4.35 (1H, m, H-2'), 3.64 (1H, m, H-3'), 3.49 (1H, m, H-4'), 3.39 (1H, d, $J=5.4$ Hz, H₂-6'a), 3.37 (1H, d, $J=5.4$ Hz, H₂-6'b), 2.27 (2H, t, $J=7.2$ Hz, H₂-2), 2.20 (2H, m, H₂-8), 2.16 (2H, m, H₂-11), 1.98 (2H, m, CH₂), 1.79 (2H, brs, CH₂), 1.64 (2H, brs, CH₂), 1.49 (2H, brs, CH₂), 1.21 (14H, brs, 7 x CH₂), 0.89 (3H, t, $J=6.3$ Hz, CH₃-18); ^{13}C NMR (DMSO- d_6) : δ 171.69 (C-1), 127.99 (C-9), 126.51 (C-10), 101.23 (C-1'), 76.86 (C-2'), 73.67 (C-3'), 72.42 (C-4'), 81.78 (C-5'), 63.32 (C-6'), 52.19 (C-2), 37.32 (CH₂), 28.95 (5 x CH₂), 28.49 (3 x CH₂), 26.71 (CH₂), 24.66 (CH₂), 22.40 (CH₂), 20.01 (CH₂),

13.61 (CH₃-18); +ve ES MS m/z (rel. int.) : 444 [M]⁺ (C₂₄ H₄₄ O₇) (5.1), 281 (27.9), 265 (21.6), 180 (19.2).

Stearyl glucoside (5)

Elution of the column with chloroform-methanol (17:3) yielded light brown sticky mass of **5**, recrystallized from methanol-acetone (1:1); 57 mg (0.054 % yield); R_f : 0.71 (chloroform-methanol 17:3); m.p. : 80-82 °C; UV λ_{max} (MeOH) : 206 nm (log ε 4.8); IR ν_{max}(KBr) : 3415, 3380, 2919, 2850, 1741, 1465, 1409, 1217, 1040, 809 cm.⁻¹; ¹H NMR (DMSO-d₆) : δ 4.95 (1H, d, J=7.1 Hz, H-1'), 4.45 (1H, m, H-5'), 4.31 (1H, m, H-2'), 4.22 (1H, m, H-4'), 3.56 (1H, m, H-3'), 3.28 (2H, brs, H₂-6), 2.30 (2H, m, H₂-2), 1.55 (2H, m, CH₂), 1.23 (28H, brs, 14x CH₂), 0.85 (3H, t, J = 6.3 Hz, Me-18); ¹³C NMR (DMSO-d₆) : 169.16 (C-1), 103.43 (C-1'), 74.60 (C-2'), 73.16 (C-3'), 71.99 (C-4'), 81.43 (C-5'), 62.16 (C-6'), 31.54 (CH₂), 30.44 (CH₂), 28.35 (12xCH₂), 25.30 (CH₂), 22.28 (CH₂), 13.69 (CH₃-18); +ve ion FAB MS m/z (rel. int.) : 446 [M]⁺, (C₂₄H₄₆O₇) (22.3), 283 (24.0), 163 (25.3).

Docosanyl glucoside (6)

Elution of the column with chloroform- methanol (4:1) produced pale yellow sticky mass of **6**, recrystallized from methanol-acetone (1:1), 27 g (0.025% yield); R_f: 0.70 (chloroform : methanol ; 4:1); m.p. : 248-249 °C; UV λ_{max} (MeOH) :211 nm (log ε 4.9); IR ν_{max} (KBr) : 3397, 3265, 2928, 2850, 1725, 1638, 1462, 1377, 1217, 1040, 729 cm.⁻¹; ¹H NMR (DMSO-d₆) : δ 4.95 (1H, d, J = 7.1 Hz, H-1') 4.58 (1H, m, H-5'), 4.43 (1H, m, H-2'), 3.67 (1H, m, H-4'), 3.56 (1H, m, H-3'), 3.13 (1H, d, J =12.0 Hz, H₂-6'a), 3.09 (1H, d, J =12.0Hz, H₂-6'b), 2.92 (1H, d, J = 9.0 Hz, H₂-2a), 2.86 (1H, d, J = 9.0 Hz, H₂-2b), 1.23 (38 H, brs, 19 x CH₂), 0.83 (3H, t, J = 6.5 Hz, Me-22); ¹³C NMR (DMSO-d₆):δ 170.88 (C-1), 103.43 (C-1'),74.60 (C-2'), 73.16 (C-3'), 71.99 (C-4'), 81.43 (C-5'), 62.16 (C-6'), 31.54 (CH₂), 30.44 (CH₂), 28.35 (20H, brs, 12 x CH₂), 28.54 (3 x CH₂), 25.30 (CH₂), 24.15 (CH₂), 22.28 (CH₂) 13.69 (CH₃-22); +ve ion FAB MS m/z (rel. int.) : 502 [M]⁺ (C₂₈H₅₄O₇) (12.3), 339 (18.5), 163 (15.6).

Palmityl Glucuronoside (7)

Elution of the column with chloroform- methanol (3:1) gave cream-coloured sticky mass of **7**, recrystallized from methanol-acetone (1:1), 76 mg (0.072 % yield); R_f : 0.41 (chloroform-methanol 3:1); m.p. : 138-140 °C; UV λ_{max} (MeOH) : 210 nm (log ε 5.2); IR ν_{max} (KBr) : 3396, 3250, 2928, 2850, 1739, 1698, 1616, 1491, 1377, 1217, 1040, 831, 729 cm.⁻¹; ¹H NMR (DMSO-d₆) :δ 4.99 (1H, d, J=7.1 Hz, H-1'), 4.85 (1H, d, J=7.5 HZ, H-1'), 4.42 (1H, m, H-5'), 4.22 (1H, m, H-2'), 3.67 (1H, m, H-4'), 3.34 (1H, m, H-3'), 2.50 (2H, brs, H₂-2), 1.23 (26H, brs, 13xCH₂),

0.85 (3H, t, J = 6.3 Hz, Me-16); ^{13}C NMR (DMSO- d_6) : δ 170.88 (C-1), 105.28 (C-1'), 74.60 (C-2'), 73.16 (C-3'), 70.57 (C-4'), 81.43 (C-5'), 181.09 (C-6'), 31.54 (CH₂), 30.44 (CH₂), 28.35 (10xCH₂), 25.30 (CH₂), 22.28 (CH₂), 14.25 (C-16); +ve FAB MS m/z (rel. int.) : 433 [M+H]⁺ (C₂₂H₄₁O₈) (16.7), 239 (15.9) 193 (19.1).

Triglycoside (8)

Elution of the column with chloroform-methanol (3:1) mixture afforded colourless crystals of **8**, recrystallized from ethanol, 73 mg (0.069 % yield); R_f: 0.4 (chloroform - methanol; 3:1); m.p. : 158-160 °C; IR ν_{max} (KBr) : 3563, 3425, 3398, 3260, 3180, 2928, 1636, 1385, 1128, 1063, 1047, 993, 912 cm⁻¹; ^1H NMR (DMSO- d_6): 5.07 (1H, d, J=7.0 Hz, H-1), 4.81(1H, dd, J=7.0, 6.8 Hz, H-2), 3.65 (1H, dd, J=5.01, 6.8 Hz, H-3), 3.55 (1H, m, H-4), 4.54 (1H, m, H-5), 3.14 (1H, d, J=9.0 Hz, H₂-6a), 3.11(1H, d, J=9.0 Hz, H₂-6b), 5.17 (1H, d, J=7.0 Hz, H₂-1'), 4.79 (1H, d, J=7.0, 6.3 Hz, H₂-2'), 3.90 (1H, dd, J=7.8, 6.3 Hz, H-3), 3.47 (1H, m, H-4'), 3.38 (2H, brs, H₂-5), 5.22 (1H, d, J=7.2 Hz, H-1'), 3.84 (1H, dd, J=7.2, 5.1 Hz, H-2'), 3.61 (1H, dd, J=5.1, 9.9 Hz, H-3'), 4.40 (1H, m, H-4'), 3.25 (2H, brs, H₂-5'); ^{13}C NMR (DMSO- d_6) : 104.12 (C-1), 77.13 (C-2), 69.93 (C-3), 71.72 (C-4), 74.38 (C-5), 60.59 (C-6), 101.12 (C-1'), 77.13 (C-2'), 69.93 (C-3'), 71.72 (C-4'), 62.20 (C-5'), 91.85 (C-1''), 72.93 (C-2''), 72.93 (C-3''), 73.41 (C-4''), 62.94 (C-5''); +veFAB MS m/z (rel. int.) : 444 [M]⁺ (C₁₆ H₂₈ O₁₄) (11.3).

Stearyl diarabinoside (9)

Elution of the column with chloroform - methanol (13:7) gave pale yellow crystalline mass of **9**, recrystallized from methanol; 53 mg (0.050 % yield); R_f: 0.71 (chloroform : methanol, 13:7); m.p. : 223-225 °C; UV λ_{max} (MeOH) : 210 nm (log ϵ 3.2); IR ν_{max} (KBr) : 3420, 3345, 2926, 2857, 1726, 1617, 1450, 1384, 1107 cm⁻¹; ^1H NMR (DMSO- d_6) : δ 4.99 (1H, d, J=7.1 Hz, H-1'), 4.85 (1H, d, J=7.5 Hz, H-1''), 4.39 (1H, d, J = 6.9, 9.6 Hz, H-2'), 3.96 (1H, m, H-2''), 3.87 (1H, m, H-3'), 3.76 (1H, m, H-3''), 3.70 (1H, m, H-4'), 3.67 (1H, m, H-4''), 3.36 (2H, brs, H₂-5'), 3.32 (2H, brs, H₂-5''), 2.77 (1H, d, J = 12.6 Hz, H₂-2a), 2.27 (1H, d, J = 15.6 Hz, H₂-2b), 2.48 (2H, m, CH₂), 1.56 (2H, m, CH₂), 1.29 (26H, brs, 13 x CH₂), 0.82 (3H, t, J = 7.2 Hz, CH₃-18); ^{13}C NMR (DMSO- d_6) : δ 172.68 (C-1), 103.16 (C-1'), 81.09 (C-2'), 70.36(C-3'), 68.75 (C-4'), 63.43 (C-5'), 100.01 (C-1''), 72.81 (C-2''), 72.27 (C-3''), 68.91 (C-4''), 61.28 (C-5''), 55.98 (C-2), 34.01 (CH₂), 34.01 (CH₂), 31.91 (CH₂), 29.74 (12 x CH₂), 22.66 (CH₂), 21.35 (CH₂), 14.10 (CH₃-18); +ve FAB MS m/z (rel. int.): 549 [M+H]⁺ (C₂₈H₅₃ O₁₀) (13.5), 399 (12.4), 283 (12.3), 267 (19.6), 265 (17.8), 149 (22.1), 133 (26.8).

Tetraglycoside (10)

Elution of the column with chloroform-methanol (1:1) mixture afforded light brown crystals of **10**, recrystallized from ethanol, 54 mg (0.051 % yield); R_f : 0.80 (chloroform : methanol; 1:1); m.p. : 268-270 °C; IR ν_{max} (KBr) : 3563, 3425, 3398, 3260, 3180, 2921, 1625, 1434, 1350, 1239, 1128, 1063, 993, 912, 859 cm^{-1} ; ^1H NMR (DMSO- d_6): δ 5.38 (1H, d, $J=7.1$ Hz, H-1), 4.81 (1H, m, H-2), 3.65 (1H, dd, $J=5.01, 7.8$ Hz, H-3), 3.54 (1H, m, H-4), 4.52 (1H, m, H-5), 3.16 (2H, d, $J=8.5$ Hz, H₂-6), 5.13 (1H, d, $J=7.0$ Hz, H-1'), 4.79 (1H, d, $J=7.0, 6.8$ Hz, H-2), 4.10 (1H, m, H-3'), 3.47 (1H, m, H-4'), 3.35 (2H, brs, H₂-5'), 5.09 (1H, d, $J=7.1$ Hz, H-1''), 4.75 (1H, m, H-2''), 3.79 (1H, m, H-3''), 3.66 (1H, m, H-4''), 3.61 (2H, brs, H₂-5''), 5.02 (1H, d, $J=7.1$ Hz, H-1'''), 3.82 (1H, m, H-2'''), 3.74 (1H, m, H-3'''), 3.79 (1H, m, H-4'''), 3.59 (2H, brs, H₂-5'''); ^{13}C NMR (DMSO- d_6): δ 103.81 (C-1), 79.93 (C-2), 69.92 (C-3), 71.72 (C-4), 74.38 (C-5), 60.59 (C-6), 104.12 (C-1'), 78.72 (C-2'), 69.92 (C-3'), 71.72 (C-4'), 62.20 (C-5'), 101.59 (C-1''), 77.13 (C-2''), 72.93 (C-3''), 69.81 (C-4''), 63.49 (C-5''), 100.16 (C-1'''), 68.50 (C-2'''), 67.37 (C-3'''), 66.57 (C-4'''), 63.49 (C-5'''); +ve FAB MS m/z (rel. int.) : 576 [M]⁺ (C₂₁H₃₆O₁₈) (10.2), 179 (11.5), 163 (15.2), 149 (21.6), 133 (16.0).

RESULTS AND DISCUSSION

Compounds **1**, **2** and **3** were the triterpenic and steroidal glycosides characterized as α -amyrin β -D-glucopyranoside, stigmasterol β -L-arabinopyranoside and β -sitosterol β -D-glucopyranoside, respectively. Compound **4**, designated as oleiyl glucoside, was obtained as a colourless crystalline mass from chloroform-methanol (9:1) eluants. It responded positively to tests of glycosides and showed IR absorption bands for hydroxyl groups (3477, 3265 cm^{-1}), ester function (1741 cm^{-1}), unsaturation (1617 cm^{-1}) and long aliphatic chain (793, 718 cm^{-1}). Its molecular weight was established at m/z 444 on the basis of mass and ^{13}C NMR spectra consistent to the molecular formula of a fatty acid glycoside, C₂₄H₄₄O₇. The ion peaks arising at m/z 265 [C₁-O fission, CH₃-(CH₂)₇CH=CH(CH₂)₇CO]⁺, 281 [O-C₁ fission, CH₃-(CH₂)₇CH=CH(CH₂)₇COO]⁺ and 180 [C₆H₁₂O₆]⁺ suggested that oleic acid was esterified with sugar unit. The ^1H NMR spectrum of **4** showed a two-proton multiplet at δ 5.28 assigned to vinylic H-9 and H-10 protons and a one-proton doublet at δ 4.96 ($J = 7.1$ Hz) ascribed to anomeric H-1'. The other sugar protons resonated between δ 4.45 – 3.37. A two-proton triplet at δ 2.27 ($J=7.2$ Hz) and two multiplets at δ 2.22 and 2.16 integrating for two protons each were ascribed to methylene H₂-2 protons adjacent to ester group and to H₂-8 and H₂-11, nearby the vinylic carbons, respectively. The other methylene protons were present as a multiplet at δ 1.98 (2H) and broad singlets at δ 1.79 (2H), 1.64 (2H), 1.49 (2H) and 1.21 (7 x CH₂). A three-proton triplet at δ

0.89 (J = 6.3 Hz) was accounted to C-18 primary methyl protons. The ^{13}C NMR spectrum of **4** displayed signals for ester carbon at 171.69 (C-1), vinylic carbons at δ 127.99 (C-9) and 126.51 (C-10), anomeric carbon at δ 101.23 (C-1'), other sugar carbons between δ 81.78 – 62.93, methylene carbons in the range of δ 55.93 – 22.59 and methyl carbon at δ 13.61 (C-18). Acid hydrolysis of **4** yielded oleic acid and D-glucose, co-TLC comparable. On the basis of spectral data analysis and chemical reactions, the structure of **4** has been formulated as *n*-octadec-9-enoyl β -D-glucopyranoside (Figure. 2).

Compound **5**, designated as stearyl glucoside, was obtained as a light brown sticky mass from chloroform-methanol (17:3) eluants. It responded positively to the tests of glycosides. Its IR spectrum displayed characteristic absorption bands for hydroxyl groups ($3415, 3380\text{ cm}^{-1}$), ester function (1741 cm^{-1}), and long chain aliphatic moiety (809 cm^{-1}). On the basis of +ve FAB mass and ^{13}C NMR spectra, its molecular weight was established as m/z 446 consistent with a molecular formula of a fatty acid glycoside, $\text{C}_{24}\text{H}_{46}\text{O}_7$. The ion fragments generating at m/z 163 [$\text{C}_6\text{H}_{11}\text{O}_5$] $^+$ and 283 [M-163] $^+$ suggested that a hexose unit was linked with stearic acid moiety. The ^1H NMR spectrum of **5** exhibited a one- proton doublet at δ 4.95 (J=7.1 Hz) assigned to H-1' anomeric protons. Four one- protons multiplets at δ 4.45, 4.31, 4.22 and 3.56 were ascribed to sugar H-5', H-2', H-4' and H-3' oxygenated methine protons, respectively. A two - proton broad signal centered at δ 3.28 was attributed to H₂-6' hydroxy-methylene protons. The remaining methylene protons appeared as multiplets at δ 2.30 (2H), 1.55 (2H) and as a broad singlet at δ 1.23 (28H). A three-proton triplet at δ 0.85 (J=6.3 Hz) was assigned to C-18 primary methyl protons. The ^{13}C NMR spectrum of **5** displayed important signals for ester carbon at δ 169.16 (C-1), anomeric carbon at δ 103.43, other sugar carbon signals between δ 81.43 - 62.16 (C-6'), methylene carbons in the range of δ 31.54-22.28 and C-18 primary methyl carbon at δ 13.69. Acid hydrolysis of **5** yielded D-glucose and stearic acid (co-TLC-comparable). On the basis of above discussion, the structure of **5** has been established as *n*- octadecanoyl- β -D-glucopyranoside (Figure 2).

Compound **6**, designated as docosanyl glucoside, was obtained as a pale yellow sticky mass from chloroform-methanol (4:1) eluants. It responded positively to the tests of glycosides and displayed IR characteristic absorption bands for hydroxyl groups ($3397, 3265\text{ cm}^{-1}$), ester group (1725 cm^{-1}) and long chain aliphatic moiety (729 cm^{-1}). On the basis of +ve FAB mass and ^{13}C NMR spectra, its molecular weight was established at m/z 502 consistent with a molecular formula of a fatty acid glycoside, $\text{C}_{28}\text{H}_{54}\text{O}_7$, The ion peaks generated at m/z 339 [$\text{CH}_3(\text{CH}_2)_{20}$

COO]⁺ due to glycosidic bond cleavage indicated the presence of C₂₂- fatty acid as the aglycone part. The ion fragment at *m/z* 163 [C₆H₁₁O₅]⁺ supported the presence of a hexose moiety in the compound. The ¹H NMR spectrum of **6** exhibited a one-proton doublet at δ 4.95 (J=7.1 Hz), assigned to H-1' anomeric proton. The other hydroxymethine protons of the sugar unit appeared between δ 4.58-3.56. Two one-proton doublets at δ 3.13(J=12.0 Hz) and 3.09(J=12.0 Hz) were ascribed to hydroxymethylene H₂-6' proton. Two one- proton doublets at δ 2.92(J=9.0 Hz) and 2.86(J=9.0 Hz) were due to methylene H₂-2 proton adjacent to the ester group. The remaining methylene protons appeared at δ 1.23 as a broad signal. A three-proton triplet at δ 0.83(J=6.5 Hz) was assigned to Me-22 primary methyl protons. The ¹³C NMR spectrum of **6** displayed important signals for ester carbon at δ 170.88(C-1), anomeric carbon at δ 103.43(C-1'), oxygenated methine carbons from δ 81.43 to 71.99, hydroxymethylene carbon at δ 62.16(C-6'), other methylene carbons between δ 31.54 – 22.28 and C-22 primary methyl carbon at δ 13.69. Acid hydrolysis of **6** yielded D-glucose (co-TLC-comparable). On the basis of above discussion, the structure of **6** has been established as n-docosanyl-β-D-glucopyranoside (Figure-2).

Compound **7**, designated as palmityl glucuronoside, were obtained as a pale yellow sticky mass from chloroform-methanol (3:1) eluants. It responded positively to the tests of glycosides and displayed characteristic IR absorption bands for hydroxyl groups (3396 cm⁻¹), ester function (1739 cm⁻¹), carboxylic group (3250, 1698 cm⁻¹) and long chain aliphatic moiety (831, 729 cm⁻¹). On the basis of +ve FAB mass and ¹³C NMR spectra, its molecular weight was established at *m/z* 433 [M+H]⁺ consistent with a molecular formula of a fatty acid glycoside, C₂₂H₄₁O₈. A prominent ion peak generated at *m/z* 239 [CH₃(CH₂)₁₄CO]⁺ due to glycosidic bond cleavage indicating the presence of palmityl group as the aglycone part. The ion fragment at *m/z* 193 [M-239, C₆H₉O₇]⁺ supported the presence of a hexose carboxylic acid moiety in **7**. The ¹H NMR spectrum of **7** exhibited a one - proton doublet at δ 4.85 (J=7.1 Hz) assigned to H-1' anomeric proton. Four one- proton multiplets at δ 4.42, 4.22, 3.67, and 3.34 were due to methine H-5', H-2', H-4' and H-3' protons, respectively. The remaining methylene protons appeared at δ 2.50 (2H) and 1.23 (26H) as broad singlets. A three - proton triplet at δ 0.85 (J=6.3Hz) was assigned to C-16 primary methyl protons. The ¹³C NMR spectrum of **7** displayed important signals for carboxylic carbon at 181.09 (C-6'), ester carbon at δ 170.88 (C-1), anomeric carbon at δ 105.28 (C-1'), oxygenated methine carbons at δ 81.43 (C-5'), 74.60 (C-2'), 73.16 (C-3') and 70.57 (C-4'), methylene carbons between δ 31.54 – 22.28 and methyl carbon at δ 14.25 (C-16). Acid hydrolysis of **7** yielded palmitic acid and D-glucuronic acid (co-TLC-comparable). On the basis

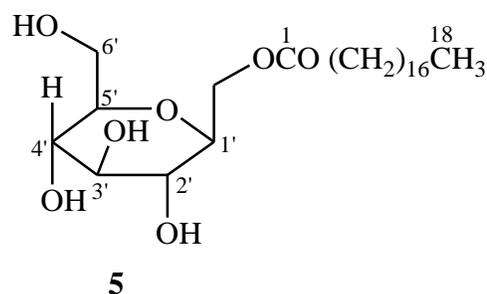
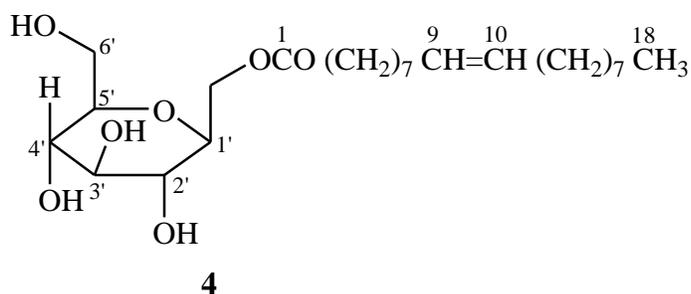
of above discussion, the structure of **7** has been established as *n*-hexadecanoyl β -D-glucuranopyranoside (Figure 2).

Compound **8**, a triglycoside, was obtained from chloroform-methanol (4:1) as a colourless crystalline mass. It gave positive tests for carbohydrates and displayed characteristic IR absorption bands for hydroxyl groups at 3563, 3425, 3398, 3260, 3180 cm^{-1} . On the basis of mass and ^{13}C NMR spectra the molecular ion peak of **8** was determined at m/z 444 corresponding to a molecular formula of a trisaccharide, $\text{C}_{16}\text{H}_{28}\text{O}_{14}$. The ^1H NMR spectrum of **8** exhibited three one-proton doublets at δ 5.07 ($J=7.0$ Hz), 5.17 ($J=7.0$ Hz) and 5.22 ($J=7.2$ Hz) assigned to three anomeric protons H-1, H-1', and H-1'', respectively. Four one-proton multiplets at δ 4.54, 3.55, 3.47 and 4.40 were accounted to carbinol H-5, H-4, H-4' and H-4'' protons, respectively. Three one – proton double doublets at δ 4.81 ($J=7.0, 6.8$ Hz), 4.79 ($J=7.0, 6.3$ Hz) and 3.84 ($J=5.1, 7.2$ Hz) were ascribed to carbinol H-2, H-2' and H-2," and their location in the deshielded region suggested (2 \rightarrow 1) glycosidic linkage. The remaining sugar protons resonated between δ 3.90-3.11. The ^{13}C NMR spectrum of **8** displayed important signals for anomeric carbons at δ 104.12 (C-1), 101.12 (C-1) and 91.85 (C-1''). The carbinol signals in the deshielded region at δ 77.13 (C-2, C-2') indicated (2 \rightarrow 1) linkages of the sugar residues. The hydroxymethylene carbons appeared at δ 60.59 (C-6). The remaining carbinol carbons of the sugar residues resonated between δ 74.38-69.13. Acid hydrolysis of **8** yielded β -D-glucose and β -L-arabinose (TLC comparable). On the basis of spectral data analysis and chemical reactions the structure of **8** has been elucidated as β -D-glucuranopyranosyl-(2 \rightarrow 1)- β -D-arabinopyranosyl-(2 \rightarrow 1)-B-D-arabinopyranoside (Figure 2).

Compound **9**, designated as stearyl diarabinoside, was obtained as a pale yellow crystalline mass from chloroform-methanol (13:7) eluents. It gave positive tests for glycosides and displayed characteristic IR absorption bands for hydroxyl groups (3420, 3345 cm^{-1}) and ester function (1726 cm^{-1}). The mass spectrum of **9** exhibited a molecular ion peak at m/z 549 $[\text{M}+\text{H}]^+$ corresponding to a molecular formula of a fatty acid diglycoside, $\text{C}_{28}\text{H}_{53}\text{O}_7$. The important ion peaks arising at m/z 267 $[\text{CH}_3 (\text{CH}_2)_{16} \text{CO}]^+$, 283 $[\text{CH}_3 (\text{CH}_2)_{16}\text{COO}]^+$, 267 $[\text{M}-283]^+$, 133 $[\text{C}_5\text{H}_9\text{O}_4]^+$, 149 $[\text{C}_5\text{H}_9\text{O}_5]^+$ and 399 $[\text{M}-149]^+$ indicated that stearic acid was linked with two C_5 sugar units. The ^1H NMR spectrum of **9** displayed two one-proton doublets at δ 4.99 ($J=7.1$ Hz) and 4.85 ($J=7.5$ Hz) assigned to anomeric H-1' and H-1'' protons, respectively. The other sugar proton signals appeared between δ 4.39 – 3.32. The signals in the range of δ 2.77 – 1.29 were associated with the methylene protons. A three-proton triplet at δ 0.82 ($J=7.2$ Hz) was ascribed to C-18 primary methyl protons. The ^{13}C NMR spectrum of **9** exhibited signals for ester carbon at

δ 172.68 (C-1), anomeric carbons at δ 103.16 (C-1') and 100.01 (C-1''), other sugar carbons in the range from δ 73.89 to 61.28, methylene carbons between δ 55.98- 21.35 and methyl carbon at 14.10 (C-18). The presence of ^1H NMR signal for H-2 in the deshielded region at δ 4.39 and ^{13}C NMR signals for C-2' at δ 81.09 suggested the attachment of another sugar at C-2'. Acid hydrolysis of **9** yielded arabinose and stearic acid (R_f comparable). On the basis of the above mentioned discussion the structure of **9** has been elucidated as *n*-octadecanoyl- β - arabinopyranosyl – (2 \rightarrow 1'') – β - arabinopyranoside. It is a new fatty acid diglycoside (Fig. 2).

Compound **10**, a tetraglycoside, was obtained from chloroform- methanol (1:1) as a light brown crystalline mass. It gave positive tests for carbohydrates and displayed characteristic IR absorption bands for hydroxyl groups at 3563, 3425, 3398, 3260, 3180 cm^{-1} . The mass spectrum of **10** showed a molecular ion peak at 576 corresponding to a molecular formula of a tetrasaccharide $\text{C}_{21}\text{H}_{36}\text{O}_{18}$. The ion fragments arising at m/z 163 [$\text{C}_6\text{H}_{11}\text{O}_5$] $^+$, 179 [$\text{C}_6\text{H}_{11}\text{O}_6$] $^+$, 133 [$\text{C}_5\text{H}_9\text{O}_4$] $^+$ and 149 [$\text{C}_5\text{H}_9\text{O}_5$] $^+$ indicated that glucose unit was attached to the arabinose chain. The ^1H NMR spectrum of **10** exhibited four one-proton doublets at δ 5.38 ($J=7.1$ Hz), 5.13 ($J=7.1$ Hz), 5.09 ($J=7.1$ Hz) and 5.02 ($J=7.1$ Hz) assigned to anomeric H-1, H-1', H-1'' and H-1''' protons, respectively. The other sugar protons appeared from δ 4.81 to 3.15. The presence of H-2, H-2' and H-2'' signals in the deshielded region at δ 4.81, 4.79 and 4.75 as multiplet suggested (2 \rightarrow 1) linkage of the sugar units. The ^{13}C NMR spectrum of **10** displayed signals for anomeric carbons at δ 103.81 (C-1), 104.12 (C-1'), 101.59 (C-1'') and 100.96 (C-1''') and other sugar carbons from δ 74.38 to 60.59. The presence of C-2, C-2' and C-1'' carbon signals in the deshielded region at δ 79.93 (C-2, C-2') and 78.13 (C-2'') supported (2 \rightarrow 1) linkage of the sugar units. Acid hydrolysis of **10** yielded D- glucose and D-arabinose. On the basis of spectral data analysis and chemical reactions, the structure of **10** was formulated as β - D- glucopyranosyl- (2 \rightarrow 1)- β - D- arabinopyranosyl-(2 \rightarrow 1)- β - D- arabinopyranosyl - (2 \rightarrow 1)- β -D- arabinopyranoside (Fig. 2). It is a new tetraglycoside isolated from a plant source for the first time.



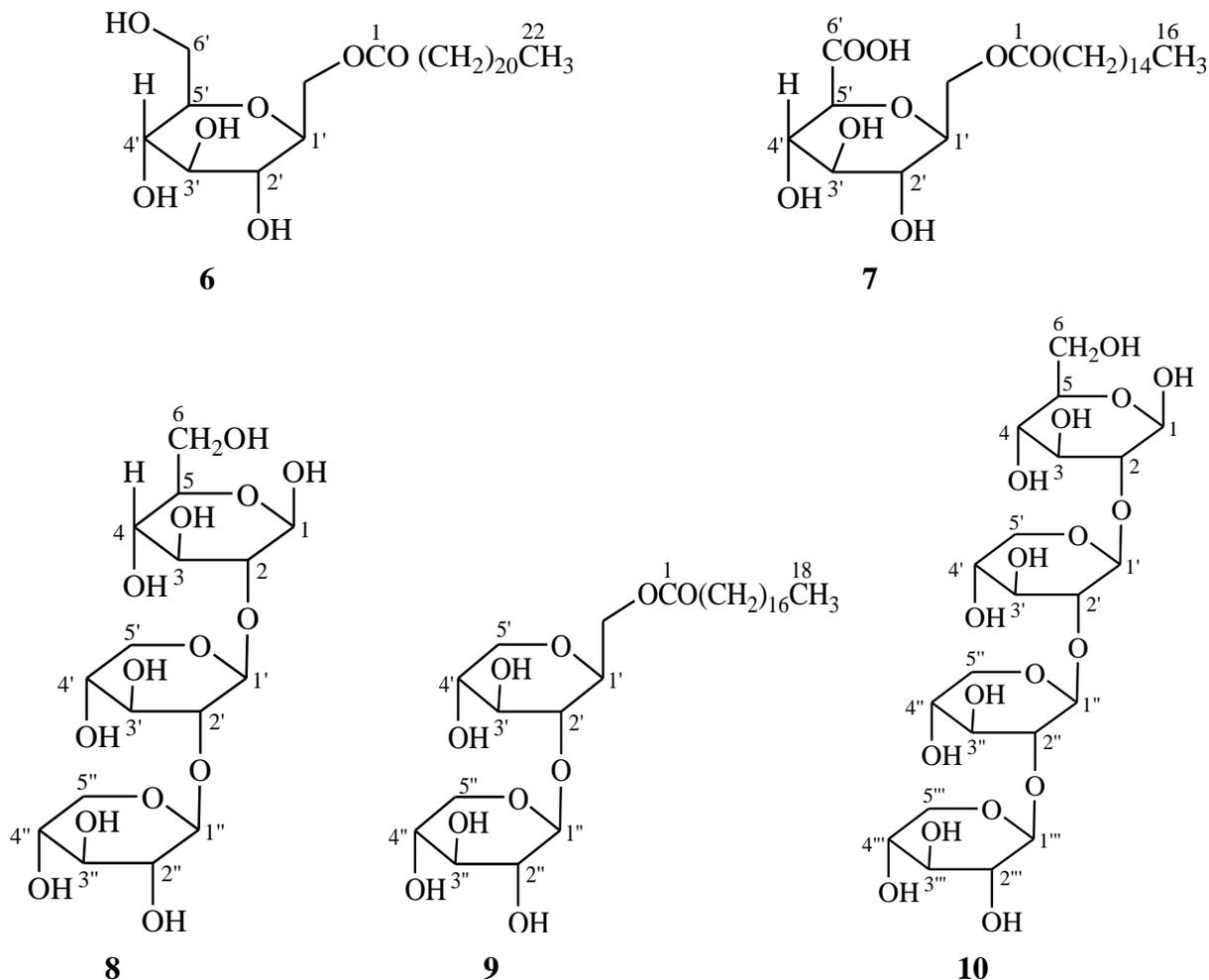


Figure. 2. Structural formulae of *n*-octadec-9-enoyl β-D-glucopyranoside (4), *n*-octadecanoyl-β-D- glucopyranoside (5), *n*-docosanyl-β-D-glucopyranoside (6), *n*-hexadecanoyl β-D-glucuronopyranoside (7), β-D-glucuronopyranosyl-(2→1)-β-D-arabinopyranosyl-(2→1)-β-D-arabinopyranoside (8), *n*-octadecanoyl- β- arabinopyranosyl – (2→1'') – β- arabinopyranoside (9) and β- D- glucopyranosyl- (2→1)- β - D- arabinopyranosyl-(2→1)-β- D- arabinopyranosyl - (2→1)- β -D-arabinopyranoside (10).

CONCLUSION

The present work characterized the triterpenic, steroidal and acyl glycosides, a triglycoside and a tetraglycoside first time from the roots of *R. communis* which may be used as chromatographic fingerprinting markers for quality control of the drug of arid region and may be responsible for medicinal uses of the roots.

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