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Original Research Article

Triterpenic and acyl glycosides from the leaves of *Centella asiatica* (L.) Urban

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ABSTRACT

Centella asiatica (L.) Urban (Apiaceae) is a small perennial, prostrate herb indigenous to India, south-eastern Asia, United States and Africa. It is used to treat anxiety, asthma, blood circulation, cancer, colds, cough, elephantiasis, epilepsy, fevers, gastrointestinal problems, hepatic, skin and urinary tract diseases, hydrocele, hypertension, hysteria, insomnia, rheumatism, scleroderma, strangury, ulcers and wounds. Phytochemical investigation of a methanolic extract of the leaves resulted in the isolation of urs-12-en-3β-ol-28-oic acid-3-*O*-β-D-glucopyranosyl-2′-arachidate (ursolic acid glucosidic arachidate, **1**), *n*-decanoyl-*O*-β-D-glucopyranosyl-(6′→1′′)-*O*-β-D-glucopyranoside (*n*-capryl diglucoside, **2**), urs-12-en-3β-ol-28-oic acid 3-*O*-β-D-xylopyranosyl-(2′→1′′)-*O*-β-D-xylopyranoside (ursolic acid 3-*O*-β-D-dixyloside, **3**), glyceryl 1-decanoyl-2-phosphate (**4**), *n*-dodecanoyl-*O*-β-D-glucopyranosyl-(6′→1′′)-*O*-β-D-glucopyranosyl-(6′′→1′′′)-*O*-β-D-glucopyranosyl-6′′′→1′′′′)-*O*-β-D-glucopyranoside (lauroyl tetraglucoside, **5**), *n*-octanoyl-*O*-β-D-glucopyranosyl-(6a→1b)-*O*-β-D-glucopyranosyl-(6b→1c)-*O*-β-D-glucopyranosyl-(6c→1d)-*O*-β-D-glucopyranosyl-(6d→1e)-*O*-β-D-glucopyranoside (caproyl pentaglucoside, **6**) and ursan-3β-ol-28-al-3-*O*-α-L-arabinopyranosyl-(2a→1b)-*O*-α-L-arabinopyranosyl-(2b→1c)-*O*-α-L-arabinopyranosyl-(2c→1d)-*O*-α-L-glucopyranosyl-(2d→1e)-*O*-α-L-glucopyranosyl-(2e→1f)-*O*-α-L-glucopyranoside (ursolic aldehyde 3-*O*-α-L-hexaglycoside, **7**). The structures of these phytoconstituents have been elucidated on the basis of spectral analysis and chemical reactions.

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1. Introduction

Centella asiatica (L.) Urban, syn. *Hydrocotyle asiatica* L. (Apiaceae), known as brahmi, gotu kola, mandukparni and Indian pennywort, is an indigenous plant to India, China, Indonesia , Australia, southeastern Asia, United States, South Africa, Madagascar, Mexico, Venezuela and Columbia. It is a prostrate, faintly aromatic, stoloniferous, perennial herb, up to 15 cm in height, with glabrous, striated stem rooting at the nodes; glabrous, sheathing, long petioled leaves; pink or purple, umbellous flowers; and globular, oblong fruits. It flourishes extensively in shady, marshy, damp and wet places forming a dense green carpet (Singh et al., 2010). The plant possesses adaptogen, anti-viral, anti-ulcerogenic, anxiolytic, carminative, cellulite, diuretic, emmenagogue, lactagogue, refrigerant, soporific, stimulant and stomachic properties. It is used to treat acne, anxiety, asthma, arthritis, blood circulation, cancer, colds, cough, diarrhea, dysentery, eczema, elephantiasis, epilepsy, fevers, gastrointestinal problems, hepatitis, hydrocele, hypertension, hysteria, insomnia, jaundice, psoriasis, rheumatism, scleroderma, strangury, ulcers, urinary tract disorders and wounds (Azis et al., 2017). Antimycobacterial (Machado et al., 2015), antibacterial (Sultan et al., 2014), antifungal (Sultan et al., 2014), antimicrobial (Idris and Nadzir, 2017; Panathula et al., 2014), anxiolytic (Wijeweera et al., 2006), antioxidant (Sultan et al., 2014), analgesic (Sultan et al., 2014), histopathological (Zheng et al., 2016), neurotrophic (Nataraj et al., 2017), antidiabetic (Fitrianda et al., 2017), anti-proliferative (Aizad et al., 2017) and anti-inflammatory (Park et al., 2017; Sultan et al., 2014) activities are among the other properties

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of this plant. It enhances the immunity of the body, memory and clarity and detoxifies the opium toxicity (Chopra et al., 1986; Gohil et al., 2010; Zheng and Qin, 2007; Quattrocchi, 2012).

The plant contains a variety of chemical compounds such as ursene-type triterpenoids asiaticoside, asiaticoside A-F, oxyasiaticoside, asiatic acid, madecassic acid, medecassoside, thankunic acid, isohankunic acid, brahmic acid, isobrahmatic acid, isothankuniside, methyl brahmate, brahmol, centellasaponins B and C , 2α,3ß,20,23-tetrahydroxy-urs-28-oic acid, 2α,3ß,23-trihydroxy-urs-20-en-28-oic acid, scheffuroside B, methyl asiatate and arabinoside; oleanene-type triterpenoids, viz., asiaticoside B, olean-13-ene, 2α,3ß,23-trihydroxy-olean-12-en-28-oic acid, terminolic acid and centellasapogenol A; dammarene-type triterpenoid saponins, namely bacosides A and B, bacopasaponins A, B, C and D, pseudojujubogenin, its glycoside and bacopasides I and II; alkaloids brahmine, nicotine, herpestine and hydrocotyline; saponins hersaponin, bacopasides I-V, bacopasaponin G, asiticoside, madecassol, madecassoside, brahmoside, brahminoside, thankuniside, isothankuniside, centellasaponin B, C and D; phenylethnoid glycosides, viz. monnierasides I-III and plantainoside B. Its essential oil is composed of terpenic acetate, β-caryophyllene, *trans*-β-farnesene, decane, cineole, germacrene D, vallerine, α-humulene, bicyclogermacrene, γ-caryophyllene, and caryophyllene oxide; flavonoids castilliferol, castillicetin, apigenin, rutin, naringin, rutin, kaempferol, catechin, quercetin, 3-glucosylquercetin, 3- glucosylkaemferol, 7-glucosylkaemferol, petuletin and kaempferol 3-*O*-β-D-glucuronide; phytosterols including campesterol, stigmasterol and β-sitosterol; and other chemical constituents like hydrocotyline, inositol, pectic acids, centelloside, hersaponin, bacogenin, monnierin, tannins, pectin, carotene, carotenoids, vitamins B, C and K, isochlorogenic acid, arabinogalactan , amino acids, centellose, chlorophyll, meso-inositol, wax, centic acid, cenellic acid, betulinic acid, indocentic acid, Indocentoic acid, euscaphic acid, bayogenin, fatty acids, centellin (6-acetoxy-trideca-1,7-dien4-yn-3-ol), asiaticin (*p*-benzoyloxy methyl-butyl benzoate), centellicin (1-(20 ,30-dihydroxypropyl)-2-en3-methyl-6-hydroxy-9-yn-undecanoate), centellasapogenol A, polyyne-alkene and polysaccharides (Qin et al., 1998; Brinkhaus et al., 2000; Shukla et al., 2000; Matsuda et al., 2001a; Matsuda et al., 2001b; Kuroda et al., 2001; Zainol et al., 2003; Jiang et al., 2005; Oyedeji and Afolayan, 2005; Siddiqui et al., 2007; Aziz et al., 2007; James and Dubery, 2009; Hashim et al., 2011; Chong and Aziz, 2011). Keeping in view the high reputation and wide application of *C. asiatica* (L.) in many indigenous systems, it has been aimed to carry out isolation and characterization of acyl and tritertpenic glycosides and glyceryl phospho-caprate from the leaves of this plant procured from Delhi, India.

2. Experimental

2.1. General procedures

Melting points were recorded using one end open capillary tubes on a thermoelectrically heated melting point M-560 apparatus (Perfit, India) without correction. UV spectra were determined with Lambda Bio 20 Spectrophotometer (Perkin Elmer, Schwerzenbach, Switzerland) in methanol. IR spectra were recorded by using KBr pellets, with Jasco FT/IR-5000 Spectrometer (FTS 135, Hong Kong). The ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR spectra were recorded on Bruker DRX-Spectrometer (Rheinstetten, 2 Germany), using $CDCl_3$ and DMSO-d_6 and TMS (Fluka analytical, Sigma-Aldrich, Netherland) as an internal standard. Mass-spectrometric detection was carried out on (Q-TOF-ESI) (Waters Corp., UK) with a +ve ESI technique. Column chromatography was performed on silica gel (Qualigens, Mumbai, India), 60-120 mesh and solvents used were purchased from Merck Specialties (E. Merck, Pvt. Ltd. New Delhi, India). The purity of the isolated compounds was checked on precoated TLC plates with Silica gel $60F_{254}$ (Merck, 0.25 mm) and the spots were visualized by exposure to iodine vapours or under UV radiations and spraying with ceric sulfate solution.

2.2. Plant material

The leaves of *C. asiatica* (L.) were collected from the herbal garden of Jamia Hamdard, New Delhi and identified by Dr. H.B. Singh, Scientist, Raw Materials Herbarium and Museum, National Institute of Science Communication and Information Resources (NISCAIR), New Delhi. A voucher specimen of the leaves was deposited in the Raw Materials Herbarium and Museum, NISCAIR, New Delhi, with a reference number NISCAIR/RHMD/Consult/09/1059/90.

2.3. Extraction and isolation

The air-dried leaves (2.0 kg) of *C. asiatica* (L.) were coarsely powdered, defatted with petroleum ether and extracted with methanol exhaustively in a Soxhlet apparatus. The combined extracts were filtered and concentrated under reduced pressure to get a dark brown viscous mass (131 g, 6.55%). Small portion of the extract was analyzed chemically to determine the presence of different chemical constituents. The dried extract (100 g) was dissolved in a minimum quantity of methanol and adsorbed on silica gel (60-120 mesh) for the preparation of a slurry. It was dried in air and chromatographed over silica gel column (1.6 m x 16 mm x 2 mm) packed in petroleum ether. The column was eluted successively in increasing order of polarity in various combinations with chloroform, chloroform-methanol (19.9: 0.1; 99: 1; 97: 3; 19: 1; 93: 7; 9: 1; 17: 3; 4:1; 3: 1; 3: 2; 2: 3, v/v) and methanol. The fractions were collected separately and matched by TLC to check

homogeneity. Similar fractions having the same Rf values were combined and crystallized. The isolated compounds were recrystallized to get pure compounds. The following compounds were isolated from the methanolic extract of the leaves of *C. asiatica* (L):

2.3.1. Ursolic acid glucosidic arachidate (**1**)

Elution of the column with chloroform-methanol (19:1) gave colourless crystals of **1**, recrystallized from chloroform-methanol (9:1), yield: 505 mg (0.50% yield), R_f 0.80 (chloroform-methanol (19:1), m.p.: 219-220 ºC; UV λ_{max} (MeOH): 211 nm (log ε 3.1), IR V_{max} (KBr): 3425, 3320, 2924, 2853, 1725, 1692, 1645, 1461, 1377, 1241, 1051, 720 cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 5.12 (1H, m, H-12), 3.70 (1H, dd, J=5.3, 8.9 Hz, H-3), 2.48 (2H, m, H_2-2''), 2.02-1.35 (23H, m, 9 x CH_2, 5 x CH), 1.32 (3H, brs, Me-23), 1.22 (34H, brs, 17 x CH_2), 1.17 (3H, d, J=6.0 Hz, Me-29), 1.03 (3H, brs, Me-25), 0.91 (3H, brs, Me-26), 0.85 (3H, t, J=7.2 Hz, Me-20''), 0.81 (3H, d, J=5.3 Hz, Me-30), 0.73 (3H, brs, Me-27), 0.53 (3H, brs, Me-24), 4.95 (1H, d, J=7.2 Hz, H-1'), 4.23 (1H, m, H-2'), 4.11 (1H, m, H-5'), 3.65 (1H, m, H-3'), 3.37 (1H, m, H-4'), 3.04 (2H, d, J=10.5 Hz, H_2-6'); ^{13}C NMR (DMSO-d_6): δ 41.63 (C-1), 27.41(C-2), 78.36 (C-3), 42.51 (C-4), 55.17 (C-5), 18.07 (C-6), 34.53 (C-7), 40.52 (C-8), 48.84 (C-9), 37.23 (C-10), 23.61 (C-11), 128.65 (C-12), 139.24 (C-13), 45.18 (C-14), 28.42 (C-15), 25.50 (C-16), 47.69 (C-17), 58.37 (C-18), 39.46 (C-19), 38.78 (C-20), 30.82 (C-21), 36.98 (C-22), 24.92 (C-23), 16.08 (C-24), 17.13 (C-25), 20.57 (C-26), 24.65 (C-27), 180.61 (C-28), 17.14 (C-29), 21.13 (C-30), 103.78 (C-1'), 76.52 (C-2'), 70.49 (C-3'), 68.15 (C-4'), 75.81 (C-5'), 61.72 (C-6'), 171.88 (C-1''), 34.53 (CH_2), 32.41 (CH_2), 29.25 (15 x CH_2), 22.16 (CH_2), 14.18 (C-20''); +ve ESI MS *m/z* (rel int.): 913 $[M+1]^+$ ($C_{56}H_{97}O_9$) (10.2), 617 (8.3), 455 (12.1), 295 (22.8), 163 (4.7).

2.3.2. *n*-Capryl diglucoside (**2**)

Further elution of the column with chloroform-methanol (19:1) gave colourless crystals of 2, recrystallized from chloroform-methanol (19:1), 160 mg (0.16 % yield), R_f: 0.91 (chloroform-methanol, 19:1), m.p.: 119-120 ºC, UV λ_{max} (methanol): 205 nm (log ε 4.1), IR V_{max} (KBr): 3425, 3260, 2927, 2854, 1720, 1444, 1380, 1071 cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 5.30 (1H, d , J=7.2 Hz, H-1'), 4.90 (1H, d, J=7.3 Hz, H-1''), 4.47 (1H, m, H-5'), 4.45 (1H, m, H-5''), 3.97 (1H, m, H-2'), 3.78 (1H, m, H-2''), 3.61 (1H, m, H-3'), 3.56 (1H, m, H-3''), 3.39 (2H, m, H-4', H-4''), 3.25 (2H, brs, H_2-6'), 3.15 (2H, brs, H_2-6''), 2.48 (2H, t, J=7.2 Hz, H_2-2), 2.24 (2H, m, H_2-3), 2.18 (2H, m, H_2-4), 1.52 (2H, m, H_2-5), 1.20 (8H, brs, 4 x CH_2), 0.82 (3H, t, J=6.2 Hz, Me-10); ^{13}C NMR (DMSO-d_6): δ 173.47 (C-1), 55.19 (C-2), 33.67 (C-3), 31.33 (C-4), 29.08 (C-5), 29.90 (C-6), 28.48 (C-7, C-8), 24.43 (C-9), 13.86 (C-10), 103.65 (C-1'), 70.18 (C-2'), 66.05 (C-3'), 64.06 (C-4'), 77.56 (C-5'), 62.98 (C-6'), 101.23 (C-1''), 69.79 (C-2''), 64.81 (C-3''), 64.06 (C-4''), 75.14 (C-5''), 60.53 (C-6''), +ve ESI MS *m/z* (rel. Int.): 497 $[M+1]^+$ ($C_{22}H_{41}O_{12}$) (6.8), 325 (4.6), 179 (10.2), 171 (8.1), 163 (3.9).

2.3.3. Ursolic acid 3-*O*-β-D-dixyloside (**3**)

Elution of the column with chloroform-methanol (9:1) afforded pale yellow beads of **3**, recrystallized from chloroform-methanol (1:1), 507 mg (0.50% yield), R_f 0.88 (chloroform-methanol, 9:1), m.p. 199-201 ºC; λ_{max} (MeOH): 220 nm (log ε 4.7); IR V_{max} (KBr): 3413, 3360, 3218, 2924, 2854, 1692, 1635, 1460, 1376, 1241, 1031 cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 5.30 (1H, m, H-12), 3.64 (1H, dd, J=4.2, 9.3 Hz, H-3), 1.26 (3H, brs, Me-23), 1.24 (3H, brs , Me-25), 0.97 (3H, brs, Me-24), 0.89 (3H, brs, Me-27), 0.85 (3H, d, J=6.1 Hz, Me-29), 0.82 (3H, d, J=6.3 Hz, Me-30), 0.73 (3H, brs, Me-26), 5.15 (1H, d, J=7.3 Hz, H-1'), 4.23 (1H, m, H-2'), 3.60 (1H, m, H-3'), 3.49 (1H, d, H-4'), 3.19 (2H, d, J=7.3 Hz, H_2-5'), 4.89 (1H, d, J=7.1 Hz, H-1''), 4.01 (1H, m, H-2''), 3.45 (1H, m, H-3''), 3.53 (1H, m, H-4''), 3.15 (2H, d, J=6.7 Hz, H_2-5''), 2.38-1.38 (23H, m, 9 x CH_2 , 5 x CH); ^{13}C NMR (DMSO-d_6): δ 46.84 (C-1), 27.24 (C-2), 77.83 (C-3), 38.68 (C-4), 54.83 (C-5), 18.13 (C-6), 32.06 (C-7), 40.08 (C-8), 49.18 (C-9), 36.38 (C-10), 23.37 (C-11), 124.84 (C-12), 139.35 (C-13), 42.16 (C-14), 28.95 (C-15), 30.24 (C-16), 48.19 (C-17), 57.38 (C-18), 39.24 (C-19), 39.52 (C-20), 29.57 (C-21), 36.92 (C-22), 28.13 (C-23), 14.89 (C-24), 16.94 (C-25), 18.14 (C-26), 23.01 (C-27), 179.06 (C-28), 15.98 (C-29), 19.55 (C-30), 100.71 (C-1'), 75.04 (C-2'), 73.68 (C-3'), 67. 06 (C-4'), 65.57 (C-5'), 97.83 (C-1''), 71.21 (C-2''), 69.97 (C-3''), 67.11 (C-4''), 63.84 (C-5''); +ve ESI MS *m/z* (ret. Int.): 721 $[M+1]^+$ ($C_{40}H_{65}O_{11}$) (26.5), 455 (6.3), 265 (9.6), 149 (11.4).

2.3.4. Glyceryl 2-phospho-1-caprate (**4**)

Elution of the column with chloroform-methanol (4:1) yielded colourless crystals of **4**, recrystallized from chloroform-methanol (1:1), 148 mg (0.15 % yield), m.p.: 89-90 ºC; R_f: 0.65 (chloroform-methanol, 4:1); UV λ_{max} (methanol) 221 nm (log ε 3.1); IR V_{max} (KBr): 3398, 2934, 1721, 1650, 1384, 1045, 923, 861 cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 4.49 (1H, m, H-2), 4.15 (2H, m, H_2-1), 3.28 (2H, d, J=5.1 Hz, H_2- 3a), 3.16 (1H, d, J=6.9 Hz, H_2- 3b), 2.48 (2H, m, H_2-2'), 1.87 (2H, m, CH_2), 1.46 (2H, m, CH_2), 1.18 (10H, brs, 5 x CH_2), 0.80 (3H, t, J=6.3 Hz, Me-10'); ^{13}C NMR (DMSO-d_6): δ 172.89 (C-1'), 72.07 (C-2), 63.29 (C-1, C-3), 33.16 (CH_2), 29.05 (6 x CH_2), 22.69 (CH_2), 14.23 (Me-10'); + ve ESI MS *m/z* (rel. int.): 327 $[M+1]^+$ ($C_{13}H_{28}O_7P$) (14.2).

2.3.5. Lauroyl tetraglucoside (**5**)

Further elution of the column with chloroform-methanol (4:1) produced pale yellow crystals of **5**, recrystallized from chloroform-methanol (1:1), 163 mg (0.16% yield), R_f: 0.67 (chloroform-methanol, 4:1), m.p.: 129-130 ºC; UV λ_{max} (methanol): 222, 256 nm (log ε 3.2, 1.8); IR V_{max} (KBr): 3510, 3397, 3265, 2929, 2850, 1722,

1643, 1403, 1044, 922, 725 cm^{-1} ; ^1H NMR (DMSO-d_6): δ 2.48 (2H, t, J=7.2 Hz, H_2-2), 1.94 (2H, m, CH_2), 1.27 (8 H, brs, 4 x CH_2), 1.15 (6 H, brs, 3 x CH_2), 0.80 (3H, t, J=6.5 Hz, Me-12), 5.08 (1H, d, J=7.3 Hz, H-1′), 5.01 (1H, d, J=7.2 Hz, H-1′′), 4.93 (1H, d, J=7.4 Hz, H-1′′′), 4.89 (1H, d, J=7.2 Hz, H-1′′′′), 4.73 (1H, m, H-5'), 4.56 (2H, m, H-5′′, H-5′′′), 4.32 (1H, m, H-5′′′′), 3.84 (1H, m, H-2′), 3.76 (1H, m, H-2′′), 3.68 (2H, m, H-2′′′, H-2′′′′), 3.51 (1H, m, H-3′), 3.48 (2H, m, H-3′′, H-3′′′), 3.43 (1H, m, H-3′′′′), 3.38 (1H, m, H-4′), 3.35 (1H, m, H-4′′), 3.32 (2H, m, H-4′′′, H-4′′′′), 3.30 (2H, d, J=8.3 Hz, H_2- 6′), 3.27 (2H, d, J=7.8 Hz, H_2-6′′), 3.19 (2H, d, J=8.5 Hz, H_2-6′′′), 3.06 (2H, d, J=9.1 Hz, H2-6′′′′); ^{13}C NMR (DMSO-d_6): d 169.83 (C-1), 33.43 (C-2), 29.56 (C-3), 29.37 (5 x CH_2), 27.38 (C-9), 25.34 (C-10), 22.68 (C-11), 14.21 (C-12), 102.83 (C-1′), 72.18 (C-2′), 68.01 (C-3′), 64.14 (C-4′), 75.93 (C-5′), 62.14 (C-6′), 98.04 (C-1''), 71.89 (C-2''), 67.64 (C-3''), 63.88 (C-4''), 75.81 (C-5''), 62.09 (C-6''), 95.23 (C-1'''), 70.18 (C-2'''), 67.45 (C-3'''), 63.71 (C-4'''), 75.31 (C-5'''), 61.43 (C-6'''), 92.14 (C-1''''), 69.73 (C-2''''), 66.58 (C-3''''), 63.29 (C-4''''), 74.92 (C-5''''), 60.17 (C-6''''); +ve ESI MS *m/z* (rel.int.) 849 $[M+1]^+$ ($C_{36}H_{65}O_{22}$) (18.2), 199 (12.4), 183 (7.6), 179 (9.1), 163 (15.3).

2.3.6. Caproyl pentaglucoside (**6**)

Further elution of the column with chloroform-methanol (4:1) afforded pale yellow crystals of **6**, recrystallized from methanol, 312 mg (0.31% yield), m.p. 144-145 ºC, R_f: 0.92 (chloroform-methanol, 4:1); UV λ_{max} (methanol): 212 nm (log ε 3.1); IR V_{max} (KBr): 3465, 3397, 3270, 2930, 2850, 1725, 1649, 1460, 1045, 924, 863 cm^{-1}; ^1H NMR-(DMSO-d_6): δ 2.48 (2H, brs, H_2-2), 2.23 (2H, m, CH_2), 1.98 (2H, m, CH_2), 1.50 (2H, m, CH_2), 1.29 (4H, brs, 2 x CH_2), 0.84 (3H, t, J=6.3 Hz, Me-8), 5.15 (1H, d, J=7.3 Hz, H-1a), 4.73 (1H, d, J=7.2 Hz, H-1b), 4.70 (1H, d, J=7.2 Hz, H-1c), 4.65 (2H, brs, H-1d, H-1e), 4.26 (1H, m, H-5a), δ 4.21(1H, m, H-5b), 4.14 (3H, m, H-5c, H-5d, H-5e), 3.70 (1H, m, H-2a), 3.65 (2H, m, H-2b, H-2c), 3.60 (2H, m, H-2d, H-2e), 3.55 (2H, m, H-3a, H-3b), 3.52 (2H, m, H-3c, H-3d), 3.49 (1H, m, H-3e), 3.45 (1H, m, H-4a), 3.41 (1H, m, H-4b), 3.39 (2H, m, H-4c, H-4d), 3.37 (1H, m, H-4e), 3.35 (2H, d, J=8.1 Hz, H_2-6a), 3.32 (2H, d, J=7.8 Hz, H_2-6b), 3.27 (2H, d, J=8.6 Hz, H_2-6c), 3.23 (2H, d, J=6.9 Hz, H_2-6d), 3.05 (2H, d, J=6.7 Hz, H_2-6e); ^{13}C NMR (DMSO-d_6): d 173.26 (C-1), 33.45 (C-2), 29.41 (C-3), 29.39 (C-4), 27.33 (C-5), 25.19 (C-6), 22.67 (C-7), 14.21 (C-8), 102.45 (C-1a), 82.51 (C-2a), 72.95 (C-3a), 68.15 (C-4a), 77.19 (C-5a), 63.42, (C-6a), 98.51 (C-1b), 82.35 (C-2b), 72.83 (C-3b), 68.27 (C-4b), 75.79 (C-5b), 62.68 (C-6b), 97.35 (C-1c), 82.35 (C-2c), 72.40 (C-3c), 69.63 (C-4c), 76.12 (C-5c), 62.13 (C-6c), 94.49 (C-1d), 81.23 (C-2d), 72.01 (C-3d), 63.52 (C-4d), 75.30 (C-5d), 61.68 (C-6d), 93.03 (C-1e), 73.53 (C-2e), 71.04 (C-3e), 63.45 (C-4e), 75.30 (C-5e), 60.66 (C-6e); +ve ESI MS *m/z* (rel. Int.): 955 $[M+1]^+$ ($C_{38}H_{67}O_{27}$) (9.3), 179 (4.8), 163 (11.6), 143 (5.7), 127 (8.1).

2.3.7. Ursolic aldehyde 3-*O*-α-*L*-hexaglycoside (**7**)

Elution of the column with chloroform-methanol (3:1) afforded pale yellow beads of **7**, recrystallized from methanol, 496 mg (0.49% yield), R_f: 0.86 (chloroform-methanol, 3:1); m.p.: 119-120 ºC, UV λ_{max} (MeOH): 210 nm (log ε 3.7); IR V_{max} (KBr): 3425, 3376, 3260, 2938, 2850, 1702, 1649, 1416, 1355, 1043, 923, 860 cm^{-1}; ^1H NMR (DMSO-d_6): δ 9.81 (3H, s, CHO-28), 3.79 (1H, dd, J=4.8, 9.1 Hz, H-3α), 1.11 (3H, brs, Me-23), 1.09 (3H, brs, Me-25), 0.99 (3H, brs, Me-24), 0.91 (3H, d, J=6.1 Hz, Me-29), 0.81 (3H, brs, Me-27), 0.76 (3H, d, J=6.3 Hz, Me-30), 0.66 (3H, brs, Me-26), 2.69-1.26 (26H, m, 10 x CH_2, 6 x CH), 5.07 (1H, d, J=6.2 Hz, H-1a), 4.98 (1H, d, J=6.7 Hz, H-1b), 4.89 (1H, d, J=6.7 Hz, H-1c), 4.93 (1H, d, J=6.8 Hz, H-1d), 4.91 (1H, d, J=6.2 Hz, H-1e), 4.87 (1H, d, J=6.3 Hz, H-1f), 4.49 (1H, m, H-5d), 4.47 (1H, m, H-5e), 4.45 (1H, m, H-5f), 4.15 (1H, m, H-2a), 4.13 (2H, m, H-2b, H-2c), 4.03 (2H, m, H-2d, H-2e), 3.87 (1H, m, H-2f), 3.61-3.34 (12H, m, H-3a to H-3f, H-4a to H-4f), 3.32 (2H, d, J=6.5 Hz, H_2-5a), 3.30 (2H, d, J=6.8 Hz, H_2-5b), 3.23 (2H, d, J=6.8 Hz, H_2-5c), 3.15 (2H, d, J=6.6 Hz, H_2-6d), 3.13 (2H, d, J=6.5 Hz, H_2-6e), 3.04 (2H, d, J=6.3 Hz, H2-6f), ^{13}C NMR (DMSO-d_6): δ 47.50 (C-1), 28.59 (C-2), 78.13 (C-3), 38.66 (C-4), 52.89 (C-5), 18.17 (C-6), 33.74 (C-7), 40.05 (C-8), 48.69 (C-9), 36.98 (C-10), 23.38 (C-11), 31.48 (C-12), 41.23 (C-13), 42.30 (C-14), 28.92 (C-15), 30.31 (C-16), 48.12 (C-17), 57.87 (C-18), 39.22 (C-19), 39.50 (C-20), 21.99 (C-21), 38.94 (C-22), 29.53 (C-23), 13.71 (C-24), 16.29 (C-25), 18.20 (C-26), 24.49 (C-27), 211.09 (C-28), 15.05 (C-29), 20.91 (C-30), 109.62 (C-1a), 82.02 (C-2a), 76.50 (C-3a), 73.33 (C-4a), 63.77 (C-5a), 108.11 (C-1b), 82.60 (C-2b), 75.74 (C-3b), 72.41 (C-4b), 63.64 (C-5b), 107.42 (C-1c), 81.72 (C-2c), 75.72 (C-3c), 70.85 (C-4c), 63.23 (C-5c), 104.40 (C-1d), 82.97 (C-2d), 69.23 (C-3d), 65.33 (C-4d), 78.38 (C-5d), 62.73 (C-6d), 102.02 (C-1e), 82.94 (C-2e), 68.40 (C-3e), 66.35 (C-4e), 77.19 (C-5e), 61.45 (C-6e), 97.85 (C-1f), 72.41 (C-2f), 67.66 (C-3f), 65.85 (C-4f), 76.75 (C-5f), 61.42 (C-6f); +ve ESI MS *m/z* (rel.int.): 1335 $[M+1]^+$ ($C_{63}H_{115}O_{29}$) (16.1), 487 (11.2), 441 (9.8), 325 (10.2), 179 (15.3), 163 (12.7).

3. Results and Discussion

3.1. Spectroscopic characteristics of compound **1**

Compound **1**, named ursolic acid glucosidic arachidate, responded positively to glycoside tests and produced effervescences with sodium bicarbonate solution. Its IR spectrum showed distinctive absorption bands for hydroxyl groups (3425, 3320 cm^{-1}), ester group (1725 cm^{-1}), carboxylic function (1692 cm^{-1}), unsaturation (1645 cm^{-1}) and long aliphatic chain (720 cm^{-1}). On the basis of mass and ^{13}C NMR spectra its molecular ion peak was determined at *m/z* 913 $[M+1]^+$ consistent to the molecular formula of a triterpenic glycosidic ester , $C_{56}H_{97}O_9$. The ion peaks arising at *m/z* 617 [$C_{36}H_{57}O_8$, M-$C_{20}H_{39}O$]$^+$, 455 [C1′-O fission, $C_{30}H_{47}O_3$]+, 295 [O

-C1'' fission, $C_{20}H_{39}O]+$ and 163 $[C_6H_{11}O_5]^+$ suggested that a triterpenic acid was linked with a hexose sugar unit which was esterified with a C_{20} fatty acid. The ^1H NMR spectrum of **1** exhibited a one-proton multiplet at δ 5.12 assigned to the vinylic H-12 proton. A one-proton doublet at δ 4.95 (J=7.2 Hz) was ascribed to anomeric H-1′ proton. A one-proton double doublet at δ 3.70 (J=5.3, 8.9 Hz) was accounted to oxymethine H-3α proton. Four one-proton multiplets at δ 4.23, 4.11, 3.65 and 3.37 and a two-proton doublet at δ 3.04 (J=10.5 Hz) were due to sugar H-2′, H-5′, H-3′ and H-4′ and hydroxymethylene H_2-6′ protons respectively. Five three-proton broad singlets at δ 1.32, 1.03, 0.91, 0.73 and 0.53, two three-proton doublets at δ 1.17 (J=6.0 Hz) and 0.81(J=5.3 Hz) and a three-proton triplet at δ 0.85 (J=7.2 Hz) were associated with the tertiary C-23, C-25, C-26, C-27 and C-24 methyl, secondary C-29 and C-30 and primary C-20'' protons, respectively, all of them were located on the saturated carbons. The other methine and methylene protons appeared from δ 2.48-1.35 (25H) and at δ 1.22 (34H). The ^{13}C NMR spectrum of **1** exhibited signals for ester carbon at δ 171.88 (C-1''), carboxylic carbon at δ 180.61 (C-28), vinylic carbons at δ 128.65 (C-12) and 139.24 (C-13), anomeric carbon at δ 103.78 (C-1′), other sugar carbons between δ 76.52-61.72 and methyl carbons from δ 24.92 to 14.18. The appearance of the sugar H-2′ proton in the deshielded region at δ 4.23 and C-2′ carbon at δ 76.52 suggested attachment of the ester function at C-2′. (Mahato and Kundu, 1994; Ali, 2001; Khan et al., 2010). Acid hydrolysis of **1** yielded ursolic acid, m.p. 284-286 ºC, D-glucose, m.p. 144 -146 ºC, $[\alpha]^{20}_D$ (+) 52.7º (conc. 10, water), R_f 0.12 (*n*-butanol-acetic acid-water (4:1:5) and arachidic acid, m.p. 75 ºC. On the basis of these evidences, the structure of **1** was established urs-12-en-3β-ol-28-oic acid-3-*O*-β-D-glucopyranosyl-2′- arachidate a new triterpenic glycosidic ester (Fig. 1).

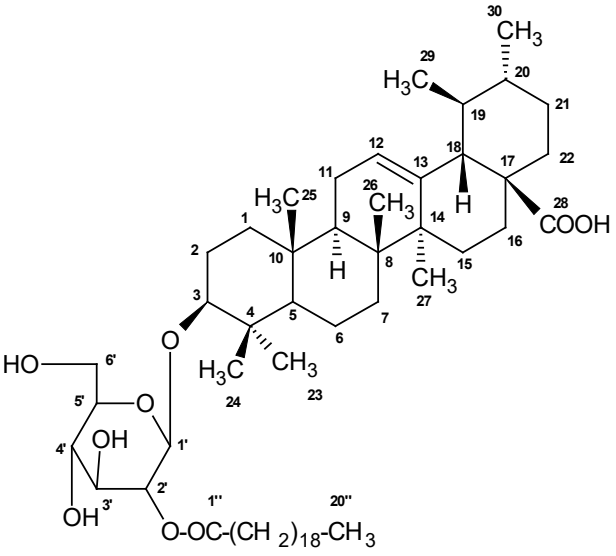

Fig. 1. Molecular structure of ursolic acid glucosidic arachidate (**1**).

3.2. Spectroscopic characteristics of compound **2**

Compound **2**, named *n*-capryl diglucoside, gave positive tests for glycosides and showed characteristic IR absorption bands for hydroxyl groups (3425, 3260 cm^{-1}) and ester function (1720 cm^{-1}). On the basis of mass and ^{13}C NMR spectra the molecular ion peak of **2** was determined at *m/z* 497 $[M+1]^+$ corresponding to the molecular formula of an acyl diglucoside, $C_{22}H_{41}O_{12}$. The ion peaks arising at *m/z* 163 $[C_6H_{11}O_5]^+$, 179 $[C_6H_{11}O_6]^+$, 325 $[[C_6H_{11}O_6\text{-}\ [C_6H_{10}O_4]^+]^+$ and 171 [M-325, CH_3-$(CH_2)_8COO]^+$ indicated that caprylic acid was esterified with a dihexoside unit. The ^1H NMR spectrum of **2** exhibited two one-proton doublets at δ 5.30 (J=7.2 Hz) and 4.90 (J=7.3 Hz) assigned to anomeric H-1′ and H-1'' protons, respectively. The other sugar protons appeared from δ 4.47 to 3.15. A three proton- triplet at δ 0.82 (J=6.2 Hz) was ascribed to primary C-10 methyl protons. The methylene protons of the acyl chain resonated between δ 2.48-1.20. The ^{13}C NMR spectrum of **2** displayed signals for ester carbon at δ 173.47 (C-1), anomeric carbons at δ 103.65 (C-1′) and 101.23 (C-1''), other sugar carbons in range from δ 77.56 to 60.53, methylene carbons between δ 55.19-24.43 and methyl carbon at δ 13.86 (C-10). The presence of the oxygenated methylene H_2-6′ protons in the deshielded region at δ 3.25 and C-6′ signal at δ 62.98 suggested attachment of the second sugar at C-6′ through (6′ →2'') linkage. Acid hydrolysis of **2** yielded capric acid (m.p., 31-32 ºC) and D-glucose, m.p. 144-146 ºC, $[\alpha]^{20}_D$ (+) 52.7º (conc. 10, water), R_f 0.12 (*n*-butanol-acetic acid-water, 4:1:5). On the basis of spectral data analysis and chemical reactions, the structure of **2** has been elucidated as *n*-decanoyl-*O*-β-D-glucopyranosyl-(6′→1'')-*O*-β-D-glucopyranoside (Fig. 2).

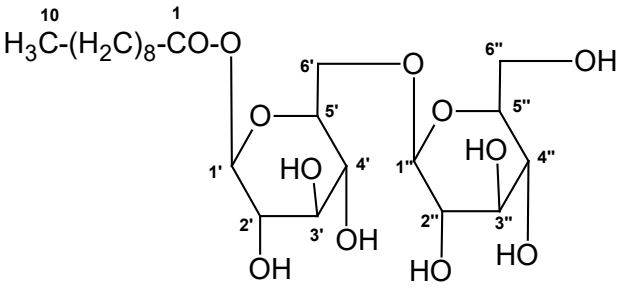

Fig. 2. Molecular structure of *n*-capryl diglucoside (**2**).

3.3. Spectroscopic characteristics of compound **3**

Compound **3**, named ursolic acid 3-*O*-β-D-dixyloside, responded positively to chemical tests of glycosides. Its IR spectrum showed absorption bands for hydroxyl groups (3413, 3360 cm^{-1}), carboxylic group (3218, 1692 cm^{-1}) and unsaturation (1635 cm^{-1}). On the basis of mass and ^{13}C NMR spectra the molecular ion peak of **3** was determined at *m/z* 721 $[M+1]^+$ corresponding to the molecular formula of a triterpenic

diglycoside , $C_{40}H_{65}O_{11}$. The ion peaks arising at *m/z* 455 [M-glycone, $C_{30}H_{47}O_3$], 265 [$C_5H_8O_4$- $C_5H_9O_4$]$^+$ and 149 [$C_5H_9O_5$]$^+$, indicated that a dipentoside unit was linked to a pentacyclic triterpenic acid. The ^{1}H NMR spectrum of **3** showed a one-proton multiplet at δ 5.30 assigned to venylic H-12 proton, a one-proton double doublet at δ 3.64 with coupling interactions of 4.2 and 9.3 Hz attributed to α-oriented oxymethine H-3 proton, five three-proton broad singlets at δ 1.26, 0.97, 1.24, 0.73 and 0.89 due to tertiary C-23, C-24, C-25, C-26 and C-27 methyl protons, respectively, two three-proton doublets at δ 0.85 (J=6.1 Hz) and 0.82 (J=6.3 Hz) accounted to secondary C-29 and C-30 methyl protons, respectively, suggesting ursane-type aglycone and the remaining methine and methylene protons between δ 2.38-1.38. Two one-proton doublets at δ 5.15 (J=7.3 Hz) and 4.89 (J=7.1 Hz) were accounted to anomeric H-1′ and H-1′′ protons, respectively. The other sugar proton appeared in the range of δ 4.23-3.15. The ^{13}C NMR spectrum of **3** exhibited forty carbon signals and the important signals appeared for vinylic carbons at δ 124.84 (C-12) and 139.35 (C-13), carboxylic carbon at δ 179.06 (C-28), anomeric carbons at δ 100.71 (C-1′) and 97.83 (C-1′′), oxymethine carbon at δ 77.83 (C-3) and other sugar carbons between δ 75.04- 63.84. The presence of H-2′ NMR signal in the deshielded region at δ 4.23 and C-2′ carbon in the deshielded region at δ 75.04 supported the existence of the second sugar unit at C-2′ through (2′→1′′) linkage. The ^{1}H NMR and ^{13}C NMR values of the triterpenic sekeleon were compared with the related ursene-type compounds (Mahato and Kundu, 1994; Ali, 2001; Khan et al., 2010). Acid hydrolysis of **3** yielded ursolic acid, m.p. 283-285 ºC and D-xylose, m.p. 144-145 ºC, $[\alpha]^{20}_D$ (+) 18.9º (conc. 10, water), R_f 0.76 (*n*-butonal: acetic acid: water (4:1:1.6) . On the basis of foregoing evidences, the structure of **3** has been formulated as urs-12-en-3β-ol-28-oic acid 3-*O*-β-D-xylopyranosyl-(2′→1′′)-*O*-β-D-xylopyranoside, a new triterpenic dixyloside (Fig. 3).

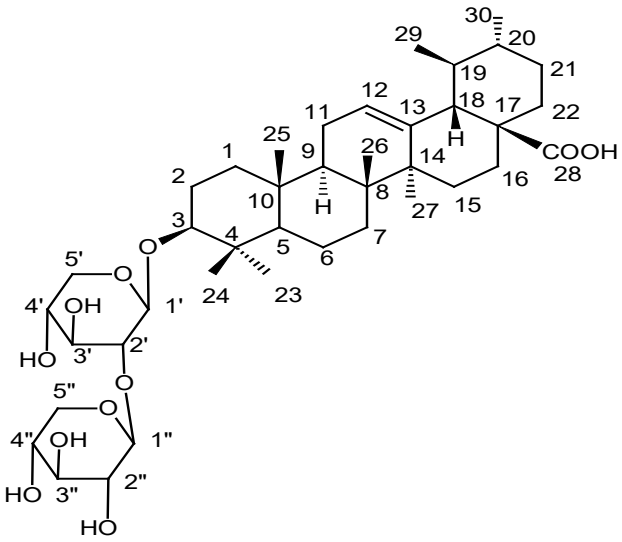

Fig. 3. Molecular structure of ursolic acid 3-*O*-β-D-dixyloside (**3**).

3.4. Spectroscopic characteristics of compound **4**

Compound **4**, glyceryl 2-phospho-1-caprate, showed IR absorption bands for hydroxyl groups (3398 cm^{-1}) and ester function (1721 cm^{-1}). The mass spectrum of **4** exhibited a molecular ion peak at *m/z* 327 [M+1]$^+$ corresponding to a molecular formula of a glyceride phosphate ($C_{13}H_{28}O_7P$). The ^{1}H NMR spectrum of **4** exhibited a one-proton multiplet at δ 4.49 ascribed to methine proton H-2, a two-proton multiplet at δ 4.15 assigned to oxymethylene H_2-1 protons and two one-proton doublets at δ 3.28 (J=5.1 Hz) and 3.16 (J=6.9 Hz) attributed to hydroxymethylene H_2-3 protons. The terminal primary methyl Me-10′ appeared at δ 0.80 as a triplet (J=6.3 Hz). The remaining methylene protons resonated as two-proton multiplets at δ 2.48, 1.87 and 1.46 and as a singlet at δ 1.18 (10 H). The ^{13}C NMR spectrum of **4** displayed an ester C-1′ carbon at δ 172.89, oxymethine carbon at δ 72.07, oxymethylene carbons at δ 63.89 (C-1, C-3) and a methyl carbon at δ 14.23 (C-10′). The methylene carbon appeared between δ 33.16-22.69. Acid hydrolysis of **4** yielded capric acid, m.p. 31-32 ºC. On the basis of the foregoing discussion, the structure of **4** has been elucidated as glyceryl 1-decanoyl -2-phosphate (Fig. 4).

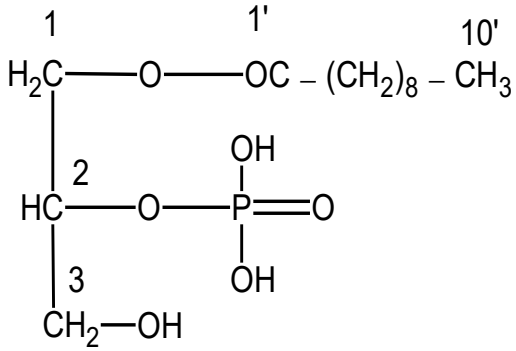

Fig. 4. Molecular structure of glyceryl-2-phospho-1-caprate (**4**).

3.5. Spectroscopic characteristics of compound **5**

Compound **5**, designated as lauroyl tetraglucoside, gave positive tests for glycosides and displayed distinctive IR absorption bands for hydroxyl groups (3510, 3397, 3265 cm^{-1}), ester function (1722 cm^{-1}) and long aliphatic chain (725 cm^{-1}). Its molecular ion peak was determined at *m/z* 849 [M+1]$^+$ on the basis of mass and ^{13}C NMR spectra consistent with a molecular formula of an acyl tetraglycoside, $C_{36}H_{65}O_{22}$ .The ion peaks arising at *m/z* 163 [$C_6H_{11}O_5$]+, 179 [$C_6H_{11}O_6$]$^+$, 183 [M-glycone, CH_3-(CH_2)10-CO]$^+$ and 199 [CH_3-(CH_2)10-COO]$^+$ indicated that lauric acid was esterified with a tetraglycoside unit containing hexose sugar units. The ^{1}H NMR spectrum of **5** exhibited a three-proton triplet at δ 0.80 (J=6.5 Hz) assigned to terminal C-12 primary methyl protons, methylene proton signals from δ 2.48 to 1.15, four one-proton doublets at δ 5.08 (J=7.3 Hz), 5.01 (J=7.2 Hz), 4.93 (J=7.4 Hz, H-1′′′), 4.89 (J=7.2 Hz,

H-1′′′′) ascribed to four anomeric signals H-1′ to H-1′′′′, and other sugar oxymethine protons as multiplets between δ 4.73-3.32 and oxymethylene protons as two-proton doublets at δ 3.30 (J=8.3 Hz, H_2- 6′), 3.27 (J=7.8 Hz, H_2-6′′), 3.19 (J=8.5 Hz, H_2-6′′′), 3.06 (J=9.1 Hz, H_2-6′′′′) . The absence of any signal beyond δ 5.08 suggested saturated nature of the molecule. The ^{13}C NMR spectrum of **5** exhibited signals for ester carbon at δ 169.83 (C-1), methyl carbon at δ 14.21 (C-12), four anomeric carbons between δ 102.83-92.14, and other sugar carbons from δ 75.93 to 60.17. The presence of the oxymethylene protons in the deshielded region at δ 3.30 (H_2- 6′), 3.27 (H_2-6′′) and 3.19 (H_2-6′′′) and their respective carbon signals at δ 62.14(C-6′), 62.09 (C-6′′) and 61.43 ((C-6′′′) suggested the attachment of the sugar units at C-6′, C-6′′ and C-6′′′ carbons through (C_6→C_1) linkages. Acid hydrolysis of **5** yielded lauric acid, m.p. 43 ºC and D-glucose, m.p. 144-146 ºC, $[\alpha]^{20}_D$ (+) 52.7º (conc. 10, water), R_f 0.12 (*n*-butanol-water- glacial acetic acid, 4:5:1). Based on the above evidences, the structure of **5** has been elucidated as *n*-dodecanoyl-*O*-β-D-glucopyranosyl-(6′→1′′)-*O*-β-D-glucopyranosyl-(6′′→1′′′)-*O*-β-D-glucopyranosyl-6′′′→1′′′′)-*O*-β-D-glucopyranoside, a new acyl tetraglucoside (Fig. 5).

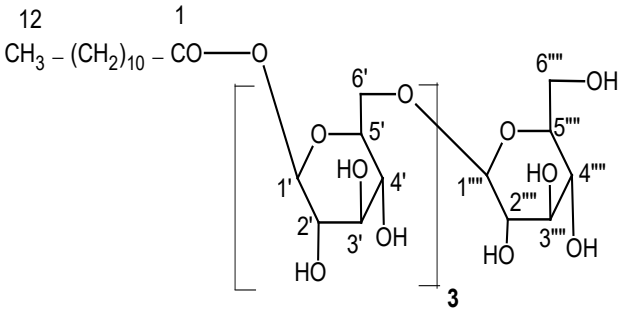

Fig. 5. Molecular structure of lauroyl tetraglucoside (**5**).

3.6. Spectroscopic characteristics of compound **6**

Compound **6**, named caproyl pentaglucoside, $[M]^+$ at *m/z* 955 $[M+1]^+$ ($C_{38}H_{67}O_{27}$), responded positively to glycoside tests and showed characteristic IR absorption bands for hydroxyl groups (3465, 3397, 3270 cm^{-1}) and ester function (1725 cm^{-1}). The ion peaks generated at *m/z* 179 $[C_6H_{11}O_6]^+$, 163 $[C_6H^{11}O_5]^+$, 143 $[CH_3(CH_2)_2COO]^+$ and 127 $[CH_3(CH_2)_2CO]^+$ suggested that a caproyl group was linked with a tetraglycoside composed of hexose units. The ^{1}H NMR spectrum **6** displayed a three-proton triplet at δ 0.84 (J=6.3 Hz) accounted to terminal C-8 primary methyl protons, methylene protons from δ 2.48 to 1.29, five anomeric protons as one-proton doublets at δ 5.15 (J=7.3 Hz, H-1a), 4.73 (J=7.2 Hz, H-1b), 4.70 (J=7.2 Hz, H-1c) assigned to H-1a, H-1b and H-1c, respectively, and as a two-proton signal at δ 4.65 due to H-1d and H-1e protons. The other sugar protons appeared as multiplets in the range of δ 4.26 to 3.37 ascribed to oxymethine protons and as two-protons doublets at δ 3.35 (J=8.1 Hz), 3.32 (J=7.8 Hz), 3.27 (J=8.6 Hz), 3.23 (J=6.9 Hz) and 3.05 (J=6.7 Hz) attributed to H_2-6a to H_2-6e protons, respectively. The absence of any signal beyond δ 5.15 supported saturated nature of the molecule. The shifting of oxymethylene H_2-6a to H_2-6d protons in the deshielded region at from δ 3.35 to 3.23 suggested (6 → 1) linkages of the sugar units. The ^{13}C NMR spectrum of **6** exhibited signals for ester carbon at δ 173.26 (C-1), methylene carbons between δ 33.45-22.67, methyl carbon at δ 14.21 (C-8), anomeric carbons at δ 102.45 (C-1a), 98.51 (C-1b), 97.35 (C-1c), 94.49 (C-1d) and 93.03 (C-1e) and other sugar carbons in the range of δ 82.51-60.66. Acid hydrolysis of **6** yielded caprylic acid, R_f 0.74 (*n*-ethyl amine) and D-glucose, m.p. 144 -146 ºC, $[\alpha]^{20}_D$ (+) 52.7º (conc. 10, water), R_f 0.12 (*n*-butanol-water-glacial acetic acid, 4:5:1). On the basis of spectral data analysis and chemical reactions, the structure of **6** has been characterized as *n*-octanoyl-*O*-β-D-glucopyranosyl-(6a→1b)-*O*-β-D-glucopyranosyl-(6b→1c)-*O*-β-D-glucopyranosyl-(6c→1d)-*O*-β-D-glucopyranosyl-(6d→1e)-*O*-β-D-glucopyranoside, a new acyl pentaglycoside (Fig. 6).

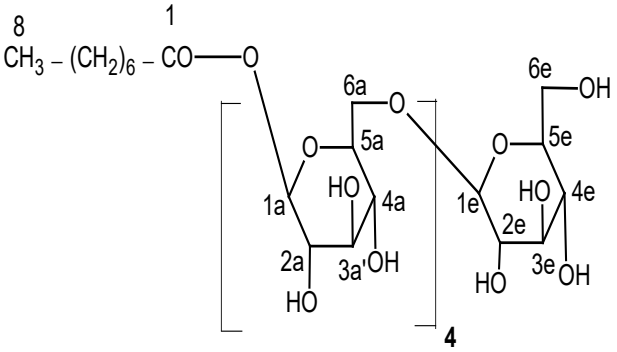

Fig. 6. Molecular structure of caproyl pentaglucoside (**6**).

3.7. Spectroscopic characteristics of compound **7**

Compound **7**, designated as ursolic aldehyde 3-*O*-α-L-hexaglycoside, gave positive tests for glycosides and exhibited characteristic IR absorption bands for hydroxyl groups (3425, 3376, 3260 cm^{-1}) and aldehyde function (1702 cm^{-1}). On the basis of mass and ^{13}C NMR spectra, the molecular weight of **7** has been established at *m/z* 1335 $[M+1]^+$ corresponding to the molecular formula of a triterpenic hexaglycoside, $C_{63}H_{115}O_{29}$. An ion fragment generating at *m/z* 441 [O-C_{1a} fission, $C_{30}H_{49}O_2]^+$ indicated a pentacyclic triterpenic aldehyde was linked to a hexaglycoside unit. The ion peaks arising at *m/z* 163 $[C_6H_{11}O_5]^+$, 179 $[C_6H_{11}O_6]^+$, 325 $[C_6H_{11}O_5$ -$C_6H_{10}O_5]^+$, and 487 $[C_6H_{11}O_5$ -$C_6H_{10}O_5$-$C_6H_{10}O_5]^+$, supported the presence of three hexose units at the terminal of the sugar unit. The ^{1}H NMR spectrum of **7** showed a one-proton singlet at δ 9.81 assigned to aldehydic H-28 proton. A one-proton double doublet at δ 3.79 (J=4.8, 9.1 Hz) was ascribed to

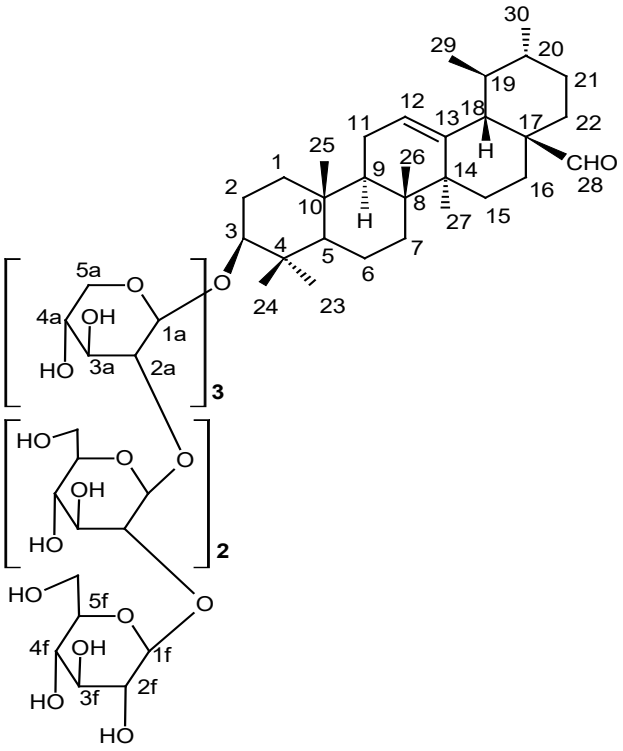

Fig. 7. Molecular structure of ursolic aldehyde 3-*O*-α-L-hexaglycoside (**7**).

α-oriented oxygenated methine H-3 proton. Two three-proton doublets at δ 0.91 (J=6.1 Hz) and 0.76 (J=6.3 Hz) and five three- proton singlets at 1.11, 0.99, 1.09, 0.66 and 0.81 were associated correspondingly with the secondary methyl H_3-29 and H_3-30 protons of ursane-type skeleton and tertiary C-23, C-24, C-25, C-26 and C-27 methyl protons, all attached to saturated carbons. The other methine and methylene protons appeared between δ 2.69- 1.26. Six one-proton doublet at δ 5.07 (J=6.2 Hz), 4.98 (J=6.7 Hz), 4.95 (J=6.7), 4.93 (J=6.8 Hz), 4.91 (J=6.2 Hz) and 4.87 (J=6.3 Hz) were attributed to α-oriented anomeric H-1a to H-1f protons, respectively. The remaining sugar protons resonated as multiplets from δ 4.49 to 3.34 ascribed to hydroxymethine and as two-proton doublets at δ 3.32 (J=6.5 Hz), 3.30 (J=6.8 Hz), 3.23 (J=6.8 Hz), 3.15 (J=6.6 Hz), 3.13 (J=6.5 Hz), 3.04 (J=6.3 Hz) accounted correspondingly to oxymethylene H_2-5a, H_2-5b and H_2-5c and hydroxymethylene H_2-6d, H_2-6e and H_2-6f protons. The ^{13}C NMR spectrum of **7** exhibited signals for aldehydic carbon at δ 211.09 (C-28), oxymethine carbon of the triterpenoid skeleton at δ 78.13 (C-3), methyl carbons from δ 29.53 to 13.71 and six anomeric carbons at δ 109.62 (C-1a), 108.11 (C-1b), 107.42 (C-1c), 104.40 (C-1d), 102.02 (C-1e) and 97.85 (C-1f). The other sugar carbons resonated from δ 82.97 to 61.42. The presence of the proton signals as multiplets in the deshielded region at δ 4.15 (H-2a), 4.13 (H-2b, H-2c) and 4.03 (H-2d, H-2e) carbon signals from δ 82.97 to 81.72 for C-2a to C-2e supported to (2→1) linkages of the sugar units. The ^{1}H and ^{13}C NMR spectral values of aglycone unit of **7** were compared with reported values of ursane-type triterpenoids (Ali, 2001; Khan et al., 2010). Acid hydrolysis of **7** yielded dihydroursolic aldehyde, *L*-glucose, m.p. 153-155 ºC, $[\alpha]^{20}_D$ (-) 51º (conc. 10, water), R_f 0.12 and L-arabinose, m.p. 160-163 ºC, $[\alpha]^{20}_D$ (+) 103º (conc. 10, water), R_f 0.18 (*n*-butanol-acetic-water, 4:1:5) . On the basis of spectral data analysis and chemical reactions, the structure of **7** has been established as ursan-3β-ol-28-al-3-*O*-α-L-arabinopyranosyl-(2a→1b)-*O*-α-L-arabinopyranosyl-(2b→1c)-*O*-α-L-arabinopyranosyl-(2c→1d)-*O*-α-L-glucopyranosyl-(2d→1e)-*O*-α-L-glucopyranosyl-(2e→1f)-*O*-α-L-glucopyranoside, a new triterpenic hexaglycoside (Fig. 7).

4. Concluding remarks

Phytochemical investigation of a methanolic extracts of the leaves of *C. asiatica* (L.) led to the isolation of acyl and ursanyl glycosides and glyceryl 2-phospho-1-caprate. This work has enhanced understanding about the phytoconstituents of the plant. These secondary metabolites can be used as analytical markers for quality control of the leaves of this plant.

Conflict of interest

The authors declare that there is no conflict of interest.

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