A NEW LANOSTANYL DIGLUCURONOSIDE FROM THE FLOWERS OF
PUNICA GRANATUM L.
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ABSTRACT
One new triterpene glycoside characterized as lanastan-3β-yl-glucuronopyranosyl-6′→1″–glucuronopyranoside has been isolated from the flowers of Punica granatum L. (Punicaceae) along with oleanolic acid acetate, n-triacontane, n-hentriacontane, tetracontyltritritric, n-tetracontane, 3-epi-α-amyrin, lanostanyl glucoside, β-sitosterol glycoside, trixyloside and hydroxynaphthoquinone as the known compounds. The structures of all the phytoconstituents have been elucidated on the basis of spectral data analysis and chemical reactions.

KEYWORDS: Punica granatum, Punicaceae, Lanostanyl diglucoside.

INTRODUCTION
Punica granatum L. (Punicaceae), commonly known as Gulnar farsi, is a large deciduous shrub or small tree up to 10 m in height with smooth dark grey bark. Pomegranate is native of Iran and is extensively cultivated as a fruit tree, as ornamental plant and for medicinal purpose in Mediterranean region India. The flowers of P. granatum are taken internally to combat diabetes, either as a single drug or in polyherbal formulations in Unani medicine. The powdered flower buds are ingested to relieve bronchitis, diarrhoea and dysentery of children. A decoction of the flowers is gargled to reduce oral and throat inflammation. The flowers are also reputed as a styptic to the gums and to allay biliousness, sore eyes, ulcers and sore throat. A paste of the flowers is applied to cure hydrocele. Modern uses of pomegranate derived products now include treatment of acquired immune deficiency syndrome (AIDS). Flower extract of P. granatum (a Unani antidiabetic medicine) lowered blood glucose in normal and alloxan induced diabetic rats. The results indicated that P. granatum extract was able to ameliorate toxicity induced by alloxan. P. granatum, a dual activator of peroxisome proliferators-activated receptor (PPAR) -α and -γ improves hyperglycemia. Gallic acid was identified as the main constituent for activity which acted through activation of PPAR-γ receptors. Administration of flowers extract in Zucker diabetic fatty rats had shown marked effect by activation of PPAR-α and thereby lowering blood glucose, circulating lipid and inhibiting its cardiac uptake. The results showed that P. granatum extract possessed antidiabetic as well as cardioprotective activities. P. granatum flower extract reduced the plasma glucose levels after sucrose loading. In vitro, P. granatum extract demonstrated a potent inhibitory effect on α-glucosidase activity. P. granatum flowers were used to evaluate the efficacy against abnormal glucose and cardiac lipid metabolism. It reduced the up-regulated cardiac mRNA expression of ET-1, ETA, inhibitor κBα and c-jun and normalized the down-regulated mRNA expression of inhibitor κBα in Zucker diabetic fatty rats. It has been reported to possess antioxidant activities. The phytochemical studies carried out so far have revealed that the flowers contain compounds which also found in peels (e.g. gallic acid) and seed (ursolic acid), and quite possibly unique, distinctive compounds as well. Further study is in progress to elucidate the chemistry of the flowers that have been ethnomedicinally isolated from the dried flowers of Punica granatum. This manuscript describes isolation of a new triterpenic glycoside along with aliphatic hydrocarbons, steroids and triterpenoids from the flowers.

MATERIAL AND METHOD
Plant material
The dried flowers of Punica granatum were purchased from Khari Baoli market of Delhi. The authenticity of the material was established by Prof.M.P.Sharma, Department of Botany, Faculty of Science, Jamia Hamdard, New Delhi. A voucher specimen No-PRL/...
recrystallized from chloroform (9:1) yielded colourless amorphous powder of m/z \( \text{(0.0058\% yield)}, R_f 0.7 \), mp 180-182°C. +veESI MS m/z (rel.int): 426 [M] \(^+\) C\(_{36}\)H\(_{50}\)O\(_2\) (1.5).

**Lanostenyl glucoside (7)**

Elution of the column with chloroform –methanol mixture (19:1) gave colourless crystals of m/z \( \text{(0.0007 % yield)}, R_f 0.7 \) (CHCl\(_3\)-acetone, 4:1), mp 201-202°C. +veESI MS m/z (rel.int): 590 [M] \(^+\) C\(_{36}\)H\(_{62}\)O\(_6\) (2.3).

**Lanostanyl diglucuroside (8)**

Further elution of the column with chloroform- methanol mixture (19:1) furnished colourless amorphous powder of m/z \( \text{(0.0008 % yield)}, R_f 0.4 \) (CHCl\(_3\)-acetone, 4:1), mp 215-216°C. UV \( \lambda \) max (MeOH): 215 nm (log \( \varepsilon \) 5.3). IR (KBr): 3451, 3260, 2955, 2845, 1737, 1737, 1700, 1601, 1390, 1310, 1280, 1199, 1087, 1005 cm\(^{-1}\).H-\(NMR\) (MeOD):\( \delta \) 5.01 (1H, brs, H-1‘), 4.91 (1H, brs, H-1′), 4.73 (4H, brs, H-2′, H-2″, H-4′, H-4″), 4.71 (2H, brs, H-5, H-5′), 4.23 (1H, dd, J = 5.4, 9.3 Hz, H-3β),3.34 (2H, brs, H-3′, H-3″), 1.32 (3H, brs, Me-28),1.28 (3H, brs, Me-30), 1.25 (3H, brs, Me-29), 1.07 (3H, brs,Me-19), 0.97 (3H, d, J = 5.68 Hz, Me-21), 0.89 (3H, d, J = 5.68 Hz, Me-26), 0.87 (3H, d, J= 7.2 Hz, Me-27), 0.76 (3H, brs, Me-18).+veESI MS m/z (rel.int): 782 [M] \(^+\) C\(_{42}\)H\(_{63}\)O\(_{13}\) (4.2).

**Hydrolysis of (8)** Compound of (8) (3 mg) was dissolved in ethanol (3 ml), dilute HCl (2 ml) added and the reaction mixture heated on a stream bath for1hr. The solvent was evaporated to dryness and the residue was dissolved in water. It was chromatographed on silica gel TLC using n- butanol- toluene- pyridine- water (5:1:3:3) as a developing solvent system. The sugar was identified as glucuronic acid, R\(_f\) 0.52.

**β-Sitosterol glycoside (9)**

Elution of the column with chloroform –methanol (4:1) mixture afforded a colourless amorphous powder of m/z \( \text{(0.0009 % yield)}, R_f 0.7 \), toluene: ethyl formate: formic acid, 5:4:1) mp 270-272°C. FAB MS m/z (rel. int.): 576[M] \(^+\) C\(_{38}\)H\(_{58}\)O\(_6\) (4.2) 400(3.1), 396 (11.5), 381 (6.7), 367 (3.6), 273 (3.0), 255 (32.5), 240 (3.8), 231(8.1), 213 (23.2), 198 (5.2), 173 (14.3), 163 (15.9), 161 (24.4), 159 (32.6), 145 (53.3), 133 (41.6), 121(32.3), 119 (32.5), 107 (53.1), 105 (50.3), 95 (49.2),93 (39.8), 83 (5.6), 71 (32.2), 69 (57.3), 67 (52.5), 55 (85.3), 43 (100).

**Isolation from petroleum ether insoluble fraction**

The petroleum ether fraction (52g) after redissolving in hot petroleum ether and concentrating to a small volume was left in a refrigerator overnight. A green solid (5g) separated out, which was filtered and washed with petroleum ether. It was recrystallized to obtain oleaonic acid acetate (1), 10 mg, mp 291-292°C. FAB MS m/z (rel.int): 498 [M]+ C\(_{32}\)H\(_{44}\)O\(_4\) (1.5).

**n-Tetracontaine (2)**

Elution of the column with petroleum ether afforded colourless amorphous powder of 2, recrystallized from chloroform-methanol(1:1), 30 mg (0.0017 % yield), mp 85-86°C R\(_f\) 0.72 (petroleum ether : acetone, 4:1).FAB MS m/z (rel.int):422 [M]+ C\(_{30}\)H\(_{62}\) (2).

**n-Henetriacontane (3)**

Further elution of the column with petroleum ether furnished colourless crystals of 3, recrystallized from acetone – methanol (1:1),mp 88-89°C, 102 mg (0.0058%yield),R\(_f\) 0.52 (petroleum ether-acetone, 4:1), mp 88-89°C. FAB MS m/z (rel. int ) :436 [M]+ C\(_{31}\)H\(_{64}\). Tetratriacontane (4)

Further elution of the column with petroleum ether gave colourless amorphous powder of 4, recrystallized from acetone – methanol (1:1), 115 mg (0.0065%yield), R\(_f\) 0.5 (petroleum ether-acetone, 4:1),mp 88-90°C. FAB MS m/z (rel.int):478 [M]+ C\(_{34}\)H\(_{70}\). n-Tetracontane (5)

Elution of the column with petroleum ether- chloroform (9:1) yielded colourless amorphous powder of 5, recrystallized from chloroform–methanol (1:1), 110 mg (0.0062 % yield), R\(_f\) 0.3 (petroleum ether-CHCl\(_3\), 7:3), mp 99-100°C. +veESIMS m/z (rel . int) : 562 [M]+ C\(_{40}\)H\(_{82}\) (1.3).

**3- Epi-α-amyrrin (6)**

Elution of the column with petroleum ether- chloroform (1:1) gave colourless crystalline powder of 6, recrystallized from acetone, 13.2 mg (0.00072 % yield),R\(_f\) 0.3, mp 180-182°C. +veESI MS m/z (rel.int): 426 [M]+ C\(_{36}\)H\(_{50}\)O\(_2\) (1.5).

**Extraction and fractionation**

The dried flowers (1.75 kg) were extracted exhaustively with ethyl alcohol in a Soxhlet apparatus. Recovery of the solvent left a brownish viscous mass (525 g). The alcoholic concentrate was extracted by refluxing with petroleum ether (60-80°C) several times to obtain petroleum ether soluble and petroleum ether insoluble fractions which constituted of 52g and 470g, respectively.
Trixyloside (10)
Elution of the column with chloroform –methanol (4:1) furnished a colourless amorphous crystalline compound 10, recrystallized from methanol; 10 mg (0.005% yield), Rf 0.6 (CHCl3-MeOH, 1:1), mp 223-225 °C. IR v max (KBr): 3450, 3360, 2924, 2935, 2850, 1460, 1375, 1329, 1092, 1023, 873 cm−1. 1H–NMR (MeOD): δ 4.88 (1H, brs, H-1′), 4.81 (1H, brs, H-1″), 4.78 (1H, brs, H-1‴), 4.75 (2H, m, H-4, H-4′), 4.71 (1H, m, H-4″), 3.80 (1H, m, H-3), 3.77 (1H, m, H-3′, H-3″), 3.65 (1H, m H-2), 3.60 (2H, m, H-2′, H-2″), 3.37 (2H, brs, H-2–5), 3.33 (2H, brs, H-2–5″), 3.31 (2H, brs, H-2–6). 13C NMR (MeOD): δ 101.53 (C-1′, C-1″), 100.26 (C-1), 71.49 (C-2), 71.43 (C-2′, C-2″) 69.85 (C-3), 69.77(C-3′, C-3″), 66.41 (C-4), 66.38 (C-4′, C-4″), 64.08 (C-5), 64.02 (C-5′, C-5″). +veESIMS m/z (rel. int.: 414 [M]+ (C15H28O13) (6.2).

Hydroxynaphthoquine (11)
Elution of the column with chloroform –methanol (7:3) yielded a pale yellow mass of 11, recrystallized from (CHCl3-MeOH, 1:1), 20 mg (0.005% yield), Rf 0.83, EtOAc: MeOH: H2O: HOAc, 5:3:1:1, mp 278-280 °C. IR v max (KBr): 3200, 1719, 1704, 1580, 1500, 1446, 1338, 195, 1112, 1055, 922, 833, cm−1. 1H NMR (DMSO-d6): 7.61 (1H, dd, J=9.0, 2.5 Hz, H-8), 7.13 (1H, dd, J=7.8, 2.7 Hz, H-6), 6.92 (1H, dd, J=7.8 Hz, 9.0 Hz, H-7), 6.73 (1H, d, J=7.8 Hz, H-2), 6.61 (1H, d, J=7.8 Hz, H-3), 13C NMR (DMSO-d6): 192.31 (C-1), 110.17 (C-2), 107.55 (C-3), 192.29 (C-4), 122.81 (C-5), 136.34 (C-6), 139.62 (C-7), 148.07 (C-8), 159.11 (C-9), 112.31 (C-10), +veESI MS m/z (rel. int.: 174 [M]+ (C26H18O15) (2.5).

RESULTS AND DISCUSSION
Compound 1,2,3,4,5,6,7,9,10,11 are the known phytoconstituents identified as olean–12–en–3β –yl acetate–28–ioic acid, n-triacontane, n-henatricontane, n-tetratricontane, n-tetracotane, urs–12–en–18–βH–3α–ol, lanast–5–en–3β–ol–3β –D–glucopyranoside, Lanastan–β–yl–glucuronopyranosyl(6′→1″)–glucuronopyranosyl, β-sitosterol β–D–glucoside, xylopyranosido–(4→1′)–xylopyranosido–(4′→1″)–xylopyranoside,9–hydroxy–1, 4-naphthoquinone, respectively, on the basis of spectral data analysis.

Lanostanyl diglucoside (8) was obtained as colourless amorphous powder from chloroform–methanol (19:1) eluant. It gave positive tests of triterpenic glycosides. Its IR spectrum exhibited absorption bands for hydroxyl (3451 cm−1), carboxyl (3260, 1700 cm−1) and ester (1737 cm−1) groups. The +ve ESI mass spectrum of 8 displayed a molecular ion peak at m/z 782 consistent with the molecular formula C42H54O13 of a triterpenic diglycoside. It indicated eight double bond equivalents; four of them were adjusted in the tetracyclic framework and the remaining four in the glucuronopyranoside moieties. The 1H NMR spectrum of 8 displayed two downfield one proton broad signals at δ 5.01 and 4.91 assigned correspondingly to H–1′ and H–1″ anomic protons. A four-carbonol protons broad signals at 4.73 was ascribed to H-2′, H-2″ H-4′ and H-4″ protons. Two two-proton broad singlets at δ 4.71 and 3.34 were assigned to rest of the glycone protons, viz. H–5′–5″ and H–3′–3″. A one-proton double doublet at δ 4.23 was assigned to oxygenated methine proton at H–3 that was placed β-orientation on the basis of its coupling constants (J = 5.4, 9.3 Hz) and biogenetic evidences. Three three-proton doublets at δ 0.97 (J = 5.68 Hz), 0.89 (J = 5.7 Hz) and 0.87 (J = 7.2 Hz) were ascribed correspondingly to Me–21, Me–26 and Me–27 secondary methyl protons whereas the remaining methyl protons resonated as three-proton broad signals at δ 1.32 (Me–28), 1.28 (Me–30), 1.25 (Me–29), 1.07 (Me–19) and 0.76 (Me–18). The presence of all methyl signals in the range δ 1.32-0.87 indicated their location on saturated carbons. The 1H– 1H COSY spectrum showed correlation on of H-3 with H-2, H-2, H-3, H-28, H-1′; H-25 with H-24 and H-27, and H-21; H-20 with H-17, H-21 and H-22. Acid hydrolysis of 8 yielded glucuronic acid (TLC comparable). The 13C NMR spectrum of 8 showed important carbon signals for carboxylic (δ 184.1), ester (δ 171.6), anomic (δ101.5) and carbinol (δ 85.2-68.3) carbons. On the basis of spectral data analysis and chemical reaction the structure of 8 has been elucidated as lanastan–3β–yl–glucuronopyranosyl(6′→1″)–glucuronopyranoside. This is a new triterpenic diglucoside.

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