INTERNATIONAL RESEARCH JOURNAL OF PHARMACY **ISSN 2230 – 8407**
Available online http://www.irjponline.com
Research Article

A NEW LANOSTANYL DIGLUCURONOSIDE FROM THE FLOWERS OF *PUNICA GRANATUM* L.

Jabbar Zoobi, Ali Mohd*
Phytochemistry Research Laboratory, Faculty of Pharmacy, Jamia Hamdard, New Delhi 110062, India

Article Received on: 22/05/11 Revised on: 29/06/11 Approved for publication: 13/07/11

*Prof Mohd Ali, Department of Pharmacognosy & Phytochemistry, New Delhi-110 062, India
Email: maliphyto@gmail.com

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-henetriacontane, tetratriacontane, n-tetracontane, 3- epi-α-amyrin, lanostenyl 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 diglucuroside.

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[1, 2]. 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[3]. Modern uses of pomegranate derived products now include treatment of acquired immune deficiency syndrome (AIDS)[4]. 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[5]. *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. [6,7] *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[8]. *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[9].It has been reported to possess antioxidant activities[10]. 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[7]. Further study is in progress to elucidate the chemistry of the flowers that have been ethnomedically 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/

JH//08/40 is deposited in the herbarium of the Department of Pharmacognosy and Phytochemistry, Jamia Hamdard, New Delhi.

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^{o}C) several times to obtain petroleum ether soluble and petroleum ether insoluble fractions which constituted of 52g and 470g, respectively.

Isolation from petroleum ether soluble 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 (5 g) separated out, which was filtered and washed with petroleum ether. It was recrystallized to obtain oleanoic acid acetate **(1)**, 10 mg, mp 291-292 ^{o}C. FAB MS m/z (rel. int): 498 $[M]^+$ ($C_{32}H_{50}O_4$) (1.5).

Isolation from petroleum ether insoluble fraction

The petroleum ether insoluble mother liquor (PFF) was evaporated to dryness to give a waxy material (45g). It was chromatographed on a column of silica gel (60-120 mesh, 900g loaded in petroleum ether. The column was eluted with petroleum ether, petroleum ether – chloroform mixtures and finally with chloroform to isolate the following compounds:

n-tetratriacontane (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 ^{o}C R_f .0.72 (petroleum ether : acetone, 4:1).FAB MS m/z (rel. int):422 $[M]^+$ ($C_{30}H_{62}$).

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 ^{o}C, 102 mg (0.0058%yield),R_f 0.52 (petroleum ether-acetone, 4:1), mp 88-89 ^{o}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 ^{o}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 ^{o}C. +veESIMS m/z (rel . int) : 562 $[M]^+$ ($C_{40}H_{82}$).(1.3).

3- Epi-α-amyrin (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 ^{o}C. +veESI MS m/z (rel.int): 426 $[M]^+$ C_{30} $H_{50}O$ (1.5).

Lanostenyl glucoside (7)

Elution of the column with of chloroform –methanol mixture (19:1) yielded colourless crystals of **7**, recrystallized from chloroform –methanol (1:1), 12 mg (0.0007 % yield), R_f 0.7 ($CHCl_3$-acetone, 4:1), mp 201-202 ^{o}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 8, recrystallized from acetone–methanol (1:1), 15 mg (0.0008 % yield), R_f 0.4 ($CHCl_3$-acetone, 4:1), mp 215-216 ^{o}C.UV λmax (MeOH): 215 nm (log ε 5.3).IR υmax (KBr):3451, 3260, 2955, 2845, 1737, 1700, 1601, 1390, 1310, 1280, 1199, 1087, 1005 cm^{-1} .^{1}H–NMR (MeOD):δ 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 $[M]^+$ ($C_{42}H_{70}O_{13}$) (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 **9**, recrystallized from methanol; 104 mg (0.0059 % yield), R_f 0.7, toluene: ethyl formate: formic acid, 5:4:1) mp 270-272 ^{o}C. FAB MS m/z (rel. int.): 576$[M]^+$ ($C_{35}H_{60}O_6$), (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).

Trixyloside (10)

Elution of the column with chloroform –methanol (4:1) furnished a lustrous colourless crystalline compound **10,** recrystallized from methanol; 10 mg (0.005% yield), R_f 0.6 ($CHCl_3$-MeOH, 1:1), mp 223-225 °C. IR υmax (KBr):3450, 3360, 3294, 2935, 2850, 1460, 1375, 1329, 1092, 1023, 873 cm^{-1} .^{1}H–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″). ^{13}C 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 $[M]^+$ ($C_{15}H_{26}O_{13}$) (6.2).

Hydroxynapthoquinone (11)

Elution of the column with chloroform –methanol (7:3) yielded a pale yellow mass of **11**, recrystallized from ($CHCl_3$-MeOH, 1:1), 20 mg (0.005% yield), R_f 0.83, EtOAc: MeOH: H_2O : HOAc, 5:3:1:1), mp 258-260 °C. IR υ_{max} (KBr) : 3200, 1719, 1704, 1620, 1580, 1500, 1446, 1338, 195, 1112, 1055, 922, 833, cm^{-1} .^{1}H 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).^{13} C NMR (DMSO-d_6): 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 $[M]^+$ ($C_6H_{10}O_3$) (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–oic acid, n-triacontane, n-henetriacontane, n-tetratriacontane, n-tetracontane, urs–12–en–18–βH–3α–ol, lanast–5–en–3β– ol–3β –D–glucopyranoside, Lanastan–3β–yl–glucuronopyranosyl–(6′→1″)–glucuronopyranoside, 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 diglucuroside (8) was obtained as colourless amorphous powder from chloroform–methanol (19:1) eluant. It gave positive tests of triterpenie glycosides. Its IR spectrum exhibited absorption bands for hydroxylic (3451 cm^{-1}), carboxlic (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 $C_{42}H_{70}O_{13}$ of a triterpenic diglycoside. It indicated eight double bond equivalents; four of them were adjusted in the tetracyclic carbon framework and the remaining four in the glucuronopyroside moieties. The ^{1}H 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″ anomeric protons. A four-carbinol 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, H–5″ and, H–3′ and H–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 ^{1}H- ^{1}H COSY spectrum showed correlation on of H-3 with H_2-2, H_2-1, H_3-28, H-1′; H-25 with H_2-24 and H_3-27, and H_3-21; H-20 with H-17, H-21 and H_2-22. Acid hydrolysis of **8** yielded glucuronic acid (TLC comparable). The ^{13}C NMR spectrum of **8** showed important carbon signals for carboxylic (δ 184.1), ester (δ 171.6), anomeric (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 diglucuronoside.

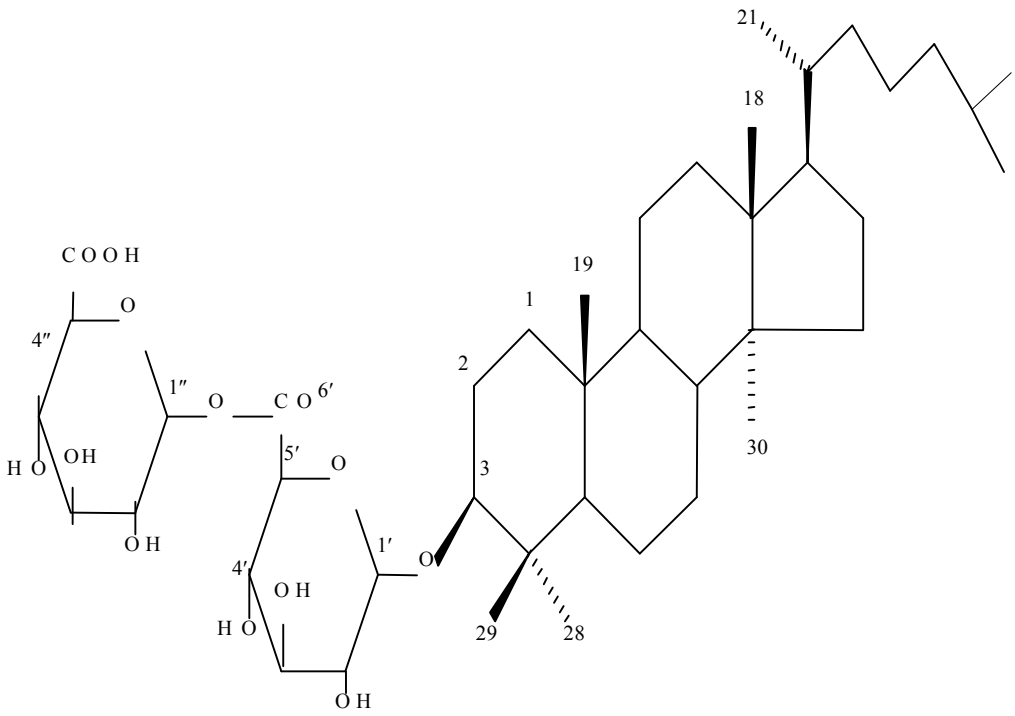
ACKNOWLEDGMENT

The authors are thankful to the Head, SAIF, Central Drug Research Institute Lucknow, for recording mass spectra of the compounds.

REFERENCES

1. Jurjani MI, Zakheera-Khwazaram-Shahi, Munshi Nawal Kishore. Lucknow Urdu translation. 1878;145.
2. Majoosi AIA, Kamilussanah, Munshi Nawal Kishore. Lucknow Urdu translation by Kantoori 1889; 145.
3. Nadkarnis AK. Popular Prakashan, Bombay. Nandkarni's Indian Material Medica 1976; 1: 1031-1035.
4. Lee J, Watson RR. Pomegranate: a role in health promotion and AIDS? In: Watson RR (Ed.), Nutrients and Foods in AIDS. *CRC* Press, Boca Raton, 1998; 179-192.
5. M.A. Jafri, M. Aslam, K. Javed, S. Singh. Effects of *Punica granatum* Linn. Flowers) on blood glucose level in normal and alloxan-induced diabetic rats. Journal of Ethnopharmacology 2000; 70: 309-314.
6. Huang TH, HPeng G, Kota BP, Li GQ, Yamahara J, Roufogalis BD, Li Y. Antidiabetic action of *Punica granatum* flower

extract: activation of PPAR-gamma and identification of an active component. Toxicology and Applied Pharmacology 2005b; 260: 160-169.
7. Kim MM, Kim S.Composition for improving oral hygiene containing *Punica granatum* L. extract. Korean Patent 2002; KR 2002066042.
8. Li Y and Lazar MA. *Punica granatum* flower extract, a potent alpha-glucosidase inhibitor, improves postprandial hyperglycemia in Zucker-diabetic fatty rats. Journal of Ethnopharmacology 2005; 99(2): 239-244.
9. Huang TH, HYeng Q, Harada M, Li GQ, Yamahara J, Roufogalis BD,Li Y.Pomegranate flower extract diminishes cardiac fibrosis in Zucker diabetic fatty rats: modulation of cardiac endothelin-1 and nuclear factor-kappa B pathways. Journal of Cardiovascular Pharmacology 2005c; 46: 856-862.
10. Gurpreet Kaur, Zoobi Jabbar, Mohammad Athar, M.S Alam.*Punica granatum* (pomegranate) flower extract possesses potent antioxidant activity and abrogates Fe-NTA induced hepatotoxicity in mice.Food and Chemical toxicology 2006; 44: 984-993.



Lanastan–3β–yl–glucuronopyranosyl–(6′→1″)–glucuronopyranoside **(8)**

Source of support: Nil, Conflict of interest: None Declared