

Phytochemical investigation of the flowers of *Punica granatum*

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Received 31 January 2005; accepted (revised) 20 July 2005

Phytochemical investigation of the flowers of *Punica granatum* L. (Punicaceae) has been carried out which leads to the isolation of four new phytoconstituents identified as 4'-hydroxy non-6'-en-yl benzoate **1**, 13-(15,19,19-trimethylcyclohex-16-en)-yl-6,10-dimethyl-tridec-10-en-3 β ,4 β ,6 α ,13 β -tetraol **2**, 3,7,8,4'-tetrahydroxy-3'-myrt-8-en-yl flavone **3** and a pentaglycoside named granatumoside **4**. The structures of these compounds have been established on the basis of spectral and chemical studies.

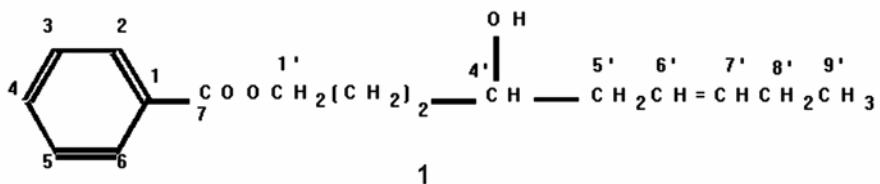
Keywords: *Punica granatum*, Phytochemical investigation, flavone, tetraol, flavone, granatumoside

IPC: Int.Cl.⁸ C07D

Punica granatum L. (Punicaceae), known as pomegranate, is a deciduous small tree, up to 8 m in height with attractive reddish scarlet edible fruits. The species originated in Iran, Afghanistan and Baluchistan, found wild in the warm valleys of the Himalayas and is cultivated throughout India¹. The dried flowers, known as Gulnar, are efficacious to treat haematuria, haemoptysis, diarrhoea, dysentery, nasal hemorrhage², and in Unani literature as a remedy for diabetes^{3,4}. Flower juice is recommended as a gargle for sore throat, in leucorrhoea, hemorrhages and ulcers of the uterus and rectum^{2,5}. The root bark and stem bark of the plant are astringent and used as an anthelmintic especially against tapeworms. Fruit rind is valued as an astringent in diarrhoea and dysentery. The powdered flower buds are useful in bronchitis. The seeds are reputed as stomachic and the pulp as cardiac and stomachic. The green leaf paste is applied to relieve conjunctivitis^{1,5}. The aqueous-ethanolic (50%, v/v) extract of the flowers leads to significant blood glucose lowering effect in normal, glucose-fed hyperglycemic and alloxan-induced diabetic rats⁶. Pelletierines, tannins, β -sitosterol, ursolic acid and aliphatic acids have been reported from different parts of the plant⁷. This paper describes the isolation and characterization of phytoconstituents from the ethanolic extract of the flowers of *P. granatum*.

Results and Discussion

Compound **1**, designated as punicanyl benzoate, was obtained as pale yellow crystalline mass from petroleum ether-chloroform (1:1) eluant. It decolourized bromine water indicating unsaturated nature of the compound. Its IR spectrum demonstrated the presence of the hydroxyl group (3413 cm^{-1}), ester group (1725 cm^{-1}), unsaturation (1631 cm^{-1}) and aromatic ring (1550, 1094 cm^{-1}). The mass spectrum of **1** exhibited a molecular ion peak at m/z 262 correspond to a benzoic acid ester $\text{C}_{16}\text{H}_{22}\text{O}_3$. The prominent ion peaks generated at m/z 77 [C_6H_5]⁺, 105 [$\text{C}_6\text{H}_5\text{CO}$]⁺, 121 [$\text{C}_6\text{H}_5\text{COO}$]⁺, 157 [M-105]⁺ and 141 [M-121]⁺ indicate the involvement of benzoic acid in the ester formation. The ion fragments at m/z 99 [$\text{C}_3\text{'-C}_4\text{' fission}$]⁺, 69 [$\text{C}_4\text{'-C}_5\text{' fission}$]⁺ and 55 [$\text{C}_5\text{'-C}_6\text{' fission}$]⁺ suggested the existence of the hydroxyl group at C-4' and olefinic linkage at Δ^6 . The ¹H NMR spectrum of **1** displayed a two-proton broad signal at δ 7.30, a two-proton broad multiplet at δ 7.21 and a one-proton multiplet at δ 6.63, all assigned to aromatic protons. Two one-proton multiplets at δ 5.31 and 5.13 were accounted to vinylic H-6' and H-7', respectively. Two one-proton doublets at δ 4.03 (J = 6.3 Hz) and 4.01 (J = 6.3 Hz) and a one-proton broad multiplet at δ 3.86 with $w_{1/2}$ 15.7 Hz were accounted to oxygenated H-1' methylene and H-4' carbinol



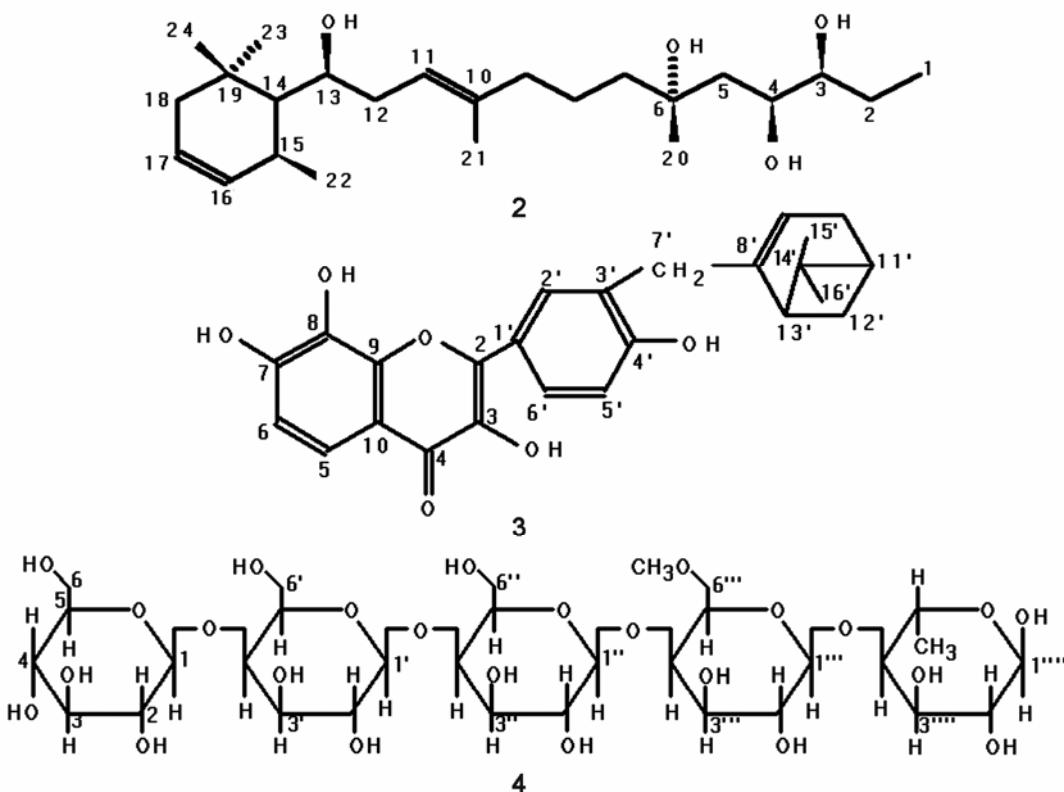
protons, respectively. A broad signal at δ 2.43 integrating for four protons was ascribed to C-5' and C-8' methylene protons adjacent to the vinylic linkage. A three-proton triplet at δ 0.83 ($J = 6.1$ Hz) was associated with C-9' primary methyl protons. The ^{13}C NMR spectrum of **1** showed signals for ester carbon at δ 171.3 (C-7), aromatic and vinylic carbons between δ 136.3- 115.6, carbinol carbon at δ 64.2 (C-1') and methyl carbon at δ 19.1 (C-9'). On the basis of foregoing spectral studies, the structure of **1** has been established as 4'-hydroxy non-6'-en-yl benzoate. This is an unreported ester from a natural or synthetic source.

Compound **2**, named granatumol, was obtained as pale yellow crystals from chloroform-methanol (9:1). It decolorized bromine water and showed IR absorption bands for hydroxyl group (3390, 3365 cm^{-1}) and unsaturation (1631 cm^{-1}). Its mass spectrum displayed a molecular peak at m/z 396 corresponding to a tetrahydroxy norsesterterpene $\text{C}_{24}\text{H}_{44}\text{O}_4$. It indicated three double bond equivalents, one in the cyclic ring and the remaining two in two vinylic linkages. Significant ion fragments at m/z 123 [$\text{C}_{13}\text{-C}_{14}$ fission] $^+$, 153 [$\text{C}_{13}\text{-C}_{12}$ fission] $^+$, 228 [M-228-Me] $^+$, 167 [$\text{C}_{12}\text{-C}_{11}$ fission] $^+$ and 213 [M-167-Me] $^+$ suggested the location of a cyclohexenyl and a hydroxyl group at C-13. The ion peaks at m/z 207, 189 [$\text{C}_9\text{-C}_{10}$ fission] $^+$, 171 [$189\text{-H}_2\text{O}$] $^+$, 175 [$\text{C}_8\text{-C}_9$ fission] $^+$, 161 [$\text{C}_7\text{-C}_8$ fission] $^+$, 249, 147 [$\text{C}_6\text{-C}_7$ fission] $^+$, 257 [$\text{C}_5\text{-C}_6$ fission-2 $\times \text{H}_2\text{O}$] $^+$, 147 [$\text{M-249-H}_2\text{O}$] $^+$ and 367 [$\text{M-C}_2\text{H}_5$] $^+$ indicated the existence of a vinylic linkage at C-10 and three hydroxyl groups at C-6, C-4 and C-3.

The ^1H NMR spectrum of **2** exhibited two one-proton doublets at δ 6.69 ($J = 9.9$ Hz) and 6.64 ($J = 9.9$ Hz) assigned to vinylic H-16 and H-17, respectively. A one-proton multiplet at δ 5.30 was attributed to vinylic H-11. Two broad multiplets at δ 3.65 (1H) and 3.37 (1H) with half width of 15.5 Hz and 16.1 Hz, respectively were ascribed correspondingly to carbinol H-13 α , H-3 α and H-4 α protons. Two one-proton doublets at δ 2.09 ($J = 7.43$ Hz) and 2.06 ($J = 16.4$ Hz) were associated with C-18 methylene protons adjacent to Δ^{14} -vinylic linkage. A

three-proton broad signal at δ 2.47 was due to C-21 methyl functionality attached to C-10 vinylic carbon. A six-proton broad signal at δ 1.21 was assigned to C-20 and C-22 methyl protons. A three-proton triplet at δ 0.83 ($J = 6.1$ Hz) was associated with C-1 primary methyl protons. The C-23 and C-24 methyl protons appeared at δ 1.19. The remaining methylene protons resonated at δ 2.23 ($\text{H}_2\text{-9}$), 1.98 ($\text{H}_2\text{-12}$) and 1.44 ($\text{H}_2\text{-5}$). The ^{13}C NMR spectrum of **2** showed signals for vinylic carbons at δ 144.3 (C-10), 129.1 (C-11), 118.3 (C-16) and 115.1 (C-17). The carbinol carbons appeared at δ 67.9 (C-3), 73.1 (C-4), 81.1 (C-6) and 76.2 (C-13). The ^1H and ^{13}C NMR values were compared with other sesterterpene molecules⁸. On the basis of foregoing account, the structure of **2** has been established as 13-(15,19,19-trimethylcyclohex-16-en)-yl-6,10-dimethyl-tridec-10-en-3 β ,4 β ,6 α ,13 β -tetraol.

Compound **3**, named punicaflavone, was obtained as a light red coloured crystalline mass from the chloroform-methanol (1:1) eluant. It responded positively to flavonoid tests and displayed UV absorption maxima at 271 and 325 nm for a flavone. The UV spectra with different shift reagents suggested the presence of the hydroxyl groups at C-3, C-7 and C-4' (ref. 9). Its IR spectrum showed absorption bands for hydroxyl (3439 cm^{-1}) and carbonyl (1680 cm^{-1}). The mass spectrum of **3** exhibited a molecular ion peak at m/z 420 consistent with the molecular formula of a bicyclic monoterpene substituted flavone, $\text{C}_{25}\text{H}_{24}\text{O}_6$. The important ion fragments arose at m/z 106 [$\text{C}_{4,10}\text{-C}_{9,0}$ fission] $^+$, and 136, 284 [$\text{C}_{3,4}\text{-C}_{9,0}$ fission] $^+$ suggesting the existence of two hydroxyl groups in ring A. The prominent ion peaks at m/z 227 [$\text{C}_{2-}\text{C}_{1}'$ fission] $^+$, 212 [217-Me] $^+$, 135, 285 [$\text{C}_{3'}\text{-C}_{7}'$ fission] $^+$, 121 [$\text{C}_{7'}\text{-C}_{8}'$ fission] $^+$ and 106 [121-Me] $^+$ indicated location of another hydroxyl group and the monoterpene moiety in ring B. The intense ion peaks at m/z 83 [$\text{C}_{10',11'}\text{-C}_{8',13'}$ fission] $^+$ and 68 [83-Me] $^+$ supported the presence of the tetracyclic ring in a myrtenol-type monoterpene. The ^1H NMR spectrum of **3** displayed three one-proton *ortho*-coupled doublets at δ 7.68 ($J = 8.55$ Hz), 6.62 ($J = 8.55$ Hz) and 6.81 ($J = 8.51$ Hz), assigned to H-5, H-6 and H-5', respectively. A one-proton *meta*-



coupled doublet at δ 7.43 ($J = 3.0$ Hz) and a one-proton multiplet at δ 7.38 were accounted to H-2' and H-6', respectively. Another one-proton multiplet at δ 5.37 was ascribed to vinylic H-9'. A six-proton broad signal at δ 1.21 was associated with C_{15'} and C_{16'} tertiary methyl functionalities. The ¹³C NMR spectrum of 3 exhibited carbon signals for the flavone moiety between δ 176.6-113.8. The vinylic carbons resonated at δ 139.2 (C-8') and 130.8 (C-9'). The upfield carbon signals in the range δ 33.6-16.3 were assigned to the remaining monoterpenic carbons. The absence of any ¹H NMR signal between δ 5.37- 2.48 and ¹³C NMR signal between δ 113.8- 37.3 ruled out the existence of any hydroxymethylene signal in the molecule and supported the carbon linkage of the monoterpenic moiety with the flavone. The ¹H-¹H-COSY spectrum showed correlation of H₂-7' with H-2', H-5', H-9', H-10', H-13' and H-12'. Based on these evidences, the structure of 3 has been established as 3,7,8,4'- tetrahydroxy- 3'- myrt-8- enyl flavone.

Compound 4, a pentaglycoside named granatumoside, was obtained as colourless crystals from methanol eluents. It responded to carbohydrate tests positively and exhibited IR characteristic bands for

hydroxyl groups (3550, 3430, 3360, and 3240 cm^{-1}). The ¹³C NMR spectrum of 4 displayed five anomeric carbon signals suggesting the pentaglycoside nature of the molecule. The ¹H NMR of 4 showed anomeric proton signals at δ 5.33 (H-1''), 5.21 (H-1), 5.06 (H-1', H-1''') and 4.90 (H-1'''). Four oxygenated methylene proton signals appeared at δ 3.26 ($J = 8.17$ Hz, H₂-6), 3.22 ($J = 8.92$ Hz, H₂-6', H₂-6'') and 3.18 ($J = 8.92$ Hz, H₂-6''). A three-proton doublet at δ 1.09 ($J = 7.1$ Hz) was ascribed to C-6'' methyl protons. A broad signal corresponding to at δ 3.52 was attributed to H-4' and methoxy protons. The remaining methine protons resonated in the range δ 4.81- 3.35. The ¹³C NMR spectrum of 4 displayed five anomeric carbon signals at δ 106.6, 104.0, 99.7, 96.98 and 102.7. Two carbon signals at δ 53.5 and 15.3 were accounted to methoxy and C-6'' methyl protons. The remaining hydroxymethylene and hydroxymethine carbon signals appeared in the range δ 78.3- 62.1. Hydrolysis of compound 4 yielded mainly D-glucose and rhamnose. Based on these evidences, the structure of 4 has been established as β -glucopyranosyl-(1 \rightarrow 4')- β -glucopyranosyl-(1' \rightarrow 4'')- β -glucopyranosyl-(1'' \rightarrow 4''')- β -6'''-methoxyglucopyranosyl-(1''' \rightarrow 4''')-rhamnopyranose.

Experimental Section

Melting points are uncorrected. ^1H and ^{13}C NMR spectra were recorded in CHCl_3 on Bruker-DRX-300 MHz and 100 MHz spectrometer respectively, using TMS as an internal standard, mass spectra on a Jeol 300 (EI/CI) system; UV spectra on a Beckman DU-64 model; and IR Spectra on a Hitachi model-270. Purity of the compounds was checked by TLC over silica gel G (Merck). The spots were visualized by exposure to UV radiation, iodine vapours and finally by spray reagent.

Plant material

The flowers of *Punica granatum* were procured from the local market of Khari Baoli, Delhi and identified by Dr. M. P. Sharma, Reader and Taxonomist, Department of Botany, Faculty of Science, Jamia Hamdard. A voucher specimen of the drug is preserved in the Phytochemistry Research Laboratory, Faculty of Pharmacy, Hamdard University.

Extraction and Isolation

The pulverized flowers (1.5 kg) were extracted exhaustively in a Soxhlet apparatus with EtOH (95%). The combined extracts were dried under reduced pressure to secure a viscous dark brown residue (200 g). The dried alcoholic extract was dissolved in minimum amount of MeOH and adsorbed on silica gel. The slurry was air-dried and chromatographed over silica gel loaded in petroleum ether (b.p. 60–80°C). The column was eluted with petroleum ether, petroleum ether- CHCl_3 (9:1, 3:1, 1:1, 1:3), CHCl_3 , $\text{CHCl}_3\text{-MeOH}$ (99:1, 98:2, 95:5, 3:1, 1:1, 1:3), and MeOH in order of increasing polarity to isolate the following compounds:

Punicanyl benzoate 1: Elution of the column with petroleum ether- CHCl_3 (1:1) afforded pale yellow coloured crystals of **1**, recrystallized from $\text{CHCl}_3\text{-MeOH}$ (1:1), 112 mg (0.007% yield), m.p. 110–111°C; IR: 3413, 2924, 2825, 1725, 1631, 1550, 1402, 1094, 794 cm^{-1} ; ^1H NMR (DMSO- d_6): δ 7.30 (2H, br s, H-2, H-6), 7.21 (2H, m, H-3, H-5), 6.63 (1H, m, H-4), 5.31 (1H, m, H-6'), 5.13 (1H, m, H-7'), 4.03 (1H, d, J = 6.3 Hz, H₂-1'a), 4.01 (1H, d, J = 6.3 Hz, H₂-1'b), 3.86 (1H, m, $w_{1/2}$ = 15.7 Hz, H-4'); 2.43 (4H, s, H₂-5', H₂-8'), 2.01 (2H, m, H₂-2'), 1.23 (2H, br s, H₂-3'), 0.83 (3H, t, J = 6.1 Hz, Me-9'); EIMS m/z (rel. int.): 262 [M]⁺ ($\text{C}_{16}\text{H}_{22}\text{O}_3$) (1:1), 157 (8.5), 141 (3.5), 121 (26.3), 109 (30.9), 105 (28.7), 99 (12.1), 81 (45.11),

77 (10.3), 69 (84.5), 55 (100); ^{13}C NMR (DMSO- d_6): 136.3 (C-1), 129.7 (C-2), 121.9 (C-3), 115.6 (C-4), 118.9 (C-5), 128.6 (C-6), 171.3 (C-7), 64.2 (C-1'), 28.9 (C-2'), 28.9 (C-3'), 81.1 (C-4'), 33.6 (C-5'), 23.6 (C-8'), 123.7 (C-6'), 124.3 (C-7'), 19.1 (C-9').

Granatumol 2: Elution of the column with CHCl_3 (9:1) yielded pale yellow crystals of **2**, recrystallized from methanol, 920 mg (0.061% yield, m.p. 121–122°C; IR: 3390, 3365, 2923, 2853, 1631, 1453, 1377, 1258, 1109 cm^{-1} ; ^1H NMR (DMSO- d_6): δ 6.99 (1H, d, J = 9.9 Hz, H-16), 6.64 (1H, d, J = 9.9 Hz, H-17), 5.30 (1H, m, H-11), 3.65 (2H, m, $w_{1/2}$ = 15.5 Hz, H-13 α), 3.37 (2H, m, $w_{1/2}$ = 16.1 Hz, H-3 α , H-4 α), 2.77 (2H, d, J = 6.9 Hz, H-15 α), 2.47 (3H, br s, Me-21), 2.23 (2H, m, H₂-9), 2.09 (1H, d, J = 7.43 Hz, H-18a), 2.06 (1H, d, J = 16.4 Hz, H-18b), 1.98 (2H, br s, H₂-12), 1.44 (2H, m, H₂-5), 1.22 (6H, br s, H₂-8, H₂-7, H₂-2), 1.21 (6H, br s, Me-20, Me-22), 1.19 (6H, br s, Me-23, Me-24), 0.83 (3H, t, J = 6.1 Hz, Me-1); EIMS m/z (rel. int.): 396 [M]⁺ ($\text{C}_{24}\text{H}_{44}\text{O}_4$) (12.3), 381 (5.1), 367 (6.3), 284 (11.3), 280 (21.1), 264 (21.7), 257 (22.3), 249 (100), 228 (9.6), 213 (14.3), 207 (21.6), 189 (17.6), 184 (14.2), 175 (11.6), 171 (13.2), 167 (11.3), 161 (13.9), 153 (32.6), 147 (20.9), 135 (33.1), 129 (26.5), 123 (92.6), 109 (30.3), 95 (32.6), 85 (11.9); ^{13}C NMR (DMSO- d_6): δ 14.6 (C-1), 24.9 (C-2), 67.9 (C-3), 73.1 (C-4), 26.9 (C-5), 81.1 (C-6), 18.6 (C-7), 26.6 (C-8), 28.6 (C-9), 144.3 (C-10), 129.1 (C-11), 29.1 (C-12), 76.2 (C-13), 34.1 (C-14), 31.3 (C-15), 118.3 (C-16), 115.1 (C-17), 29.0 (C-18), 49.6 (C-19), 24.7 (C-20), 22.1 (C-21), 24.7 (C-22), 22.13 (C-23), 26.7 (C-24).

Punicaflavone 3: Elution of the column with $\text{CHCl}_3\text{-MeOH}$ (1:1) furnished light red coloured crystals of **3**, recrystallized from MeOH , 35 mg (0.002% yield), m.p. 294–95°C, UV λ_{max} (MeOH) 271, 325 nm ($\log \epsilon$ 7.1, 3.2); ($\text{MeOH} + \text{NaOMe}$) 270, 370 nm; ($\text{MeOH} + \text{NaOAc}$) 280, 326 nm; ($\text{MeOH} + \text{NaOAc} + \text{H}_3\text{BO}_3$) 272, 330 nm; ($\text{MeOH} + \text{AlCl}_3$) 270, 380 nm; IR (KBr): 3439, 2922, 2851, 1680, 1620, 1555, 1408, 1098, 794 cm^{-1} ; ^1H NMR (DMSO- d_6): δ 7.68 (1H, d, J = 8.55 Hz, H-5), 7.43 (1H, d, J = 3.0 Hz, H-2'), 7.38 (1H, m, H-6'), 6.81 (1H, d, J = 8.51 Hz, H-5'), 6.62 (1H, d, J = 8.55 Hz, H-6), 5.37 (1H, m, H-9'), 2.48 (2H, br s, H₂-7'), 2.26 (1H, dd, J = 7.1 Hz, 4.4 Hz, H-13'), 1.97 (1H, m, H-11'), 1.60 (2H, m, H₂-10'), 1.21 (6H, br s, Me-15', Me-16'), 0.83 (2H, dd, J = 6.1 Hz, H₂-12'); EIMS m/z (rel. int.): 420 [M]⁺ ($\text{C}_{25}\text{H}_{24}\text{O}_6$) (6.1), 285 (4.3), 284 (4.8), 256 (5.5), 227 (6.3), 212 (7.5), 155 (34.3), 136 (14.5), 135 (12.6),

121 (15.3), 108 (24.7), 106 (12.6), 83 (48.3), 68 (74.1), 55 (100); ^{13}C NMR (DMSO- d_6): δ 146.3 (C-2), 138.2 (C-3), 176.6 (C-4), 127.0 (C-5), 113.8 (C-6), 159.1 (C-7), 130.8 (C-8), 145.1 (C-9), 113.9 (C-10), 125.2 (C-1'), 113.8 (C-2'), 128.6 (C-3'), 151.3 (C-4'), 114.3 (C-5'), 127.0 (C-6'), 37.3 (C-7'), 139.2 (C-8'), 130.8 (C-9'), 29.1 (C-10'), 37.3 (C-11'), 26.0 (C-12'), 31.3 (C-13'), 33.6 (C-14'), 21.6 (C-15'), 16.3 (C-16').

Granatumoside 4: Elution of the column with MeOH yielded colourless crystals of **4**, recrystallized from EtOH, 750 mg (0.05% yield); m.p. 295–96°C; IR: 3550, 3430, 3360, 3240, 2957, 2848, 1640, 1457, 1393, 1239, 1064, 1006, 922, 774 cm^{-1} ; ^1H NMR (DMSO- d_6) δ 5.33 (1H, br s, H-1''), 5.21 (1H, d, J = 5.4 Hz, H-1), 5.06 (2H, br s, H-1', H-1'''), 4.90 (1H, br s, H-1''''), 4.81 (3H, m, H-4', H-4'', H-4'''), 4.53 (2H, m, H-4, H-4'''), 3.80 (1H, d, J = 7.06 Hz, H-2), 3.76 (1H, d, J = 7.06 Hz, H-2'), 3.73 (1H, d, J = 7.06 Hz, H-2''), 3.69 (1H, d, J = 10.04 Hz, H-2'''), 3.64 (1H, d, J = 1.04 Hz, H-2''''), 3.52 (4H, br s, H-4', OMe), 3.38 (5H, m, H-3, H-3', H-3'', H-3'''), 3.36 (2H, m, H-5, H-3'''), 3.35 (2H, m, H-5', H-5''), 3.33 (2H, m, H-5'', H-5'''), 3.26 (2H, d, J = 8.17 Hz, H₂-6), 3.22 (4H, d, J = 8.92 Hz, H₂-6', H₂-6''), 3.18 (2H, d, J = 8.92 Hz, H₂-6'''), 1.09 (3H, d, J = 7.1 Hz, Me-6'''); ^{13}C NMR (DMSO- d_6): δ 106.6 (C-1), 72.68 (C-

2), 69.4 (C-3), 76.8 (C-4), 82.84 (C-5), 63.5 (C-6), 104.0 (C-1'), 72.2 (C-2'), 69.0 (C-3'), 75.89 (C-4'), 81.63 (C-5'), 63.0 (C-6'), 99.7 (C-1''), 72.0 (C-2''), 68.4 (C-3''), 75.46 (C-4''), 80.48 (C-5''), 62.6 (C-6''), 96.98 (C-1'''), 70.5 (C-2'''), 64.3 (C-3'''), 74.99 (C-4'''), 78.3 (C-5'''), 62.1 (C-6'''), 53.5 (OMe), 102.7 (C-1''''), 70.35 (C-2''''), 64.8 (C-3''''), 73.58 (C-4''''), 77.3 (C-5''''), 15.3 (C-6'''').

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