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# NEW FLAVONOIDS FROM *Punica granatum* FLOWERS

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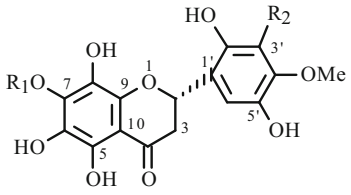
*Two new flavonoids were isolated from the flowers of* Punica granatum *Linn. (Punicaceae) along with the known compounds ellagic acid, gallic acid, sucrose, and gallic acid glycoside. The structures of the new flavonoids have been characterized as 5,6,7,8,2′,3′,5′-heptahydroxy-4′-methoxyflavanone (punicaflavanol) and 5,6,7,8,2′,5′-hexahydroxy-4′-methoxyflavanone-7-β-D-xylopyranoside (granatumflavanyl xyloside) on the basis of spectral data analyses and chemical reactions.*

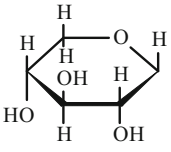
**Keywords**: *Punica granatum*, Punicaceae, flavonoids, structure elucidation.

*Punica granatum* Linn. (Punicaceae) is a large deciduous shrub or small tree, up to 10 m in height, that grows well in the warm valleys and hills of the Himalayas and is cultivated throughout India [1]. In traditional Ayurvedic medicine, all parts of *P. granatum* are used for the treatment of various disorders [2]. The flowers of *P. granatum* are used in folk medicine for the treatment of bronchitis, diarrhea, dysentery, ulcers, hepatic damage, sore eyes, and diabetes [3, 4]. Phytochemical investigation of *P. granatum* flowers showed the presence of various phenolic compounds, pentacyclic triterpenes, and sterols [5, 6]. The present paper describes the isolation and characterization of two new flavonoids (**1**, **2**) from the flowers of *P. granatum* along with the known compounds ellagic acid (**3**), gallic acid (**4**), sucrose (**5**), and gallic acid glucoside (**6**).




**1, 2**

**1:** $R_1 = H, R_2 = OH$

**2:** $R_1 =$  , $R_2 = H$

Compound **1**, named punicaflavanol, was obtained as pale yellow crystals from chloroform–methanol (19:1) eluats. It responded positively to the tests of flavonoids. The UV spectrum of **1** displayed absorption maxima at 281 and 363 nm, indicating the flavanol type nature of the molecule. It showed shift of bands with sodium methoxide, suggesting presence of the free hydroxyl groups in the molecule. The shift of band II to 316 nm on addition of sodium acetate suggested the presence of the 7-hydroxyl group with the 5-hydroxyl group. The shift of band II to 293 nm on addition of sodium acetate and boric acid indicated the location of A-ring *o*-dihydroxyl groups. The appearance of band II at 301 nm on addition of $AlCl_3$ indicated the existence of the 5-hydroxyl group in the flavanone. The shift of band II to 293 nm on addition of $AlCl_3$+HCl supported the A-ring *o*-dihydroxy groups [7–9]. Its IR spectrum displayed the characteristic absorption bands for the hydroxyl groups (3561, 3509, 3136 $cm^{-1}$) and the carbonyl group (1696 $cm^{-1}$). The mass spectrum of **1** exhibited a molecular ion peak at *m/z* 366, pointing to the molecular formula $C_{16}H_{14}O_{10}$. The retro-Diels-Alder fragmentation of ring C yielded the diagnostic peaks at *m/z* 184 and 182, supporting the presence of four hydroxyl groups in ring A and three hydroxyl and one methoxyl groups in ring B, respectively.

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TABLE 1. $^{13}C$ NMR Values of Punicaflavanol (**1**) and Granatumflavanyl Xyloside (**2**) (DMSO-$d_6$, δ, ppm)

| **1** | | **2** | |
|---|---|---|---|
| C atom | $\delta_C$ | C atom | $\delta_C$ |
| 2 | 78.81 | 2 | 78.82 |
| 3 | 36.96 | 3 | 37.01 |
| 4 | 193.06 | 4 | 193.14 |
| 5 | 160.14 | 5 | 160.21 |
| 6 | 145.82 | 6 | 140.40 |
| 7 | 172.53 | 7 | 172.63 |
| 8 | 138.44 | 8 | 139.65 |
| 9 | 158.73 | 9 | 159.21 |
| 10 | 108.13 | 10 | 108.16 |
| 1′ | 114.97 | 1′ | 138.52 |
| 2′ | 148.05 | 2′ | 143.65 |
| 3′ | 140.37 | 3′ | 115.02 |
| 4′ | 149.67 | 4′ | 149.73 |
| 5′ | 143.60 | 5′ | 148.16 |
| 6′ | 112.97 | 6′ | 121.28 |
| OMe | 51.98 | 1″ | 101.23 |
| | | 2″ | 72.61 |
| | | 3″ | 73.54 |
| | | 4″ | 70.16 |
| | | 5″ | 61.17 |
| | | OMe | 51.98 |

The generation of important ion fragments at *m/z* 140 $[C_{10,4} - C_{9,O}$ fission$]^+$, 188 $[C_{3,4} - C_{O,2}$ fission$]^+$, and 155, 211 $[C_2 - C_1'$ fission$]^+$ also supported the substitution pattern. The ion fragments at *m/z* 338 $[366 - CO]^+$, 323 $[338 - Me]^+$, and 351 $[M - Me]^+$ arose from the removal of the carbonyl group and the methyl group from the molecular ion peak [9]. The $^1H$ NMR spectrum of **1** exhibited a one-proton broad signal at δ 7.30 ascribed to H-6′, suggesting the 2′,3′,4′,5′-tetraoxygenated pattern of ring B [10, 11]. An ABX system with resonances as double doublets at δ 5.32 (J = 2.5, 13.1 Hz), 2.97 (J =2.5, 13.1 Hz) and 2.81 (J = 13.1, 17.2 Hz) was characteristic of H-2*β*, H-3ax, and H-3eq of the flavanone moiety [7]. A three-proton broad signal at δ 3.62 was attributed to the methoxyl protons attached at C-4′. The $^{13}C$ NMR spectrum of **1** (Table 1) displayed important signals for the C-4 carbonyl carbon (δ 193.06) and the flavanone carbon between δ 172.53–36.96. The signals at δ 51.98 confirmed the existence of one methoxyl group in the molecule. The $^1H$ NMR and $^{13}C$ NMR values were compared with a flavanone molecule like hesperetin [9]. The DEPT spectrum of **1** showed the presence of one methoxyl, one methylene, two methine, and twelve quaternary carbons. In the HMBC spectrum of **1**, H-2 correlated to C-1′, C-2′, C-6′, and C-4; and H-6′ interacted with C-1′, C-2′, and C-2. In the CD spectrum, a negative cotton effect at 301 nm (Δ – 11.2) and a positive cotton effect at 332 (Δ + 6.41) indicated the *S*-configuration at C-2 [12, 13]. On the basis of spectral data analysis, the structure of **1** has been elucidated as 5,6,7,8,2′,3′,5′-heptahydroxy-4′-methoxyflavanone.

Compound **2**, named granatumflavanyl xyloside, was obtained as light yellow crystals from chloroform–methanol (23:2) eluats. It responded positively to the tests of flavonoids. The UV spectrum of **2** displayed absorption maxima at 277 and 363 nm, indicating the flavanol type nature of the molecule. It showed the shift of bands with sodium methoxide, suggesting the free hydroxyl group in the molecule. The decreasing intensity of band II with time on addition of sodium methoxide indicated A-ring dihydroxy groups. A small shift of band II (+ 22 nm) on addition of sodium acetate supported the location of the sugar moiety at C-7. A positive shift of + 12 nm in band II on addition of sodium acetate and boric acid indicated A-ring *o*-di-hydroxy groups. A shift of + 14 nm in band II on addition of $AlCl_3$ suggested the A-ring-di-hydroxy group [7–9].

Its IR spectrum displayed the characteristic absorption band for the hydroxyl group (3373, 3160 $cm^{-1}$), carbonyl group (1700 $cm^{-1}$), and unsaturation (1593 $cm^{-1}$). The mass spectrum of **2** exhibited a molecular ion peak at *m/z* 482 pointing to the molecular formula of flavonoid glycoside $C_{21}H_{22}O_{13}$. The generation of ion peaks at *m/z* 133 $[C_5H_9O_4]^+$ and 149 $[C_5H_9O_5]^+$ indicated that a $C_5$-sugar unit was attached to the flavanone. The retro-Diels-Alder fragmentation of ring C yielded the diagnostic peaks at *m/z* 316 $[C_{12}H_{12}O_{10}]^+$ and 166 $[C_9H_{10}O_3]^+$, supporting the presence of four hydroxyl groups and a $C_5$ sugar unit in ring A and two hydroxyl and one methoxyl groups in ring B, respectively. The ion peaks arising at *m/z* 272 $[C_{10,4} - C_{9,O}$ fission$]^+$,

140 [272 – $C_5H_9O_4$]$^+$, and 122 [272 – $C_5H_{10}O_5$]$^+$ also suggested the location of the sugar moiety in ring A. The ion peaks formed at *m/z* 210 [M – 272]$^+$, 182 [$C_{9,O} - C_{3,4}$ fission], and 168 [$C_{9,O} - C_{2,3}$ fission] supported the existence of two hydroxyl and one methoxy groups in ring B [9]. The $^1$H NMR spectrum of **2** exhibited two one-proton broad signals at δ 7.45 and δ 7.29 ascribed to *p*-coupled H-3′ and H-6′, establishing the 2,4,5-trioxygenated ring B [10, 11]. An ABX system with double doublets at δ 5.31 (J = 2.7, 13.5 Hz), 2.97 (J =2.7, 13.5 Hz), and 2.81 (J = 13.5, 16.9 Hz) was characteristic of H-2*β*, H-3ax, and H-3eq of the flavanone moiety [7]. A one-proton doublet at δ 5.28 (J = 7.1 Hz) was ascribed to the anomeric proton H-1″. A one-proton doublet at δ 4.24 with coupling constant 7.2 Hz was ascribed to H-2″. Two one-proton doublet signals at δ 3.05 (J = 10.8 Hz) and δ 3.01 (J = 10.8 Hz) were attributed correspondingly to oxygenated methylene protons $H_2$-5″a and $H_2$-5″b, respectively. The remaining carbinol protons of the sugar units appeared as multiplets at δ 4.05 (1H) and δ 3.45 (1H). A three-proton broad signal at δ 3.62 was ascribed to methoxyl protons attached at C-4′. The $^{13}$C NMR spectrum of **2** displayed important signals for the C-4 carbonyl carbon (δ 193.14), flavanone carbons between δ 172.63–37.01, anomeric carbons at δ 101.23 (C-1′), and sugar carbons between δ 73.54–61.17. The DEPT spectrum of **2** showed the presence of one methoxyl, two methylene, seven methine, and eleven quaternary carbons. In the HMBC spectrum of **2**, H-2 correlated with C-1′, C-2′, C-6′, and C-4; H-3′ interacted with C-1′, C-2′, and C-4′; and H-1″ interacted with C-2″, C-3″, and C-7. In the CD spectrum, a negative cotton effect at 303 nm (Δ – 12.9) and a positive cotton effect at 335 (Δ + 7.15) indicated the *S*-configuration at C-2 [12, 13]. Acid hydrolysis of flavonoid glycosides gave flavanone and sugar as xylose (TLC comparable). On the basis of spectral data analyses and chemical reactions, the structure of **2** has been elucidated as 5,6,7,8,2′,5′-hexahydroxy-4′-methoxy- flavanone-7-*β*-D-xylopyranoside.

## EXPERIMENTAL

**General Procedure.** Melting points were determined on a Perfit melting point apparatus and are uncorrected. FT IR: Jasco FT/IR-5000; UV: Lambda Bio 20 spectrophotometer, MeOH; $^1$H NMR (400 MHz): Advance DRY 400, Bruker Spectrospin, $CDCl_3$; $^{13}$C NMR (75 MHz): Advance DRY 100, Bruker Spectrospin, $CDCl_3$ with TMS as an internal standard; MS: FAB ionization on JEOL-JMS-DX 303; CC: Silica gel (Qualigens), 60–120 mesh; TLC: silica gel G (Qualigens). Spots were visualized by exposure to iodine vapors, UV radiation, and by spraying reagents.

**Plant Material.** The flowers of *Punica granatum* were purchased from the Khari Baoli Market of Delhi and identified by Dr. M. P. Sharma, taxonomist, Department of Botany, Jamia Hamdard, New Delhi. A voucher specimen, No. PRL/JH/05/21, was deposited in the Herbarium of the Phytochemical Research Laboratory, Faculty of Pharmacy, Jamia Hamdard, New Delhi.

**Extraction and Isolation.** The dried flowers (800 g) were coarsely powdered and exhaustively extracted with methanol. The combined extracts were concentrated on a steam-bath and dried under reduced pressure to get 420 g of dark brown mass. The viscous dark brown mass was dissolved in a little quantity of methanol and adsorbed on silica gel (60–120 mesh) for the preparation of slurry. It was dried in air and chromatographed over a silica gel column packed in petroleum ether. The column was eluted with petroleum ether, chloroform, and methanol successively in order of increasing polarity.

**Punicaflavanol (1).** Elution of the column with chloroform–methanol (19:1) mixture gave pale yellow crystals of **1**, recrystallized from methanol, 825 mg (0.103% yield), mp 258–260°C; CD (*c* 0.26, MeOH): ($\Delta\varepsilon_{301}$ – 11.2), ($\Delta\varepsilon_{301}$ + 6.41); UV (MeOH, $\lambda_{max}$, nm): 281, 363 (log ε 5.3, 2.6); UV (MeOH + NaOMe, $\lambda_{max}$, nm): 279, 329 (log ε 2.7, 3.6); UV (MeOH + $AlCl_3$, $\lambda_{max}$, nm): 301, 374 (log ε 5.6, 2.4); UV (MeOH + $AlCl_3$+HCl, $\lambda_{max}$, nm): 293, 362 (log ε 5.4, 2.5); UV (MeOH + NaOAc, $\lambda_{max}$, nm): 316, 381 (log ε 3.9, 1.8); UV (MeOH + NaOAc + $H_3BO_3$, $\lambda_{max}$, nm): 293, 364 (log ε 4.1, 1.8); IR (KBr, $\nu_{max}$, cm$^{-1}$): 3561, 3509, 3136, 2922, 2855, 1696, 1593, 1387, 1296, 1207, 1095; $^1$H NMR (DMSO-$d_6$, δ, ppm, J/Hz): 7.30 (1H, br.s, H-6′), 5.32 (1H, dd, J = 2.5, 13.1, H-2*β*), 3.62 (3H, br.s, OMe), 2.97 (1H, dd, J = 2.5, 13.1, H-3ax), 2.81 (1H, dd, J = 13.1, 17.2, H-3eq); $^{13}$C NMR (DMSO-$d_6$): see Table 1; +ve FAB MS *m/z* (rel. int.): 366 [M]$^+$ ($C_{16}H_{14}O_{10}$) (13.6), 351 (18.5), 338 (29.8), 323 (26.6), 211 (16.3), 188 (36.1), 184 (15.3), 182 (21.7), 178 (37.3), 155 (26.2), 140 (23.7), 77 (100).

**Granatumflavanyl Xyloside (2).** Elution of the column with chloroform–methanol (23:2) mixture gave light yellow crystals of **2**, recrystallized from methanol, 630 mg (0.078% yield), mp 252–254°C; UV (MeOH, $\lambda_{max}$, nm): 277, 363 (log ε 5.7, 2.6); UV (MeOH + NaOMe, $\lambda_{max}$, nm): 282, 341 (log ε 2.3, 2.1); UV (MeOH + $AlCl_3$, $\lambda_{max}$, nm): 291, 374 (log ε 5.9, 3.1); UV (MeOH + NaOAc, $\lambda_{max}$, nm): 299, 386 (log ε 1.3, 0.7); UV (MeOH + NaOAc + $H_3BO_3$, $\lambda_{max}$, nm): 289, 368 (log ε 1.3, 0.8); IR (KBr, $\nu_{max}$, cm$^{-1}$): 3373, 3160, 2928, 1700, 1593, 1445, 1397, 1338, 1199, 1094, 1040, 983, 923; $^1$H NMR (DMSO-$d_6$, δ, ppm, J/Hz): 7.45 (1H, br.s, H-3′), 7.29 (1H, br.s, H-6′), 5.31 (1H, dd, J = 2.7, 13.5, H-2*β*), 5.28 (1H, d, J = 7.1, H-1″), 4.24

(1H, d, J = 7.2, H-2″), 4.05 (1H, m, H-3″), 3.62 (3H, br.s, OMe), 3.45 (1H, m, H-4″), 3.05 (1H, d, J = 10.8, $H_2$-5″a), 3.01 (1H, d, J = 10.8, $H_2$-5″b), 2.97 (1H, dd, J = 2.7, 13.5, H-3ax), 2.81 (1H, dd, J = 13.5, 16.9, H-3eq); $^{13}$C NMR (DMSO-$d_6$): see Table 1; +ve FAB MS *m/z* (rel. int.): 482 $[M]^+$ ($C_{21}H_{22}O_{13}$) (3.1), 316 (19.8), 272 (23.1), 210 (10.7), 182 (21.1), 168 (25.9), 166 (25.2), 149 (26.5), 140 (41.3), 133 (73.1), 122 (70.6), 140 (41.3).

**Hydrolysis of 2.** Compound **2** (20 mg) was dissolved in MeOH, 2 N HCl (1:1) was added, and the mixture heated to half volume. The solution was extracted with EtOAc (3 × 10 mL), and the organic phase was washed with $H_2O$ (2 × 10 mL), dried over anhydrous $Na_2SO_4$, and evaporated to give an aglycone, mp 237–238°C, $^1$H NMR (DMSO-$d_6$, δ, ppm, J/Hz): 7.41 (1H, br.s, H-3′), 7.26 (1H, br.s, H-6′), 5.35 (1H, dd, J = 2.7, 13.5, H-2*β*), 3.59 (3H, br.s, OMe), 2.95 (1H, dd, J = 2.7, 13.5, H-3ax), 2.81 (1H, dd, J = 13.7, 17.5, H-3eq). +ve FAB MS *m/z* (rel. int.): 350 $[M]^+$ ($C_{16}H_{14}O_9$) (16.8). The aqueous phase was concentrated and analyzed by paper chromatography along with standard samples of monosacchrides. *n*-Butanol–ethanol–water (4:1:2.2) was used as the developing solvent system. The paper was sprayed with aniline hydrogen phthalate. The sugar was identified as D-xylose.

**Ellagic Acid (3)**. Elution of the column with a chloroform–methanol (9:1) mixture yielded cream colored crystals of **3**, recrystallized from pyridine, 585 mg (0.074% yield), mp 360°C (decomp.); UV (MeOH, $\lambda_{max}$, nm): 254 (log ε 5.5); IR (KBr, $\nu_{max}$, $cm^{-1}$): 3474, 3376, 3155, 1723; +ve FAB MS *m/z* (rel. int.): 302 $[M]^+$ ($C_{14}H_6O_8$) (39.3).

**Gallic Acid (4)**. Further elution of the column with a chloroform–methanol (9:1) mixture furnished colorless crystals of **4**, recrystallized from methanol, 465 mg (0.058 % yield), mp 235–239°C; lit. mp 325–240°C (The Merck Index, 2001); IR (KBr, $\nu_{max}$, $cm^{-1}$): 3556, 3350, 3076, 1697; +ve FAB MS *m/z* (rel. int.): 170 $[M]^+$ ($C_7H_6O_5$).

**Sucrose (5).** Elution of the column with a chloroform–methanol (8:2) mixture gave colorless crystals of **5** recrystallized from methanol, 752 mg (0.941% yield), mp 160–165°C (decomp.); $[\alpha]_D^{25}$ + 66.13° (26, $H_2O$); IR (KBr, $\nu_{max}$, $cm^{-1}$): 3410, 3286.

**Gallic Acid Glucoside (6)**. Elution of the column with a chloroform–methanol (1:1) mixture gave light brown crystals of **6**, recrystallized from methanol, 633 mg (0.079% yield), mp 195–196°C; UV (MeOH, $\lambda_{max}$, nm): 221, 254 (log ε 3.2, 5.3); IR (KBr, $\nu_{max}$, $cm^{-1}$): 3510, 3335, 3000.

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