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Chemical constituents of ornamental pomegranate and its antioxidant and anti-inflammatory activities in comparison with edible pomegranate

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Abstract
Chromatographic fractionation of the alcoholic extract of leaves of *Punica granatum* L. var. *nana* (Punicaceae) resulted in the isolation and identification of nine compounds, three of them are firstly reported in the family: β-amyrin (1), corosolic acid (4) and luteolin-7-*O*-(6``-*O*-galloyl)-β-d-glucoside (9) in addition to β-sitosterol (2), ursolic acid (3), friedelin (5), β-sitosterol-3-*O*-β-d-glucopyranoside (6), 2-methyl-pyran-4-one-3-*O*-β-d-glucopyranosid (7), luteolin-4'-*O*-β-d-glucoside (8). Their structures were established by various spectroscopic methods (^{1}H NMR, ^{13}C NMR, HSQC, HMBC and UV spectroscopy) as well as comparison with authentic samples. Biological studies of different extracts of *P. granatum* L. var. *nana* (ornamental pomegranate) in comparison with *P. granatum* L. (edible pomegranate) showed that the extracts of edible pomegranate have slightly higher antioxidant activity than the corresponding extracts of the ornamental one using DPPH assay, while the extracts of ornamental pomegranate have higher anti-inflammatory activity than corresponding extracts of the edible one using carrageenan-induced rat hind paw edema model.

Keywords: ornamental pomegranate, anti-inflammatory, antioxidant, *Punica granatum* L. var. nana.

Introduction
Punica granatum L. var. *nana* is a member of family Punicaceae, which is unusual in having the sole genus *Punica,* a genus of large shrubs or small trees with 2 species [1]. One is *P. protopunica* Balf. S., which is found wild in Socotra island and the other one is *P. granatum* (edible pomegranate), cultivated in tropical and subtropical parts of the world for its edible fruits [2]. The root and stem bark and to lesser extent the fruit rind of pomegranate have been commonly used as vermifugal or taenicidal agents. Also, pomegranate was found to have astringent effect. It was used in the form of an aqueous decoction for dysentery, diarrhea, also the fruit juice was used stomach ulcers, while the fruit rind extract is useful in stopping women's bleeding [3, 4]. It served as a remedy for snakebites, burns and leprosy [3, 5]. Pomegranate flowers were used for treatment of diabetes mellitus [5]. The fruit rind was also used as a tanning material [6]. *P. granatum* L. var. *nana* is a dwarf variety of pomegranate, popularly planted in gardens as an ornamental plant [7]. It is a deciduous shrub and differs from edible pomegranate in being shorter in height (3-4 m), having orange flowers and numerous petals. Also, the fruits are smaller in size and not edible [8]. This study describes the isolation and identification of the main constituents of the leaves of the ornamental pomegranate in addition to evaluation of the antioxidant and anti-inflammatory activities of different extracts in comparison with the edible one.

Materials and Methods
Equipment
Melting points are carried out on an Electrothermal 9100 Digital Melting Point apparatus (Electrothermal Engineering Ltd, Essex, England). UV spectra were recorded in MeOH on Ultrospec 1000, UV-VIS spectrometer, Pharmacia Biotech, Cambridge, England. NMR spectra (chemical shifts in ppm, coupling constants in Hz) were recorded on JEOL ALPHA-500 spectrometer, Bruker Advanc III 400 MHz spectrophotometer (Bruker BioSpin AG, Fällanden, Switzerland), and Agilent INOVA 600AS instrument (600, Japan) using DMSO-d_6, $CDCl_3$, and C_5D_5N as solvents. Column chromatographic separation was performed on silica gel 60 (0.04–0.063 mm, Merck), RP-18 (0.04–0.063 mm, Merck).

TLC was performed on precoated TLC plates silica gel 60 F254 (0.2 mm, Merck). The solvent systems used for TLC analysis include *n*-hexane: acetone (80:20, S1), CH_2Cl_2: MeOH (90:10, S2), CH_2Cl_2: MeOH (80:20, S3).

Plant material

Leaves and stem bark of *P. granatum* L. var. *nana* (ornamental pomegranate) and *P. granatum* L. (edible pomegranate) were collected during the flowering stage (April to July 2011) from the Experimental Station of Agriculture and from the Faculty of Pharmacy garden of medicinal plants, Assiut University, Assiut, Egypt. The plants were identified and authenticated by Dr. Naeem E. Keltawy, Professor of Ornamental Horticulture and Floriculture, Faculty of Agriculture, Assiut University. Voucher specimens (No 45) were kept in the herbarium, Pharmacognosy Department, Faculty of Pharmacy, Assiut University.

Chemicals

DPPH (2,2-Diphenyl-1-picryl-hydrazine) and carrageenan were purchased from Aldrich Co., USA, tween 80% (Sigma Chemical Co., St. Louis, USA), Normal saline 0.9% (El-Nasr pharmaceutical and chemical Co., Egypt), Indomethacin (El-Nile Co., Egypt).

Extraction and isolation

The air-dried powdered leaves of *P. granatum* L. var. *nana* (4.5 kg) were exhaustively extracted with 70% ethanol by maceration at room temperature. The ethanolic extract was concentrated under reduced pressure to obtain a viscous residue (700 g). This residue was subjected to solvent fractionation using *n*-hexane, $CHCl_3$ and EtOAc. Each fraction was separately concentrated to give 92, 53 and 205 g respectively and 300 g remaining aqueous extract. The *n*-hexane fraction (50 g) was subjected to VLC using *n*-hexane: acetone in a gradient elution manner, where five subfractions (HL-I-:HL-V) were obtained. Subfraction HL-II (12 g) eluted with *n*-hexane-acetone (95:5) was chromatographed on silica gel column using *n*-hexane-acetone in a gradient elution manner to give three compounds 1, 2 and 3. Subfraction HL-V (8 g) was subjected to silica gel CC using *n*-hexane-acetone in a gradient elution manner to give compound 4. The chloroform fraction (50 g) was subjected to VLC using CH_2Cl_2-MeOH in a gradient elution manner, where four subfractions (CH-I: CH-IV) were obtained. Subfraction CH-III (3 g, eluted with CH_2Cl_2-MeOH 90:10) was subjected to silica gel CC using CH_2Cl_2-MeOH in a gradient elution manner to give compounds 5 and 6. The EtOAC fraction (25 g) was subjected to silica gel CC using gradient system of CH_2Cl_2-MeOH, where five subfractions (E-I: E-V) were obtained. Fraction E-II (2.5 g) was subjected to silica gel CC using CH_2Cl_2-MeOH in a gradient elution manner followed by preparative G_{60} F_{254} TLC using CH_2Cl_2- MeOH (80:20) to give compound 7. Subfraction E-IV (0.5 g) eluted with CH_2Cl_2- MeOH (85:15) was rechromatographed on medium pressure Rp-18 column using (H_2O-MeOH 60:40) to give compound 8. The fraction E-VI (0.5 g) eluted with (CH_2Cl_2- MeOH 70:30), by repeated crystallization from MeOH gave compound 9.

Acid hydrolysis

Few milligrams of compounds 6, 8 and 9 were separately dissolved in 5 ml MeOH and an equal volume of 10% H_2SO_4 v/v was added. The mixture was refluxed for 3 hrs, then cooled. The hydrolyzate was shaken with ethyl acetate 3 times, distilled off and the aglycone was subjected to TLC using $CHCl_3$: MeOH (85:15) as solvent system. The acidic solution was then neutralized with barium carbonate, concentrated and spotted alongside authentic sugars on Whatman No. 1 sheets using *n*- butanol-acetic acid-water (4:1:2, v/v/v) as a solvent system.

Antioxidant activity

Antioxidant activity was determined by the DPPH method [9]. 0.2 ml of methanolic solutions of the total ethanolic leaves and stem bark extracts of both ornamental pomegranate (LOP, SOP) and edible pomegranate (LEP, SEP) and leaves fractions; including *(n*-hexane, chloroform, ethyl acetate and aqueous) were mixed with 2ml of methanolic solution of $DPPH^{\cdot}$ (100 µM). Similarly 0.2 ml of methanolic solutions of quercetin in a concentration range of (1000-5 µg/ml) is mixed with 2 ml $DPPH^{\cdot}$ and used as a positive control. The experiment was carried out in two phases; the first phase involved concentrations of (1000, 500, 250, 100 and 50 µg/ml) for *n*-hexane and chloroform fractions and the second phase involved concentrations of (100, 50, 40, 20, 10, 5 µg/ml) for total ethanolic leaves and stem bark extracts of ornamental and edible pomegranate, ethyl acetate and aqueous fractions of ornamental pomegranate. A mixture of 0.2 ml of methanol and 2 ml of methanolic solution of $DPPH^{\cdot}$ (100 µM) served as blank. After mixing, all the solutions were incubated in dark for 30 minutes and absorbance was measured at 517 nm. The experiment was performed in triplicates and percent of antioxidant activity was calculated as follows:

$$\text{Antioxidant activity} = \frac{\text{Absorbance of blank} - \text{Absorbance of test}}{\text{Absorbance of blank}} \times 100$$

Pharmacological study

Animals

Adult male albino rats (each 100-120 g) were used. All animal procedures were conducted in accordance with the Good Laboratory Practice (GLP) according to internationally valid guidelines and regulations of the World Health Organization (WHO) [10, 11].

Preparation of extracts for administration

Weighed amount (1 g) of each extract was separately taken and solubilized in 3% (*v/v*) tween 80 in normal saline and the volume was completed to 10 ml by normal saline. A control solution was prepared using the same amount of tween 80 in normal saline (negative control).

Acute toxicity

The acute toxicity tests (LD_{50}) of the total ethanolic extracts of leaves and stem bark of (ornamental pomegranate) in comparison with (edible pomegranate) leaves and stem barks extracts were determined according to the procedure described by [12]. The animals were divided into 4 groups (3 rats/group)

for each given dose. Doses of 1, 2, 3, 4 and 5 g/kg were administered intraperitoneally (i.p.).

Anti-inflammatory activity

Carrageenan-induced rat hind paw edema model described by [13] was performed, where ten groups (6 rats/ group) were used. The first group was kept as a negative control, injected intraperitoneally by 3% tween 80 in normal saline, while the second group injected by indomethacin (8mg/Kg) as a positive control. The other groups were separately intraperitioneally injected with different extracts at a dose of 400 mg/kg of the body weight. After 30 minutes from administration, the inflammation was induced by injection of the carrageenan suspension in the right paw while the left one was injected by an equal volume of saline solution. The percentage of inhibition was calculated.

Statistical analysis

Data were analyzed using student's T-test and the values were expressed as mean ± S.E. (n = 6 animals).

Results

Nine compounds were isolated from the ethanolic leaves extract of *P. granatum* L. var. *nana*.

Compound 1: White fine needles (40 mg, acetone), m.p. 198-200 °C; R_f 0.58 (S1). Mixed melting point as well as co-chromatography with authentic sample, compound 1 was identified as **β-amyrin**.

Compound 2: White needles (80 mg, acetone), m.p. 136-140 °C; R_f 0.44 (S1). Mixed melting point as well as co chromatography with authentic sample, compound 2 was identified as **β-sitosterol**.

Compound 3: Colourless needles (100 mg, MeOH), m.p. 290-292 °C; R_f 0.3 (S1); NMR spectral data: see Tables 1, 2. These data are in a good agreement with **ursolic acid** [14, 15].

Compound 4: White crystals (6 mg, MeOH), m.p. 251-254 °C; R_f 0.47 (S2); NMR spectral data: see Tables 1, 2. By comparison with the reported data [15, 16], compound 4 was identified as corosolic acid.

Compound 5: White needles (35 mg, MeOH), m.p. 262-265 ºC; R_f 0.3 (S1); NMR spectral data: see Tables 1, 2. By comparison with the reported data [15, 17], compound 5 was identified as friedelin**.**

Table 1: ^{1}H NMR spectral data of compounds 3-5.

H	Compound 3 (100 MHz, C_5D_5N)	Compound 4 (150 MHz, DMSO-d_6)	Compound 5 (100 MHz, $CDCl_3$)
2	-----	3.4, 1 H *	2.32, 1 H, *m* 2.4, 1 H, *m*
3	3.46, 1 H, *t*, J= 8.4 Hz	2.74, 1 H, *d*, J= 9.6 Hz	-----
4	-----	-----	2.26, 1 H, *q*, J= 6.4 Hz
12	5.47, 1 H, *br s*	5.14, 1 H, *t* like, J= 3.6 Hz	-----
18	1 H, *d*, J= 10.4 Hz	2.11, 1 H, *d*, J= 11.4 Hz	-----
23	1.27, 3 H, *s*	0.92, 3 H, *s*	0.90, 3 H, *d*, J= 6.8 Hz
24	0.84, 3 H, *s*	0.71, 3 H, *s*	0.74, 3 H, *s*
25	1.03, 3 H, *s*	0.92, 3 H, *s*	0.91, 3 H, *s*
26	1.01, 3 H, *s*	0.74, 3 H, *s*	1.03, 3 H, *s*
27	1.23, 3 H, *s*	1.04, 3 H, *s*	1.07, 3 H, *s*
28	-----	-----	1.19, 3 H, *s*
29	1.00, 3 H, *d*, J= 5.6 Hz	0.82, 3 H, *d*, J= 6 Hz	0.97, 3 H, *s*
30	0.99, 3 H, *d*, J= 5.6 Hz	0.91, 3 H, *d*, J= 6 Hz	1.02, 3 H, *s*
Other methylenes and methines	1.33-2.32, *m*	1.09-1.9, *m*	1.28-2, *m*

* Overlapped with the solvent signal

Table 2: ^{13}C NMR spectral data of compounds 3-5

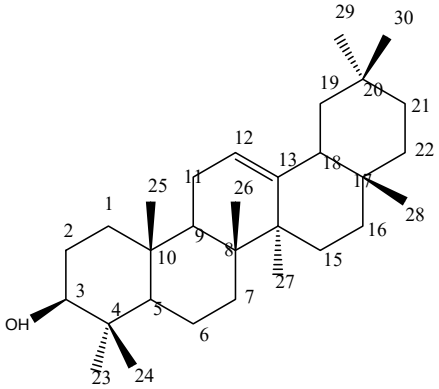
C	Compound 3 100 MHz, C_5D_5N)	Compound 4 (150 MHz, DMSO-d_6)	Compound 5 (100 MHz, $CDCl_3$	C	Compound 3 (100 MHz, C_5D_5N)	Compound 4 (150 MHz, DMSO-d_6)	Compound 5 (100 MHz, $CDCl_3$)
	40.33	46.78	22.29	16	26.17	25.59	36.01
1	29.36	67.1	41.54	17	49.38	47.03	30
2	79.39	82.22	213.29	18	54.8	52.33	42.78
3	40.64	38.46	58.23	19	40.75	38.46	35.35
4	57.07	54.7	42.16	20	40.66	38.39	28.18
5	20.04	17.98	41.29	21	32.33	30.14	32.77
6	34.83	32.59	18.24	22	38.71	36.26	39.26
7	41.21	38.88	53.1	23	30.08	28.79	6.84
8	49.29	46.94	37.44	24	17.86	16.39	14.67
9	38.5	37.53	59.47	25	16.94	16.9	17.96
11	24.89	22.9	35.69	26	18.8	16.96	20.27
12	125.06	124.44	30.51	27	25.18	23.23	18.68
13	140.52	138.18	39.7	28	181.2	178.19	32.1
14	43.75	41.66	38.3	29	18.71	17.13	35.04
15	29.94	27.46	32.42	30	22.69	21.03	31.79

Compound 6: White powder (25 mg, MeOH)), m.p. 278ºC; Rf 0.46 (S2); ^{1}HNMR (DMSO-d_6, 600 MHz): δH 0.69 (3H, *s*, H-18), 0.79 (3H, *d*, J = 6.6 Hz, H-27), 0.81 (3H, *d*, J = 7.2 Hz, H-26), 0.82 (3H, *t*, J = 7.2 Hz, H-29), 0.90 (3H, *d*, J = 6.6 Hz, H-21), 0.96 (3H, *s*, H-19), 1.12-2.37 (*m*, Other-methylene and methine protons), 2.89–3.64 (5H, m, sugar protons), 3.44 (1H, *m*, H-3), 4.22 (1H, *d*, J = 7.8 Hz, H-1'), 5.32 (1H, *t* like, J = 2.4 Hz, H-6); By co chromatography with authentic sample and comparison with the reported data [18], compound 6 was identified as *β*-sitosterol-3-O-*β*-glucopyranoside

Compound 7: White needles (10 mg, MeOH), m.p. 261-262ºC; Rf 0.48 (S3); ^{1}HNMR (DMSO-d_6, 500 MHz): δH 2.36 (3H, *s*, H-7), 3.08-3.63 (5H, *m*, sugar protons), 4.74 (1H, *d*, J = 7.5 Hz, H-1'), 6.42 (1H, *d,* J = 6 Hz, H-5), 8.12 (1H, *d*, J = 6 Hz, H-6) ; ^{13}C-NMR (DMSO-d_6, 125 MHz): δC 161.3 (C-2), 141.8 (C-3), 174.2 (C-4), 116.2 (C-5), 155.6 (C-6), 15.2 (C-7), 103.6 (C-1'), 73.9 (C-2'), 76.3 (C-3'), 69.6 (C-4'), 77.4 (C-5'), 60.8 (C-6'). By comparison with the reported data [19], compound 7 was identified as 2-Methyl-pyran-4-one-3-*O*-β-d-glucopyranoside.

Compound 8: Yellow powder (25 mg, MeOH), m.p. 266-268ºC; Rf 0.43 (S3); UV (MeOH): λmax 256, 337; +NaOMe: 256, 375; +$AlCl_3$: 274, 380; +$AlCl_3$/HCl: 274, 380; +NaOAc: 274, 345; +NaOAc/H_3BO_3: 256, 338 nm; ^{1}HNMR (DMSO-d_6, 400 MHz): δH 3.39-3.80 (5H, *m*, sugar protons), 4.95 (1H, *d*, J=7.2 Hz, H-1''), 6.25 (1H, *d*, J=1.6 Hz, H-6), 6.55 (1H, *d*, J=1.6 Hz, H-8), 6.87 (1H, *s*, H-3), 7.30 (1H, *d,* J=8.4 Hz, H-5'), 7.56 (1H, *d*, J= 2.0 Hz, H-2'), 7.58 (1H, *dd*, J= 8.4, 2.0 Hz, H-6'), 12.9 (1H, *s*, 5-OH); ^{13}C-NMR (DMSO-d_6, 100 MHz): δC 164.75 (C-2), 104.24 (C-3), 182.24 (C-4), 161.89 (C-5), 99.39 (C-6), 163.64 (C-7), 94.50 (C-8), 157.81 (C-9), 104.42 (C-10), 125.13 (C-1'), 114.03 (C-2'), 147.36 (C-3'), 149.00 (C-4'), 116.42 (C-5'), 118.92 (C-6'), 101.58 (C-1''), 73.68 (C-2''), 77.74 (C-3''), 70.20 (C-4''), 76.26 (C-5''), 61.14 (C-6''). By comparison with the reported data [20], compound 8 was identified as luteolin-4'-*O*-β-d-glucoside.

Compound 9: Yellow powder (28 mg, MeOH); Rf 0.35 (S3); UV (MeOH): λmax 269, 348; +NaOMe: 269, 406; +$AlCl_3$: 274, 421; +$AlCl_3$/HCl: 274, 377; +NaOAc: 269, 359; +NaOAc/H_3BO_3: 269, 378 nm; ^{1}HNMR (DMSO-d_6, 400 MHz): δH 3.08-3.5 (3H, *m*, sugar protons), 4.2, 4.268 (2H, overlapped with anomeric proton signal, H-6''a, H-6'' b), 4.2 (1H, *d*, J=6.8 Hz, H-1''), 6.20 (1H, *d*, J=2 Hz, H-6), 6.45 (1H, *d*, J=2 Hz, H-8), 6.68 (1H, *s*, H-3), 6.9 (1H, *d,* J=8 Hz, H-5'), 6.95 (2H, *s,* H-2''', H-6'''), 7.39 (1H, *d*, J= 2.0 Hz, H-2'), 7.4 (1H, *dd*, J= 8, 2.0 Hz, H-6'); ^{13}C-NMR (DMSO-d_6, 100 MHz): δC 164.35 (C-2), 102.58 (C-3), 182.12 (C-4), 161.92 (C-5), 99.28 (C-6), 164.54 (C-7), 94.3(C-8), 157.74 (C-9), 104.14 (C-10), 121.94 (C-1'), 113.79 (C-2'), 146.18 (C-3'), 150.15(C-4'), 116.47 (C-5'), 119.44 (C-6'), 103.31 (C-1''), 73.84 (C-2''), 76.85 (C-3''), 69.91 (C-4''), 74.08 (C-5''), 64.1 (C-6''), 120.2 (C-1'''), 108.9 (C-2''', C-6'''), 146.00 (C-3''', C-5'''), 138.78 (C-4'''), 166.26 (C-7'''). By comparison with the reported data [21], compound 9 was identified as luteolin-7-*O*-(6``-*O*-galloyl)-β-d-glucoside.



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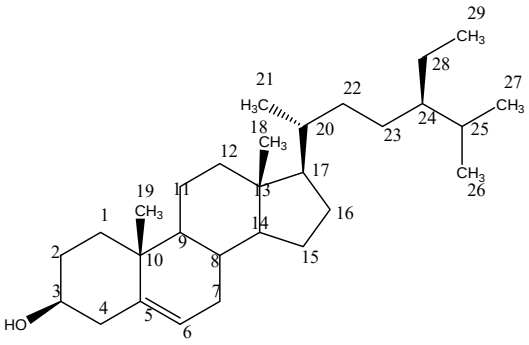

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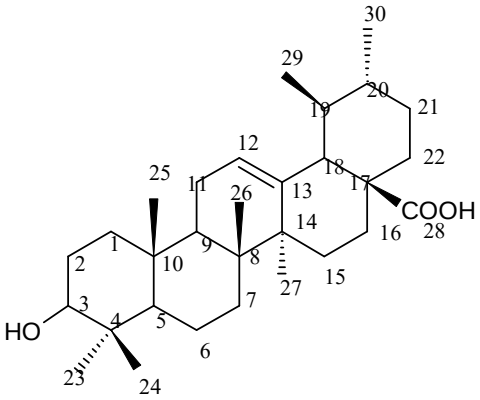

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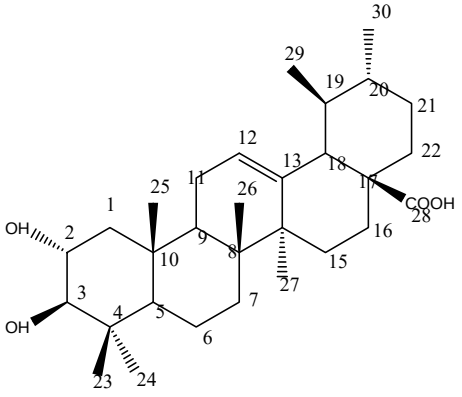

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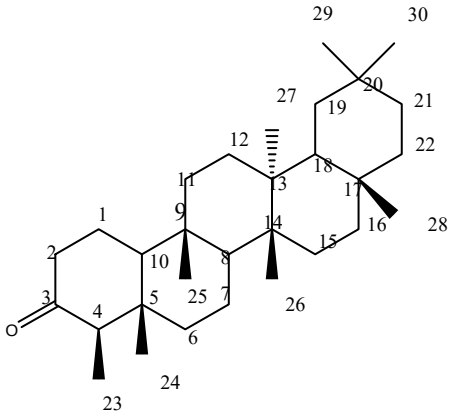

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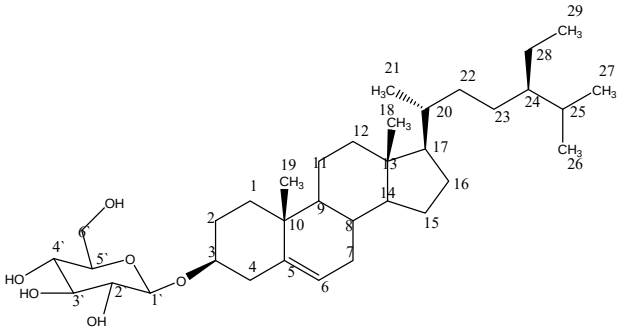

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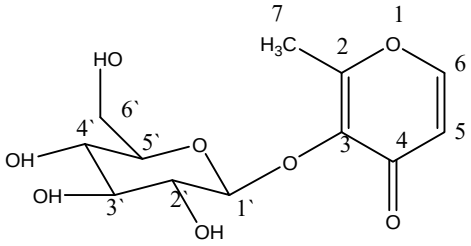

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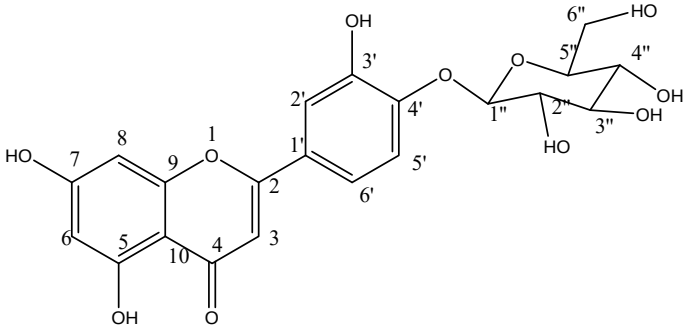

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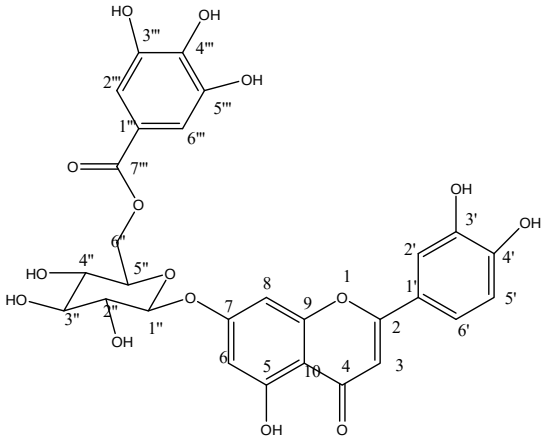

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Fig 1: Structures of the isolated compounds (1-9) from *P. granatum* L. var. *nana*

Biological Activity

Antioxidant activity

The total ethanolic extracts of LEP and SEP have slightly higher antioxidant activity than the corresponding extracts of LOP and SOP. Considering the fractions of LOP, the ethyl acetate and aqueous fractions had the highest antioxidant activity which was comparable to the total ethanolic extract of LOP (Figure 2) while *n*-hexane and chloroform fractions did not show antioxidant activity.

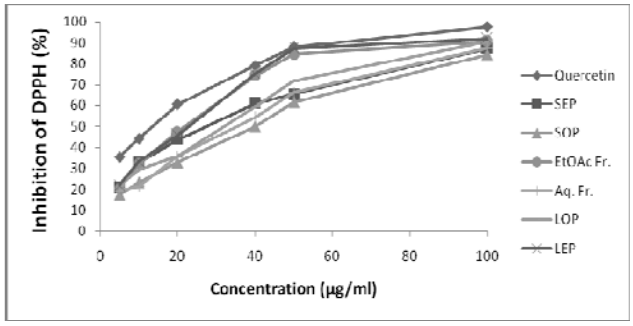

Fig 2: Antioxidant activity of different extracts of ornamental and edible pomegranate

Pharmacological study

Acute toxicity

No signs of toxicity were observed in rats given both ornamental and edible pomegranate leaves extracts at the chosen doses level (5 g/kg), while for the stem bark extracts, toxicity signs appear at 4 g/kg dose level for both ornamental and edible pomegranate stem bark extracts. The LD_{50} of both ornamental and pomegranate stem bark extracts was found to be 3.5 g/kg**.**

Anti-inflammatory activity

The tested extracts and fractions possess varying significant anti-inflammatory activity. The total ethanolic extract of SOP showed higher percentage of edema inhibition in comparison with the total ethanolic extract of SEP through the whole 4 hrs. The total ethanolic extract of LOP showed higher percentage of edema inhibition in the 3rd and 4th hrs than the total ethanolic extract of LEP. The *n*-hexane and aqueous fractions of LOP showed a potent anti-inflammatory activity comparable to that of indomethacin. The results are listed in Table 3.

Table 3: Percentage of anti-inflammatory activity of the different extracts of ornamental and edible pomegranate carrageenan-induced hind paw edema model in rats

Group	Dose (mg/kg)	Time after phlogistic agent administration (hours)				
		1/2	1	2	3	4
		% inhibition of inflammation				
Indomethacin	8	21.7±0.13***	32.5±0.33***	41.2±0.14***	50.4±0.4***	50.9±0.3***
Total ethanolic extract of SEP	400	15.6±0.12***	29.3±0.11***	36.6±0.32***	43.6±0.2***	35.6±0.16*
Total ethanolic extract of SOP	400	24±0.02***	30.9±0.02***	42.6±0.3***	50.4±0.28***	42.2±0.07*
Total ethanolic extract of LEP	400	17.7±0.16***	19.5±0.19***	47.7±0.28***	50.4±0.07***	43±0.04*
Total ethanolic extract of LOP	400	12.1±0.32**	27.5±0.03***	34.9±0.26***	58.6±0.14***	58.3±0.22**
n-Hexane fr of LOP	400	16.7±0.17***	34.5±0.25***	38.1±0.17***	52.6±0.13***	56.6±0.52**
Chloroform fr. of LOP	400	16.8±0.12***	23.8±0.26***	31.9±0.16**	45.7±0.48***	49.6±0.49**
Ethyl acetate fr. of LOP	400	17.6±0.22***	32.3±0.32***	33.7±0.3**	45.6±0.5***	36.9±0.03*
Aqueous fr. of LOP	400	18.4±0.24***	27.5±0.12***	34.2±0.12***	49.9±0.31***	54.2±0.23***

Data are expressed as mean ±S.E, n=6

Differences with respect to the control group were evaluated using the student t-test.

(*P<0.05, ** P<0.01, *** P<0.001)

Discussion

Chemical investigation of ornamental pomegranate leaves resulted in isolation and identification of nine compounds including three compounds that are firstly reported in the family Punicaceae (β-amyrin, corosolic acid and luteolin-7-*O*-(6``-*O*-galloyl)-β-d-glucoside*)*, five compounds firstly reported in the plant (β-sitosterol, ursolic acid, friedelin, *β*-sitosterol-3-*O*-*β*-glucopyranoside and luteolin-4`-*O*-β-d-glucoside and one previously isolated compound (2-Methyl-pyran-4-one-3-*O*-β-d-glucopyranoside).

The tested extracts and fractions showed significant antioxidant activity using DPPH assay. The extracts of edible pomegranate have slightly higher antioxidant activity than the corresponding extracts of the ornamental one. The antioxidant activity is possibly due to presence of phenolic compounds as flavonoids, anthocyanins and tannins [22, 23]. In addition they showed varying significant anti-inflammatory activity using carrageenan-induced edema model for inflammation, the extracts of ornamental pomegranate have higher anti-inflammatory activity than corresponding extracts of the edible one. Carrageenan model of inflammation is said to be biphasic with the first phase attributed to the release of histamine, serotonin and kinins in the first hour, while the second phase is attributed to release of prostaglandins and lysosome enzymes [24]. The tested extracts inhibited both phases of inflammation. The anti-inflammatory activity may be attributed to presence of flavonoids, sterols, triterpenes like ursolic acid, anthocyanins and tannins [25-27].

Conclusion

Nine compounds were isolated from the ethanolic leaves extract of *P. granatum* L. var. *nana*, including triterpenes, sterols and glycosides. Biological screening of different extracts of ornamental pomegranate for both antioxidant and anti-inflammatory activities revealed significant activity in comparison with edible one suggesting its use as a valuable source of constituents of potential medicinal use.

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