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Bioactive Potential of pomegranate (*Punica granatum* L.) Extracts from Sonora, Mexico: Toward the Valorization of Peel, Seed, and Juice Fractions

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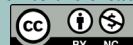
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Abstract. Pomegranate (*Punica granatum* L.) is widely recognized for its abundance of bioactive compounds with potential health benefits. However, information regarding the chemical composition and biological properties of pomegranates cultivated in northwestern Mexico remains scarce. This study aimed to characterize the chemical profile and evaluate the antioxidant, antiproliferative, antibacterial, and α -glucosidase inhibitory activities of ethanolic and methanolic extracts from peel, seed, and juice of *P. granatum* fruits grown in Sonora, Mexico. Fruits collected from northwestern Mexico were separated into peel, juice, and seed fractions and extracted with ethanol or methanol. Extracts were characterized by total phenolic and flavonoid contents, and by ellagic acid content determined by HPLC-DAD. Antioxidant activity was assessed by the DPPH radical scavenging assay; antiproliferative activity

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was evaluated by the MTT assay on HeLa, PC3, and L929 cell lines; antibacterial activity against *Escherichia coli* and *Staphylococcus aureus* was determined by the broth microdilution method; and α -glucosidase inhibition was analyzed spectrophotometrically. Peel extracts exhibited the highest phenolic (up to 251 mg GAE/g DE) and flavonoid contents and the strongest antioxidant activity ($EC_{50} = 14.59 \pm 1.04 \mu\text{g/mL}$). They also displayed pronounced antiproliferative activity, particularly against PC3 cells ($IC_{50} = 187.82 \pm 32.22 \mu\text{g/mL}$), and selective antibacterial activity against *S. aureus*, achieving complete inhibition at 800 $\mu\text{g/mL}$. Methanolic peel extracts showed complete α -glucosidase inhibition across all tested concentrations. Peel extracts of *P. granatum* from Sonora exhibited superior chemical richness and multifunctional bioactivity, highlighting their potential as sustainable sources of natural antioxidants, antimicrobials, and hypoglycemic agents for functional food and nutraceutical applications.

Resumen. La granada (*Punica granatum* L.) es ampliamente reconocida por su abundancia de compuestos bioactivos con potenciales beneficios para la salud. Sin embargo, la información sobre la composición química y las propiedades biológicas de las granadas cultivadas en el noroeste de México es limitada. El objetivo de este estudio fue caracterizar el perfil químico y evaluar las actividades antioxidante, antiproliferativa, antibacteriana y la inhibición de α -glucosidasa de extractos etanólicos y metanólicos obtenidos de la cáscara, semillas y jugo de frutos de *P. granatum* cultivados en Sonora, México. Los frutos recolectados en el noroeste de México se separaron en fracciones de cáscara, jugo y semillas, y se sometieron a extracción con etanol o metanol. Los extractos se caracterizaron mediante contenido total de fenoles, contenido de flavonoides y cuantificación de ácido elágico por HPLC-DAD. La actividad antioxidante se evaluó mediante el ensayo de captura del radical DPPH; la actividad antiproliferativa se determinó mediante el método MTT en las líneas celulares HeLa, PC3 y L929; la actividad antibacteriana contra *Escherichia coli* y *Staphylococcus aureus* se evaluó mediante microdilución en caldo; y la inhibición de α -glucosidasa se analizó espectrofotométricamente. Los extractos de cáscara mostraron los mayores contenidos de fenoles (hasta 251 mg GAE/g DE) y flavonoides, así como la actividad antioxidante más alta ($EC_{50} = 14.59 \pm 1.04 \mu\text{g/mL}$). También presentaron marcada actividad antiproliferativa, particularmente contra células PC3 ($IC_{50} = 187.82 \pm 32.22 \mu\text{g/mL}$), además de actividad antibacteriana selectiva contra *S. aureus*, logrando una inhibición completa a 800 $\mu\text{g/mL}$. Los extractos metanólicos de cáscara mostraron inhibición total de α -glucosidasa en todas las concentraciones evaluadas. Los extractos de cáscara de *P. granatum* provenientes de Sonora exhibieron una notable riqueza química y bioactividad multifuncional, destacando su potencial como fuentes sostenibles de antioxidantes naturales, agentes antimicrobianos e hipoglucemiantes para aplicaciones en alimentos funcionales y nutraceuticos.

Introduction

Pomegranate (*Punica granatum* L.) is a typical crop of Asian and Mediterranean countries, the Middle East, and some regions of the United States of America [1,2]. In Mexico, the main *P. granatum* L. producing states are Morelos, Hidalgo, Oaxaca, and Guanajuato [3]. In recent years, the State of Sonora, has shown a remarkable increase in pomegranate cultivation, establishing itself as an emerging region in the production of this fruit. The main production areas are located in the northern part of the state, where climatic and soil conditions are favorable for its cultivation. The fruit of *P. granatum* L. is composed of approximately 80 % water and 15 % carbohydrates, mainly sugars (such as fructose, sucrose, and glucose), and the remainder is represented by fiber, vitamins (such as vitamin C), and natural bioactive compounds such as polyphenols [4]. The chemical composition of the fruit of *P. granatum* L. varies depending on the variety and growing conditions. The peel, which represents the majority of the fruit's weight, is rich in phenolic compounds such as phenolic acids, flavonoids, and tannins, which contribute to the pomegranate's biological activity. Flavonoids, particularly the anthocyanins present in the arils, contribute to the red color and offer antioxidant properties [5,6].

The fruit of *P. granatum* possesses a pleasant flavor and an attractive color, in addition to various health-promoting properties. Its seeds, which represent approximately 10 % of the fruit, are thick and woody in texture, while the fleshy or pulpy part represents approximately 50 %. The seeds are prismatic, very juicy, and have colors ranging from intense ruby red to white, with a characteristic sweet and sour flavor. In many countries, the

pomegranate and its different parts are still used in traditional medicine to treat various pathologies. For example, in Morocco, dried pomegranate peels is used to control diarrhea and dental plaque [7]. Furthermore, pomegranate has been used medicinally as a blood purifier and as an herbal remedy for the prevention of influenza, mouth ulcers, parasitic infections, gastrointestinal ulcers, and upper respiratory tract infections [8,9].

Bioactive compounds present in pomegranate are primarily secondary metabolites (such as steroids, alkaloids, phenols, and tannins), whose dietary consumption is highly relevant. These compounds offer significant therapeutic potential, serving as natural alternatives to synthetic drugs. They exhibit antimicrobial activity and could be effective in combating infectious diseases, particularly those caused by multidrug-resistant pathogenic bacteria with uncontrolled acquired resistance, which pose significant challenges for global health and biomedical research [10].

Several studies have demonstrated the efficacy of *P. granatum* fruit in treating of type 2 diabetes mellitus, attributed to its rich content of dietary fiber, minerals, vitamins, and other natural bioactive compounds beneficial for glycemic regulation [11]. In various animal models, the antidiabetic effects of *P. granatum* L. extracts have been linked to the activation of the peroxisome proliferator-activated receptor (PPAR- γ); a key transcription factor involved in glucose metabolism [12]. Furthermore, pomegranate juice, which is particularly rich in punicalagin, has been reported to stimulate insulin secretion by pancreatic β -cells, suggesting its potential as an adjunct in antidiabetic therapy [13]. The seeds of *P. granatum* also constitute an excellent source of dietary fiber and can be used as a functional food ingredient to alleviate constipation, promote healthy bowel movements, reduce cholesterol levels, and support blood glucose control [14]. The aim of this study was to determine the chemical composition and evaluate the biological activities of ethanolic and methanolic extracts of pomegranate fruit cultivated and marketed in the northern region of the state of Sonora, Mexico (30°37'N, 110°43'O, 31°13'N, 110°55'O).

Experimental

Sample collection and extract preparation

Pomegranates (*Punica granatum* L.) were collected between August and September 2023 from local markets in two nearby localities in Sonora, Mexico: Magdalena (30°37'49"N, 110°58'10"O; 30.6303, -110.9694) and Ímuris (30°46'38"N, 110°51'42"O; 30.7772, -110.8617). A representative sample of 10–15 fruits per locality was combined to form a pooled batch. Fruits were manually separated into peel, juice, and seeds. For each fruit fraction (peel, seeds, and juice), 10 g of sample were mixed separately with methanol or ethanol at a 1:10 (w/v) ratio. The mixtures were extracted under continuous agitation at 150 rpm for 7 days in the dark. After extraction, the solvent was removed using a rotary evaporator at 35 °C under vacuum and 150 rpm until dry extracts were obtained.

Determination of total phenolic content

The total phenolic content (TPC) was determined using the Folin–Ciocalteu method, as previously described by Singleton and Rossi (1965) [15], with minor modifications. Briefly, 10 μ L of each sample (stock solution at 2.5 mg/mL) were mixed with 60 μ L of sodium carbonate solution (7 % w/v), 40 μ L of Folin–Ciocalteu reagent (0.2 N), and 90 μ L of Milli-Q water in a 96-well microplate. The reaction mixture was incubated in the dark for 1 h at room temperature, and absorbance was measured at 750 nm using a Multiskan EX microplate reader (ThermoLabSystem, Waltham, MA, USA). A calibration curve was prepared using gallic acid (0.006–0.8 mg/mL), and results were expressed as mg of gallic acid equivalents per gram of dry extract (mg GAE/g DE).

Determination of flavonoid content

The total flavonoid content (TFC) was determined using the aluminum chloride colorimetric method [16] with slight modifications. Briefly, 10 μ L aliquot of each extract (stock solution at 2.5 mg/mL) was mixed with 130 μ L of methanol and 10 μ L of AlCl₃ solution (5 % w/v) in a 96-well microplate. The plate was incubated in the dark for 30 min at room temperature, and absorbance was measured at 412 nm using the same microplate reader (Multiskan EX, ThermoLabSystems, Waltham, MA, USA). A calibration curve was prepared with quercetin (0.2–1 mg/mL), and results were expressed as mg of quercetin equivalents per gram of dry extract (mg QE/g DE).

Determination of ellagic acid by HPLC-UV-DAD

The pomegranate extracts were passed through a 0.22 μm nylon membrane filter prior to chromatographic analysis. An aliquot of 50 μL (stock solution at 100 $\mu\text{g/mL}$) was injected into a high-performance liquid chromatography system equipped with a diode array detector (HPLC-DAD, 1260 Infinity, Agilent Technologies, Santa Clara, CA, USA) for the identification and quantification of ellagic acid. Separation was achieved on a C18 column (25 cm $\times$ 4 mm, 5 μm ; SupelcosilTM LC-18, Supelco, Bellefonte, PA, USA) using a mobile phase consisting of solvent A (water containing 5 % formic acid) and solvent B (methanol) at a constant flow rate of 1.0 mL/min. A linear gradient elution program was applied as follows: 100–98 % A (0–2 min), 98–68 % A (2–32 min), 68–60 % A (32–40 min), 60–5 % A (40–50 min) and held at 5 % A for an additional 5 min[17].

Detection was carried out at 366 nm and 378 nm. Ellagic acid was identified by comparing retention times and UV spectra with those of analytical standards, and quantification was based on a calibration curve prepared with ellagic acid standards (Sigma-Aldrich, St. Louis, MO, USA). Results were expressed as milligrams of ellagic acid per gram of dry extract (mg/g DE), reported as the mean $\pm$ standard deviation ($n = 3$).

Antioxidant activity by DPPH assay

The antioxidant activity of *P. granatum* methanolic and ethanolic extracts was determined using the DPPH radical scavenging assay as previously described[18] with slight modifications. Briefly, 100 μL of each sample prepared in methanol at different concentrations (3.12–400 $\mu\text{g/mL}$) were mixed with 100 μL of a 300 μM DPPH solution in a 96-well microplate (Costar, Corning, NY, USA). The mixtures were incubated in the dark for 30 minutes at room temperature, and the absorbance was measured at 517 nm using a microplate reader (Multiskan GO, Thermo Scientific, Waltham, MA, USA). Ascorbic acid (70 μM) was used as a positive control. The radical scavenging activity was expressed as the percentage decrease in absorbance relative to the control. The EC₅₀ value, defined as the extract concentration required to inhibit 50 % of the DPPH radical, was calculated by plotting the percentage of inhibition against extract concentration. Linear regression was performed using the concentration range that showed a linear response.

Antiproliferative activity assay

For antimicrobial assays, dried extracts were reconstituted in DMSO to prepare stock solutions at 40 mg/mL. In all assays, the final concentration of DMSO did not exceed 1 %. DMSO at the same final concentration was included as a vehicle control, and viability was calculated relative to the viability observed in the DMSO control. The cytotoxic activity of *P. granatum* extracts was evaluated using the MTT colorimetric assay on HeLa (human cervix adenocarcinoma), PC3 (human prostate adenocarcinoma), and L929 (mouse fibroblast) cell lines [19]. Cells were cultured in D5F medium and maintained at 37 °C in a humidified incubator with 5 % CO₂ until reaching 70–90 % confluence. A 50 μL aliquot of cell suspension (7,500 cells) was seeded into each well of a 96-well flat-bottom microplate (Corning, NY, USA) and incubated for 24 h under standard culture conditions. The extracts were tested at concentrations ranging from 50 to 400 $\mu\text{g/mL}$ by adding 50 μL of each sample per well, followed by a 24 h incubation. Four hours before the end of the incubation period, the medium was replaced with 100 μL of fresh D5F medium, and 10 μL of MTT solution (5 mg/mL) was added. After an additional 4 h incubation, the resulting formazan crystals were solubilized with 100 μL of DMSO. Absorbance was measured at 570 nm with a reference wavelength of 630 nm using a Multiskan GO microplate reader (Thermo Fisher Scientific, Waltham, USA).

Antibacterial activity assay

The antibacterial activity of *P. granatum* extracts was evaluated using the broth microdilution method following the Clinical and Laboratory Standards Institute (CLSI) guidelines [20]. The extracts were suspended in Mueller–Hinton broth and adjusted to final concentrations ranging from 100 to 800 $\mu\text{g/mL}$. A 100 μL aliquot of each concentration was dispensed into the wells of a 96-well microplate. The reference strains *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923 were subcultured on Mueller–Hinton agar, and bacterial suspensions were adjusted to a 0.5 McFarland standard ($\approx 1 \times 10^8$ CFU/mL). The inoculum was then diluted 20-fold, and 10 μL of the diluted suspension was added to each well containing the extracts. Plates were incubated at 37 °C for 24 h, and bacterial growth was measured spectrophotometrically at 620 nm using a

Multiskan GO microplate reader (Thermo Fisher Scientific, Waltham, MA, USA). Bacterial viability (%) was calculated by comparing the optical density of treated wells with that of the untreated control.

α -glucosidase inhibition assay

The α -glucosidase inhibitory activity was evaluated according to a previously described method [21]. Briefly, 50 μ L of phosphate-buffered saline (PBS pH 6.9) were mixed with 10 μ L of *P. granatum* extract at concentrations of 40, 60, 80, and 100 μ g/mL, followed by the addition of 50 μ L of α -glucosidase enzyme solution (0.5 U/mL) in a 96-well plate. The mixture was incubated for 10 min at 37 °C, after which 50 μ L of *p*-nitrophenyl- α -D-glucopyranoside (*p*-NPG, 5 mM) were added as substrate. The reaction was allowed to proceed for 20 min at 37 °C and stopped by adding 50 μ L of sodium carbonate solution (40 mg/mL). Absorbance was measured at 405 nm using a Multiskan GO microplate reader (Thermo Fisher Scientific, Waltham, MA, USA). Acarbose was used as the reference inhibitor.

Statistical analysis

Statistical analyses were performed using GraphPad Prism version 9.3.0 (GraphPad Software, San Diego, CA, USA). Data are presented as mean $\pm$ standard deviation. Two-way ANOVA followed by Šidák's multiple comparisons test was used for comparisons involving multiple treatments and concentrations. In the antiproliferative assay, comparisons between ethanolic and methanolic peel extracts were performed separately for each cell line using an unpaired Welch's *t*-test. Differences were considered statistically significant at *p* < 0.05.

Results

Determination of total phenolic and flavonoid contents and HPLC-based detection of ellagic acid in *P. granatum* extracts

We assessed the chemical composition of *P. granatum* peel, juice, and seed extracts by quantifying total phenolics and flavonoid contents, as well as by high performance liquid chromatography (HPLC) analysis. Table 1 summarizes the results for both ethanolic and methanolic extracts. Peel extracts contained the highest levels of phenolic compounds, flavonoids, and ellagic acid, followed by seeds and juice extracts. No significant differences were observed between ethanolic and methanolic extracts. HPLC analysis identified and quantified ellagic acid as the predominant compound, and the chromatographic profiles clearly differentiated peel, juice, and seed extracts. Among these, peel extracts (both ethanolic and methanolic) exhibited the greatest molecular diversity and concentration (Supplementary Material 1).

Table 1. Chemical composition of *P. granatum* L. extracts

Sample	Total Phenolics (mgEGA/g of extract)	Total Flavonoids (mgEQ/g of extract)	Ellagic acid (mg/g of extract)
EtOH-Peel	251.34 $\pm$ 8.40	31.10 $\pm$ 3.24	13.11 $\pm$ 0.32
MetOH-Peel	229.00 $\pm$ 11.14	39.05 $\pm$ 0.95	8.06 $\pm$ 0.09
EtOH-Seed	76.18 $\pm$ 5.16	< 30	1.40 $\pm$ 0.01
MetOH-Seed	74.16 $\pm$ 5.53	< 30	ND
EtOH-Juice	11.44 $\pm$ 1.25	< 30	1.69 $\pm$ 0.01
MetOH-Juice	15.78 $\pm$ 1.00	< 30	1.43 $\pm$ 0.03

Data represent the mean of three independent experiments $\pm$ standard deviations. ND: not detected.

Antioxidant activity

We assessed the antioxidant potential of peel, juice, and seed extracts of *P. granatum* using the DPPH assay. Table 2 presents the EC₅₀ values obtained for both ethanolic and methanolic extracts. Peel extracts exhibited the strongest antioxidant activity, with EC₅₀ values of 14.59 ± 1.04 µg/mL (ethanolic) and 15.80 ± 0.47 µg/mL (methanolic). Seed extracts showed moderate activity, with EC₅₀ values of 254.87 ± 13.99 µg/mL and 230.08 ± 14.73 µg/mL for ethanolic and methanolic extracts, respectively. In contrast, both juice extracts displayed weak antioxidant activity, with EC₅₀ values exceeding 400 µg/mL (Fig. 1).

Table 2. Antioxidant activity of *P. granatum* L. extracts by DPPH.

<i>P. granatum</i> extract	EC ₅₀ (µg/mL)
Ethanolic	
Peel	14.59 ± 1.04^a
Seed	254.87 ± 13.9^b
Juice	>400
Methanolic	
Peel	15.80 ± 0.47^a
Seed	230.08 ± 14.73^b
Juice	>400

Data represents the mean of three independent experiments $\pm$ standard deviations. Statistical comparisons were performed between fruit fractions within each solvent using an unpaired *t*-test. Different superscript letters within the same solvent indicate statistically significant differences between peel and seed extracts ($p < 0.05$). Extracts labeled with “a” showed significantly lower EC₅₀ values, indicating higher DPPH radical scavenging activity, whereas extracts labeled with “b” showed significantly higher EC₅₀ values, indicating lower DPPH radical scavenging activity.

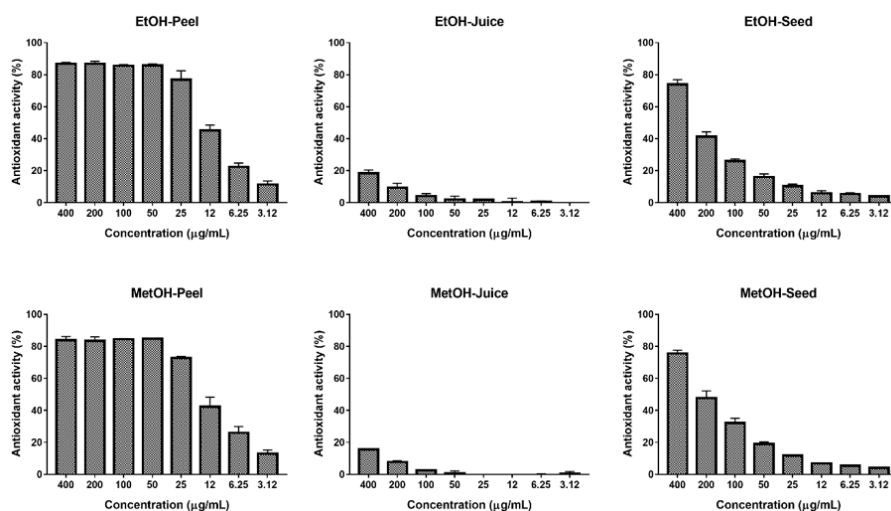


Fig. 1. Antioxidant activity of *P. granatum* L. extracts by DPPH radical. Data represent the mean of three independent experiments $\pm$ standard deviations.

Antiproliferative activity

We evaluated the antiproliferative activity of *P. granatum* extracts against two cancerous cell lines (HeLa and PC3) and one non-cancerous cell line (L929). Table 3 presents the IC₅₀ values for all extracts. Peel extracts exhibited the greatest antiproliferative activity among the tested extracts. Against HeLa cells, the ethanolic and methanolic peel extracts showed IC₅₀ values of 411.87 ± 70.03 µg/mL and 297.83 ± 39.32 µg/mL, respectively. Against PC3 cells, the corresponding IC₅₀ values were 187.82 ± 32.22 µg/mL and 204.85 ± 25.21 µg/mL, indicating greater susceptibility of PC3 cells compared to HeLa. In contrast, seed and juice extracts exhibited weak activity, with IC₅₀ values ≥ 400 µg/mL. For the non-cancerous L929 cells, all extracts presented high IC₅₀ values (>400 µg/mL), suggesting low cytotoxicity toward normal cells.

Table 3. Antiproliferative activity of *P. granatum* L. extracts against HeLa, PC3 and L929 cell lines.

Sample	IC ₅₀ (µg/mL)		
	HeLa	PC3	L929
EtOH-Peel	411.87 ± 70.03	187.82 ± 32.22	>400
MetOH-Peel	297.83 ± 39.32	204.85 ± 25.21	>400
EtOH-Seed	>400	>400	>400
MetOH-Seed	>400	>400	>400
EtOH-Juice	>400	>400	>400
MetOH-Juice	>400	>400	>400

Data represent the mean of three independent experiments $\pm$ standard deviations. Statistical comparisons were performed between ethanolic and methanolic peel extracts within each cell line using an unpaired Welch's *t*-test. No statistically significant differences were observed between extracts ($p > 0.05$).

Antibacterial activity

We evaluated the antibacterial potential of the extracts against *Escherichia coli* (Gram-negative) and *Staphylococcus aureus* (Gram-positive) clinically relevant bacterial models. As shown in Fig. 2, *E. coli* viability ranged from 60 % to 90 % across all treatments, with ethanolic and methanolic peel extracts displaying the highest activity at 800 µg/mL.

In contrast, *S. aureus* was more susceptible to the extracts. Both ethanolic and methanolic peel extracts completely inhibited bacterial growth at 800 µg/mL and reduced viability to approximately 20 % at the lowest tested concentration (100 µg/mL). Seed extracts also displayed inhibitory effect against *S. aureus* at 800 µg/mL, with viabilities of ~50 % for the ethanolic seed extract and ~30 % for the methanolic extracts. In comparison, neither ethanolic nor methanolic juice extracts exhibited measurable antibacterial activity.

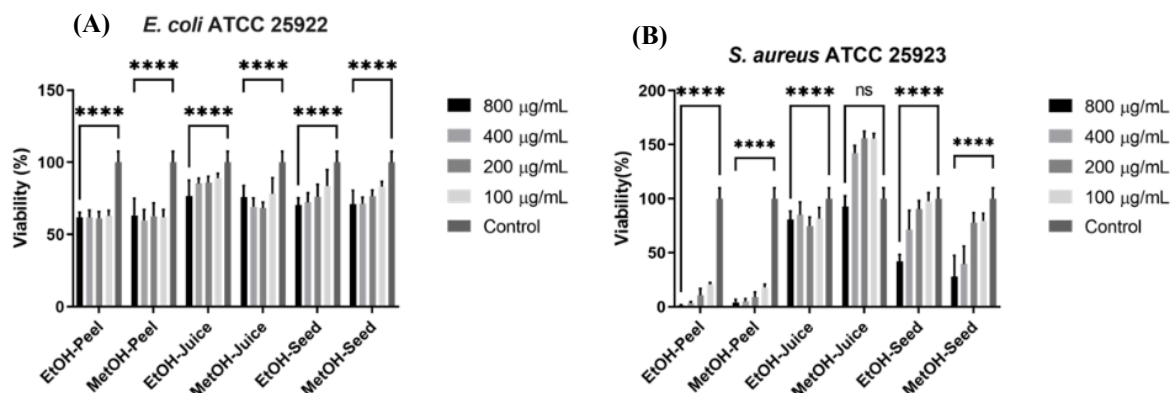


Fig. 2. Antibacterial activity of *P. granatum* L. extracts against (A) *Escherichia coli* ATCC 25922 and (B) *Staphylococcus aureus* ATCC 25923. Data represent the mean of two independent experiments $\pm$ standard deviations. Statistical analysis was performed using two-way ANOVA followed by Šídák's multiple comparisons test. For each extract, only the highest concentration tested (800 µg/mL) was compared with the untreated control. Asterisks indicate statistically significant differences; **** $p < 0.0001$.

α -glucosidase activity

As shown in Fig. 3, the methanolic peel extract produced complete inhibition of α -glucosidase at all tested concentrations (100–40 µg/mL), with minimal variability among replicates. The ethanolic peel extract also displayed strong inhibition (80–90 % inhibition) at 100–60 µg/mL but decreased to ~60 % at 40 µg/mL. These results indicate that the methanolic peel extract achieved maximal inhibition effect even at the lowest concentration tested, suggesting an IC_{50} value below 40 µg/mL, whereas the ethanolic extract displayed a concentration-dependent response.

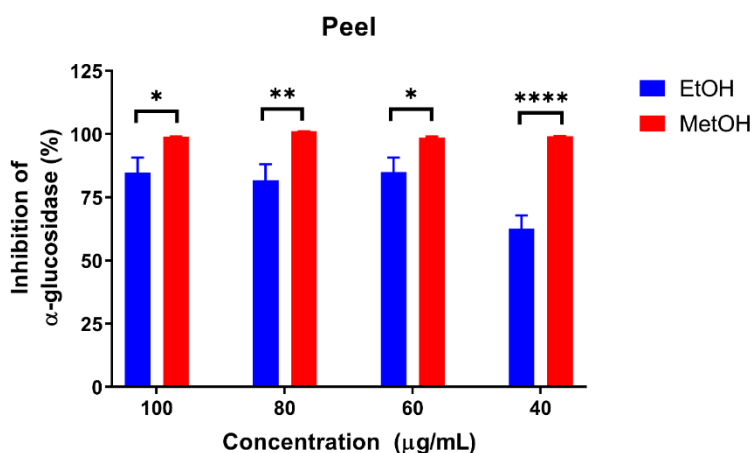


Fig. 3. α -glucosidase inhibition of *P. granatum* L. peel extracts. Data represent the mean of two independent experiments $\pm$ standard deviations. Statistical analysis was performed using two-way ANOVA followed by Šídák's multiple comparisons test. Asterisks indicate statistically significant differences between compared groups: $p < 0.05$ (*), $p < 0.01$ (**), and $p < 0.0001$ (***).

Discussion

Chemical composition of peel, seed, and juice

Our analysis showed that pomegranate peel extracts contained the highest levels of total phenolics and flavonoids, as well as the highest concentration of ellagic acid, which was the only individual compound identified and quantified by HPLC-DAD in the present study. Seed extracts showed intermediate values, whereas juice extracts generally presented the lowest levels. This compositional hierarchy is consistent with previous reports describing the peel as the pomegranate fraction with the greatest accumulation of phenolic compounds [22,23].

Previous studies have reported that pomegranate peel is particularly rich in hydrolysable tannins, including punicalagins and punicalin, as well as phenolic acids and flavonoids, whereas juice and seeds may show distinct profiles associated with anthocyanins and lipidic components, respectively [22,23]. In agreement with our results, Barathikannan et al. (2016) reported that methanolic peel extracts contained substantially higher total phenolic content than seed and juice fractions [24]. Thus, the higher total phenolic and flavonoid contents observed in peel extracts from Sonoran fruits are consistent with the general pattern of phenolic enrichment reported for this fruit fraction.

Environmental and geographical conditions are known to influence phenolic distribution. Studies conducted in Israel and Iran have shown that fruits from cooler climates accumulate greater amounts of anthocyanins and vitamin C than those from warmer, arid environments, underscoring the strong climatic modulation of fruit composition [25,26]. Overall, our data from Sonoran fruits fit with the established trend of phenolic enrichment in the peel, while also suggesting that regional conditions and processing factors may contribute to the composition observed. Further chromatographic or mass spectrometry-based analyses would allow a more comprehensive characterization of additional compounds reported in pomegranate extracts.

Antioxidant activity

The superior antioxidant activity of peel extracts, evidenced by EC₅₀ values as low as 14–16 µg/mL, correlates directly with their elevated phenolic content. This observation is consistent with previous reports that consistently associate the antioxidant capacity of pomegranate with its phenolic richness, particularly in the peel fraction [23,27]. Ellagitannins and ellagic acid are recognized as the major contributors to this effect, although catechins, anthocyanins, and other flavonoids may also play synergistic antioxidant actions [28].

Methodological parameters can also influence variations among studies. For example, ultrasound-assisted extraction has been shown to yield peel extracts with >90 % DPPH radical inhibition, surpassing conventional maceration methods [29]. In our case, the methanolic peel extract showed particularly strong scavenging activity at the lowest concentrations tested, likely due to the polarity of solvents and its efficiency in recovering phenolic constituents. Overall, these findings reinforce the strong relationship between high phenolic content and antioxidant efficacy in *P. granatum* extracts.

Antiproliferative effects

The antiproliferative assays demonstrated that peel extracts showed the greatest, although moderate, inhibitory effects on cancer cell viability, with PC3 prostate carcinoma cells showing greater sensitivity than HeLa cervical carcinoma cells. These findings are consistent with prior studies showing that pomegranate peel and seed extracts inhibited cancer cell growth through mechanisms involving reactive oxygen species (ROS) generation, cell cycle arrest, and activation of pro-apoptotic pathways [30]. Nasr et al. (2023), further reported modulation of key apoptotic regulators such as p53, Bax, and caspase-3, along with downregulating of the antiapoptotic protein Bcl-2, supporting the reported multitargeted effects of pomegranate-derived compounds in cancer models [30]. It is important to highlight that, all extracts displayed limited cytotoxicity toward non-cancerous L929 fibroblasts, corroborating previous observations that pomegranate phenolic compounds may exert greater effects on malignant cells than on non-cancerous cells [30]. Overall, these findings support the potential of phenolic-rich peel fractions as candidates for further investigation as phenolic-rich extracts with moderate antiproliferative activity and low cytotoxicity toward L929 cells.

Antibacterial activity

Our antibacterial assays revealed that *S. aureus* was more susceptible to the extracts than *E. coli*, with ethanolic and methanolic peel extracts achieving complete inhibition at 800 µg/mL. This Gram-specific pattern aligns with numerous studies reporting that Gram-positive bacteria are generally more sensitive to pomegranate derived compounds (Kumar et al., 2023; Kupnik et al., 2021). Peel extracts are consistently identified as the most effective fraction, primarily attributed to the presence of punicalagins, ellagic acid, and related hydrolysable tannins that disrupt bacterial membranes, proteins and metabolic enzymes [28,31,32]. Our results fall within the inhibitory ranges documented in previous broth microdilution studies, which often report minimum inhibitory concentration (MIC) values between 0.2 and 1 mg/mL for pomegranate peel extracts against Gram-positive pathogens [33]. These results confirm the strong antibacterial potential of peel extracts and underscore their particular relevance for food preservation and natural antimicrobial formulations. Recent studies have further elucidated the molecular mechanisms underlying the antibacterial activity of *P. granatum*. Phytochemicals such as tannins, flavonoids, and phenolic acids disrupt bacterial physiology through multiple pathways, including inhibition of nucleic acid and protein synthesis, interference with membrane integrity, and enzyme inactivation. Computational studies have shown that epicatechin, kaempferol, and apigenin from *P. granatum* establish stable interactions with CTX-M-9 β -lactamase, effectively blocking its catalytic site and counteracting a key resistance mechanism in multidrug-resistant *E. coli*. Complementary *in vitro* assays support this evidence, demonstrating that pomegranate extracts exert broad-spectrum antibacterial activity through both membrane perturbation and targeted inhibition of bacterial resistance enzymes [34,35].

α -Glucosidase inhibitory (hypoglycemic) activity

The methanolic peel extract completely inhibited α -glucosidase activity at all tested concentrations, whereas ethanolic peel extract maintained strong activity but declined to ~60 % inhibition at 40 µg/mL. These results are consistent with previous studies identifying ellagic acid, catechins, vanillin, and vanillic acid as major contributors to α -glucosidase and α -amylase inhibition [21]. Reported IC₅₀ values for pomegranate peel extracts vary widely depending on solvent and extraction conditions, ranging from approximately 10 µg/mL for acetone and aqueous extracts to as high as 1000 µg/mL for methanolic extracts [36,37]. The difference observed between methanolic and ethanolic preparations in our study likely reflects solvent-specific efficiencies in recovering these bioactive phenolics. Overall, our results indicate that phenolic-rich pomegranate peel fractions can inhibit α -glucosidase activity, suggesting their potential relevance for modulating carbohydrate digestion. However, since hypoglycemic activity cannot be concluded from this assay alone, further studies using complementary enzymatic, cellular, and *in vivo* models are required to confirm their biological relevance for diabetes management.

Valorization of peel and seed by-products

The consistent superiority of the peel extract across all biological assays underscores its high potential for valorization. The peel constitutes nearly 50 % of the total fruit mass and is commonly discarded as agro-industrial waste, despite being the most concentrated source of bioactive compounds [23,28]. Recent studies have demonstrated the successful incorporation of pomegranate peel powder into functional foods such as cereal bars, cheese, yogurts, and beverages, resulting in enhanced antioxidant capacity and improving product stability [38,39]. Beyond the food sector, pomegranate peel has been investigated for applications in active packaging films, natural dyes, adsorbents, and even bioenergy production, reflecting its versatility within sustainable and circular bioeconomy frameworks [40,41]. The present findings further justify the development of peel-derived functional ingredients and nutraceuticals formulations, offering an environmental responsible strategy for the revalorization of pomegranate byproducts.

Limitations and perspectives

A limitation of this study is that the chromatographic analysis was restricted to ellagic acid, leaving other major constituents such as punicalagins, gallic acid and anthocyanins uncharacterized. Comprehensive metabolomic profiling would enable a more detailed understanding of the relationships between chemical composition and bioactivity. In addition, climatic and agronomic factors in Sonora may influence phenolic accumulation differently from those in Mediterranean or Asian regions, suggesting the need for comparative studies under varying environmental conditions [25,26].

Despite these constraints, the consistency of our results with previously published data supports the conclusion that pomegranate peel extract represents the most bioactive fraction, displaying strong antioxidant, antiproliferative, antibacterial, and α -glucosidase inhibitory effects. Collectively, these findings highlight its potential as a high value ingredient for functional food development and strengthen the case for large scale valorization of pomegranate byproducts.

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References

1. Ambigaipalan, P.; de Camargo, A. C.; Shahidi, F. *J. Agric. Food Chem.* **2016**, 64, 6584–6604. DOI: <http://dx.doi.org/10.1021/acs.jafc.6b02950>
2. Liu, Y.; Seeram, N. P. *J. Sep. Sci.* **2018**, 41, 3022–3033. DOI: <http://dx.doi.org/10.1002/jssc.201800480>
3. <https://www.gob.mx/agricultura/articulos/que-hay-detras-de-la-produccion-de-granada?idiom=es>, accessed in October, 2025.
4. Vučić, V.; Grabež, M.; Trchounian, A.; Arsić, A. *Curr. Pharm. Des.* **2019**, 25, 1817–1827. DOI: <http://dx.doi.org/10.2174/1381612825666190708183941>
5. Bielli, P.; Calabrese, L. *Cell. Mol. Life Sci.* **2002**, 59, 1413–1427. DOI: <http://dx.doi.org/10.1007/s00018-002-8519-2>
6. Hegazi, N. M.; El-Shamy, S.; Fahmy, H.; Farag, M. A. *J. Food Compos. Anal.* **2021**, 97, 103773. DOI: <http://dx.doi.org/10.1016/j.jfca.2020.103773>
7. Sabraoui, T.; Khider, T.; Nasser, B.; Eddoha, R.; Moujahid, A.; Benbachir, M.; Essamadi, A. *Int. J. Food Sci.* **2020**, 2020, 8885889. DOI: <http://dx.doi.org/10.1155/2020/8885889>
8. Pathak, P. D.; Mandavgane, S. A.; Kulkarni, B. D. *Rev. Chem. Eng.* **2015**, 31. DOI: <http://dx.doi.org/10.1515/revce-2014-0041>
9. Sorrenti, V.; Randazzo, C. L.; Caggia, C.; Ballistreri, G.; Romeo, F. V.; Fabroni, S.; Timpanaro, N.; Raffaele, M.; Vanella, L. *Front. Microbiol.* **2019**, 10, 660. DOI: <http://dx.doi.org/10.3389/fmicb.2019.00660>
10. Shrinet, K.; Singh, R. K.; Chaurasia, A. K.; Tripathi, A.; Kumar, A., in: *Natural Bioactive Compounds*, Elsevier, **2021**, 337–362. DOI: <http://dx.doi.org/10.1016/B978-0-12-820655-3.00017-3>
11. Banihani, S.; Swedan, S.; Alguraan, Z. *Nutr. Res.* **2013**, 33, 341–348. DOI: <http://dx.doi.org/10.1016/j.nutres.2013.03.003>
12. Huang, T.; Peng, G.; Kota, B.; Li, G.; Yamahara, J.; Roufogalis, B.; Li, Y. *Toxicol. Appl. Pharmacol.* **2005**, 207, 160–169. DOI: <http://dx.doi.org/10.1016/j.taap.2004.12.009>
13. Koren-Gluzer, M.; Aviram, M.; Meilin, E.; Hayek, T. *Atherosclerosis*. **2011**, 219, 510–518. DOI: <http://dx.doi.org/10.1016/j.atherosclerosis.2011.07.119>
14. Ko, K.; Dadmohammadi, Y.; Abbaspourrad, A. *Foods*. **2021**, 10, 657. DOI: <http://dx.doi.org/10.3390/foods10030657>
15. Singleton, V. L.; Rossi, J. A. *Am. J. Enol. Vitic.* **1965**, 16, 144–158.

16. Popova, M.; Bankova, V.; Butovska, D.; Petkov, V.; Nikolova-Damyanova, B.; Sabatini, A. G.; Marcazzan, G. L.; Bogdanov, S. *Phytochem. Anal.* **2004**, *15*, 235–240. DOI: <http://dx.doi.org/10.1002/pca.777>
17. Cantos, E.; García-Viguera, C.; de Pascual-Teresa, S.; Tomás-Barberán, F. A. *J. Agric. Food Chem.* **2000**, *48*, 4606–4612. DOI: <http://dx.doi.org/10.1021/jf0002948>
18. Usia, T.; Banskota, A. H.; Tezuka, Y.; Midorikawa, K.; Matsushige, K.; Kadota, S. *J. Nat. Prod.* **2002**, *65*, 673–676. DOI: <http://dx.doi.org/10.1021/np010486c>
19. Grunberger, D.; Banerjee, R.; Eisinger, K.; Oltz, E. M.; Efros, L.; Caldwell, M.; Estevez, V.; Nakanishi, K. *Experientia* **1988**, *44*, 230–232. DOI: <http://dx.doi.org/10.1007/BF01941717>
20. Clinical and Laboratory Standards Institute. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard*, 9th ed.; CLSI: Wayne, PA, **2012**; Vol. 32.
21. Laaraj, N.; Bouhrim, M.; Kharchoufa, L.; Tiji, S.; Bendaha, H.; Addi, M.; Drouet, S.; Hano, C.; Lorenzo, J. M.; Bnouham, M.; Mimouni, M. *Foods* **2022**, *11*, 1353. DOI: <http://dx.doi.org/10.3390/foods11091353>
22. Derakhshan, Z.; Ferrante, M.; Tadi, M.; Ansari, F.; Heydari, A.; Hosseini, M. S.; Conti, G. O.; Sadrabad, E. K. *Food Chem. Toxicol.* **2018**, *114*, 108–111. DOI: <http://dx.doi.org/10.1016/j.fct.2018.02.023>
23. Kumar, S.; Singh, V.; Johar, V.; Varma, S.; Thakur, R.; Banerjee, D. *J. Food Chem. Nanotechnol.* **2023**, S588–S593. DOI: <http://dx.doi.org/10.17756/jfcn.2023-s1-074>
24. Barathikannan, K.; Venkatadri, B.; Khushro, A.; Al-Dhabi, N. A.; Agastian, P.; Arasu, M. V.; Choi, H. S.; Kim, Y. O. *BMC Complement. Altern. Med.* **2016**, *16*, 264. DOI: <http://dx.doi.org/10.1186/s12906-016-1237-3>
25. Borochoy-Neori, H.; Judeinstein, S.; Harari, M.; Bar-Ya'akov, I.; Patil, B. S.; Lurie, S.; Holland, D. *J. Agric. Food Chem.* **2011**, *59*, 5325–5334. DOI: <http://dx.doi.org/10.1021/jf2003688>
26. Ghasemi-Soloklui, A. A.; Kordrostami, M.; Gharaghani, A. *Sci. Rep.* **2023**, *13*, 15899. DOI: <http://dx.doi.org/10.1038/s41598-023-42749-z>
27. Kupnik, K.; Primožič, M.; Vasić, K.; Knez, Ž.; Leitgeb, M. *Plants* **2021**, *10*, 1554. DOI: <http://dx.doi.org/10.3390/plants10081554>
28. Magangana, T. P.; Makunga, N. P.; Fawole, O. A.; Opara, U. L. *Molecules* **2020**, *25*, 4690. DOI: <http://dx.doi.org/10.3390/molecules25204690>
29. Habchi, C.; Badran, A.; Srouf, M.; Daou, A.; Baydoun, E.; Hamade, K.; Hijazi, A. *Processes* **2023**, *11*, 1111. DOI: <http://dx.doi.org/10.3390/pr11041111>
30. Nasr, M.; Naeem, S. A.; El-Shenbaby, I.; Mohamed, F. M. A.; Mahmoud, S. M.; Abuamara, T. M. M.; Abd-Elhay, W. M.; Elbayoumy, F. M. A. E.; Elkot, A.; Shikhon, T.; Abo-Akrab, M.; Doma, M. A.; Hasan, A. *J. Exp. Pharmacol.* **2023**, *15*, 191–205. DOI: <http://dx.doi.org/10.2147/JEP.S404321>
31. Shabir, I.; Dar, A. H.; Dash, K. K.; Manzoor, S.; Srivastava, S.; Pandey, V. K.; Shams, R.; Bashir, I.; Khan, S. A.; Mukarram, S. A.; Kovács, B. *Appl. Food Res.* **2024**, *4*, 100572. DOI: <http://dx.doi.org/10.1016/j.afres.2024.100572>
32. Silva, C. R. e A.; Vidal, C. S.; Filho, S. M. de A.; Agatão, I. M.; Berbert, L. C.; Salles, J. B. de; Cardoso, A. M.; Kuster, R. M.; Victório, C. P.; Assis, M. C. de. *Molecules* **2025**, *30*, 2387. DOI: <http://dx.doi.org/10.3390/molecules30112387>
33. Fawole, O. A.; Makunga, N. P.; Opara, U. L. *BMC Complement. Altern. Med.* **2012**, *12*, 200. DOI: <http://dx.doi.org/10.1186/1472-6882-12-200>
34. Tasnim, F.; Hosen, M. E.; Hasan, A. R.; Hasan, M. T.; Rahaman, M. M.; Hossain, M. M.; Jubayed, M. A. Al; Aktaruzzaman, M.; Altayyar, M.; Yihune, E.; Islam, M. T.; Zaki, M. E. *A. Sci. Rep.* **2025**, *15*. DOI: <http://dx.doi.org/10.1038/s41598-025-02065-0>

35. Scaglione, E.; Sateriale, D.; Mantova, G.; Di Rosario, M.; Continisio, L.; Vitiello, M.; Pagliarulo, C.; Colicchio, R.; Pagliuca, C.; Salvatore, P. *Front. Microbiol.* **2024**, *15*, 1383027. DOI: <http://dx.doi.org/10.3389/fmicb.2024.1383027>
36. Kam, A.; Li, K. M.; Razmovski-Naumovski, V.; Nammi, S.; Shi, J.; Chan, K.; Li, G. Q. *Phytother. Res.* **2013**, *27*, 1614–1620. DOI: <http://dx.doi.org/10.1002/ptr.4913>
37. Shi, R.; Zhou, N.; Zhang, H.; Gong, M.; Han, L. *Front. Nutr.* **2022**, *9*, 1014862. DOI: <http://dx.doi.org/10.3389/fnut.2022.1014862>
38. Kandyli, P.; Kokkinomagoulos, E. *Foods.* **2020**, *9*, 122. DOI: <http://dx.doi.org/10.3390/foods9020122>
39. Costa, N. H. B.; Amadio, G. B.; Souza-Santos, Y. J.; Carvalho, R. A.; Vanin, F. M. *Food Sci. Nutr.* **2025**, *13*, e70690. DOI: <http://dx.doi.org/10.1002/fsn3.70690>
40. Bertolo, M. R. V.; Martins, V. C. A.; Horn, M. M.; Brenelli, L. B.; Plepis, A. M. G. *Carbohydr. Polym.* **2020**, *228*, 115386. DOI: <http://dx.doi.org/10.1016/j.carbpol.2019.115386>
41. Dhande, D. Y.; Nighot, D. V.; Sinaga, N.; Dahe, K. B. *Renew. Sustain. Energy Rev.* **2021**, *149*, 111349. DOI: <http://dx.doi.org/10.1016/j.rser.2021.111349>