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Chemical Characterization and Antibacterial Activity of Aqueous Extracts from Dried Pomegranate Peels and Pressed Seed Residues

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Abstract

This study investigated the proximate chemical composition and qualitative bioactive profile of dry pomegranate peels and pressed seed residues (obtained after juice extraction), while evaluating the antibacterial activity of both their fresh and dry aqueous extracts. Methodology involved applying standard and modified AOAC methods on the dry plant parts to determine basic components (moisture, ash, fiber, fat, protein, and reducing sugars). Aqueous extraction using distilled water was performed on the dry materials for chemical screening of nine bioactive groups, and on both fresh and dry forms for the antibacterial assay against *Escherichia coli*, *Klebsiella*, and *Streptococcus* species. Results revealed that dry peels contained 8.00 % ± 0.01 moisture, 4.9 % ± 0.07 ash, 21.00 % ± 0.77 fiber, 5.00 % ± 0.97 fat 6.14 %, ± 0.09 protein, and 7.00 % ± 0.89 reducing sugars, while dry pressed seed residues contained 14.50 % ± 0.75 moisture, 3.60 % ± 0.25 ash, 37.00 % ± 1.02 fiber, 10.00 % ± 0.98 fat, 8.76 % ± 0.05 protein, and 20.00 % ± 0.35 reducing sugars. Qualitative screening of the dry parts confirmed the presence of nine bioactive groups; based on a semi-quantitative assessment of reaction color intensity, phenols were highest (+++) in pressed seed residues, followed by peels (++). Notably, the antibacterial assay demonstrated potent growth inhibition against most of the tested pathogenic bacterial strains across the evaluated

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extracts. Overall, these positive findings strongly support utilizing dry pomegranate residues as potent and sustainable natural sources for functional food and pharmaceutical industries, particularly for their application as antibacterial agents.
Keywords: Pomegranate residues, pomegranate peels, pressed seed residues, phenols, Aqueous extract, proximate composition, antimicrobial, Klebsiella.

التوصيف الكيميائي والنشاط المضاد للبكتيريا للمستخلصات المائية لقشور وبذور الرمان الجافة

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الملخص

تناولت هذه الدراسة تقييم التركيب الكيميائي الأساسي والمحتوى النوعي للمركبات الحيوية الفعالة لقشور وبذور الرمان الجافة (المتحصل عليها كمخلفات بعد عصر العصير)، مع تقييم النشاط المضاد للبكتيريا لمستخلصاتها المائية الطازجة والجافة على حد سواء. واشتملت منهجية العمل على تطبيق طرق الجمعية الرسمية للكيميائيين التحليليين المعمليين (AOAC) القياسية والمعدلة على الأجزاء النباتية الجافة لتحديد المكونات الأساسية (الرطوبة، الرماد، الألياف، الدهون، البروتين، والسكريات المختزلة). تم إجراء الاستخلاص المائي باستخدام الماء المقطر على المواد الجافة للكشف الكيميائي عن تسع مجموعات حيوية فعالة، وعلى كلا الصورتين (الطازجة والجافة) لإجراء اختبار النشاط المضاد للبكتيريا ضد سلالات *Escherichia coli* و*Klebsiella* و*Streptococcus*. أظهرت النتائج أن القشور الجافة تحتوي على رطوبة بنسبة 8.00% ± 0.01، ورماد بنسبة 4.9% ± 0.07، وألياف بنسبة 21.00% ± 0.77، ودهون بنسبة 5.00% ± 0.97، وبروتين بنسبة 6.14% ± 0.09، وسكريات مختزلة بنسبة

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7.00% ± 0.89. وفي المقابل، احتوت بقايا البذور المعصورة الجافة على رطوبة بنسبة 14.50% ± 0.75، ورماد بنسبة 3.60% ± 0.25، وألياف بنسبة 37.00% ± 1.02، ودهون بنسبة 10.00% ± 0.98، وبروتين بنسبة 8.76% ± 0.05، وسكريات مختزلة بنسبة 20.00% ± 0.35. وأكّد الفحص النوعي للأجزاء الجافة وجود المجموعات الحيوية التسعة؛ واستناداً إلى التقييم شبه الكمي لكثافة لون التفاعل، سجلت الفينولات أعلى ظهور (+++) في بقايا البذور المعصورة تليها القشور. ومن الجدير بالذكر أن اختبار النشاط المضاد للبكتيريا أظهر تثبيطاً قوياً لنمو معظم السلالات البكتيرية الممرضة المختبرة عبر المستخلصات المعنية.

الكلمات المفتاحية :مخلفات الرمان، قشور الرمان، بقايا البذور المعصورة، الفينولات، المستخلص المائي، التركيب الكيميائي الأساسي، مضاد للميكروبات، كليبسيلا.

Introduction:

The pomegranate plant (*Punica granatum* L.), belonging to the family *Punicaceae* and now widely classified under *Lythraceae*, holds substantial commercial and therapeutic value due to its exceptionally rich profile of natural phytochemicals. It serves as a potent source of polyphenols, flavonoids, and anthocyanins, which contribute to its prominent antioxidant capabilities and characteristic pigmentation (Todaro et al., 2016). Extensive literature has verified that various anatomical parts of the pomegranate house a diverse array of bioactive constituents, notably hydrolysable tannins and phenolic acids (Viuda-Martos et al., 2012). Recent pharmacological investigations indicate that these polyphenolic complex matrices exert neuroprotective effects, positively influence the blood-brain barrier to enhance cerebral blood flow (Aleksandrova et al., 2023), modulate gut microbiota dynamics, and suppress systemic inflammation and oxidative stress (Panchal et al., 2022). Furthermore, agro-industrial byproducts of pomegranate processing, such as peels and pressed seed residues, are increasingly recognized for their capacity to suppress critical enteric pathogens, including *Salmonella* and *Escherichia coli*, mitigating environmental waste while serving as natural antimicrobials (Gullon et al., 2016).

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Despite the abundance of literature characterizing the generic attributes of Punica granatum, a distinct knowledge gap persists regarding the utilization of pressed seed residues (obtained as a byproduct after juice extraction) in comparison to peels. Crucially, prior studies have predominantly focused on organic or single-state extracts, leaving a scarcity of comparative baseline data regarding the differential antimicrobial performance of purely aqueous extracts utilized in their fresh versus dehydrated (dry) states. The stability and retention of complex active groups following drying processes remain poorly understood, particularly concerning their targeted efficacy against opportunistic Gram-negative pathogens like *Klebsiella* species.

To address this empirical gap, the present study establishes a comprehensive chemical and biological baseline profile for regional pomegranate residues. The primary objective is to execute a rigorous proximate chemical characterization of dry pomegranate peels and pressed seed residues utilizing validated standard and modified AOAC protocols, combined with a broad qualitative phytochemical screening of nine major bioactive families. Concurrently, this work explicitly compares the qualitative antibacterial potency of both fresh and dry aqueous extracts against key pathogenic strains (*Escherichia coli, Klebsiella* species, and *Streptococcus* species) utilizing a direct microbial inhibition assay based on visual turbidity monitoring. This integrated approach not only provides necessary reference data but also evaluates the functional feasibility of incorporating green, water-extracted pomegranate residues into circular-economy food systems and eco-friendly pharmaceutical industries.

Materials and Methods

Sample Collection and Preparation:

Pomegranate (*Punica granatum* L.) fruits were purchased from local retail markets in Sabratha, Libya, between September 2025 and May 2026. To ensure a highly representative and accurate data profile, a total of 65 samples were collected and analyzed. The fruits were thoroughly washed with distilled water, and the peels were

manually separated from the pressed residues. The arils were then mechanically pressed to extract the juice completely, and the resulting pressed seed residues (juice-free pomace) were collected. Both tissues (peels and pressed seed residues) were then oven-dried at 40 °C and finely ground into a powder. To eliminate individual variability among the fruits, a single homogeneous pooled sample was prepared by thoroughly mixing equal amounts of powder from all processed fruits. From this grand pooled batch, independent representative samples were drawn for analysis. To ensure data accuracy and reproducibility, all chemical analyses, phytochemical screenings, and antibacterial assays were performed in triplicate ($n = 3$).

Phytochemical Analysis of Peels and Pressed seed residues:

This section details the screening and detection of active functional groups for specific bioactive compounds present in the investigated samples.

Aqueous Extract of Pomegranate Samples (Pressed seed residues and Peels):

Water was selected as the extraction solvent based on critical scientific, economic, and ethnomedicinal considerations. Its high polarity and dielectric constant ensure the efficient dissolution of hydrophilic compounds abundant in pomegranate residues, such as tannins and other phenolic compounds. Strategically, utilizing water aligns with Green Chemistry principles by offering a non-toxic, sustainable, and cost-effective process that avoids hazardous organic residues. Crucially, this choice mirrors traditional medicine and routine domestic applications, where water is universally employed to prepare remedies. Investigating aqueous extracts directly validates these empirical folk practices, bridging the gap between historical ethnomedicinal knowledge and modern pharmaceutical applications (Ahmed et al., 2026; Sun et al., 2025; Tamborlin et al., 2020).

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Preparation of Aqueous Extract of Pomegranate Samples (Pressed seed residues and Peels):

For the qualitative phytochemical screening, aqueous extracts of dry pomegranate peels and pressed seed residues were prepared through a controlled maceration process. A 2 g sample of each plant powder was accurately weighed and transferred into a 100 mL beaker containing 50 mL of distilled water, establishing a concentration of 4% (w/v). This concentration was systematically chosen to yield a clear, light-colored filtrate, thereby preventing the intense natural pigments of the pomegranate matrix from interfering with the visual colorimetric and precipitation endpoints of the chemical screening reagents. The mixture was continuously stirred using a magnetic stirrer for 4 hours at an ambient temperature of 25 °C. Following extraction, the suspension was subjected to centrifugation (at 2000 rpm for 20 min) to isolate the clear supernatant, which was subsequently utilized as the stock filtrate for the qualitative bioactive screening experiments.

Qualitative Screening of Bioactive Compounds:

To evaluate the chemical profile of the obtained aqueous extract, the following qualitative tests were conducted. These screening analyses were performed to detect the presence of major secondary metabolites, including tannins, flavonoids, saponins, and alkaloids, following standard established protocols. The specific experimental procedures for each qualitative test are outlined below, and the corresponding results are comprehensively summarized in Table 1.

Detection of Glycosides: Glycosides were detected using Benedict's test to screen for reducing sugars following acid hydrolysis. The formation of a reddish-brown (brick-red) precipitate after heating in a water bath indicated a positive (Oshah, Z. et al., 2019).

Detection of Alkaloids: A 1 mL aliquot of Dragendorff's reagent was added to a test tube containing 5 mL of the aqueous extract. The appearance of an orange-red precipitate confirmed a positive test result (Kancherla et al., 2019).

Detection of Flavonoids: Flavonoids were screened by mixing 2 mL of the aqueous extract with 5 mL of dilute ammonia solution in a test tube. A yellow coloration was observed in the pressed seed residues extract, while an orange coloration appeared in the peel extract. Upon adding a few drops of concentrated sulfuric acid, resulting in the decolorization of the solution, which indicates a positive result (Ghamiaa et al., 2023).

Detection of Phenols: To assess the phenolic content, a 3 mL aliquot of the aqueous extract was transferred into a test tube, followed by the addition of 2 mL of ferric chloride solution. The emergence of a bluish-green coloration confirmed a positive reaction (Ghamiaa et al., 2023).

Detection of Resins: Resins were detected according to the following procedure: 1g of the pomegranate sample powder was placed into a test tube with 5ml of Ethanol then incubated in a water bath for 2 minutes. Following this, a few drops of acidified water were added to the alcoholic extract. The development of a distinct turbidity in the tube served as evidence of a positive test (Shaikh & Patil, 2020).

Detection of Terpenoids and Steroids: A 1 mL aliquot of the aqueous extract was placed in a test tube, to which 2 mL of chloroform, along with a few drops of acetic acid and sulfuric acid, were added. The appearance of a purple ring indicated a positive test for terpenoids, whereas the formation of a blue ring after allowing the mixture to stand for 12 hours confirmed a positive test for steroids (Ghamiaa et al., 2023).

Detection of Saponins: A 5 g of powder sample was mixed with 5 mL of distilled water in a test tube and shaken vigorously. The persistent formation of a stable foam for 6 minutes under cold conditions indicated a positive result (Oncho et al., 2021).

Detection of Tannins: Tannins were screened by taking a 2 mL aliquot of the aqueous extract, to which a few drops of lead acetate were added. The appearance of a white precipitate confirmed a positive reaction (Baloch et al., 2024).

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Quantitative Determination of Proximate Chemical Composition:

The basic chemical constituents of the sample were quantitatively evaluated. The full profile of these primary components is illustrated in Table 2 and graphically represented in Figure 1.

Estimation of Protein Content:

Total nitrogen and crude protein contents in dried pomegranate peels (0.2056 g) and pressed seed residues (0.4304 g) were determined using a modified Kjeldahl digestion method combined with spectrophotometric quantification. Samples were digested with concentrated H_2SO_4 in the presence of a catalyst, and subsequently diluted to a total volume of 100 mL. For the spectrophotometric analysis, the peel digest was diluted 5*X*, whereas the pressed seed residue digest underwent a 50*X* dilution (achieved by sequential 5*X* and 10*X* dilutions). Diluted aliquots were reacted with Nessler's reagent, and the absorbance was recorded at 420 nm using a spectrophotometer. Nitrogen concentrations (x, in ppm) were derived from the absorbance values (y) using the linear regression equation obtained from an ammonium sulfate standard curve (1–6 ppm) plotted in Figure 2: $y = 0.0989x + 0.0107$ ($R^2 = 0.9982$). The actual nitrogen concentration was calculated by multiplying the measured concentration (x) $x = (y - 0.0107) / 0.0989$) by the respective dilution factor (*DF*). The total mass of nitrogen (g) was computed as follows:

$$\textbf{Mass of N (g)} = \frac{\textbf{Actual Conc. (mg/L)} \times \textbf{Total Volume (L)}}{\textbf{1000}}$$

Finally, the percentages of nitrogen and crude protein were quantified sequentially using the following equations (Koch & McMeekin, 2002): (Koch & McMeekin, 2002).

$$\%\textbf{Nitrogen} = \frac{\text{Mass of N (g)}}{\text{Sample Weight (g)}} \times 100$$

$$\%\, Crude\ Protein = \%\, Nitrogen \times 6.25$$

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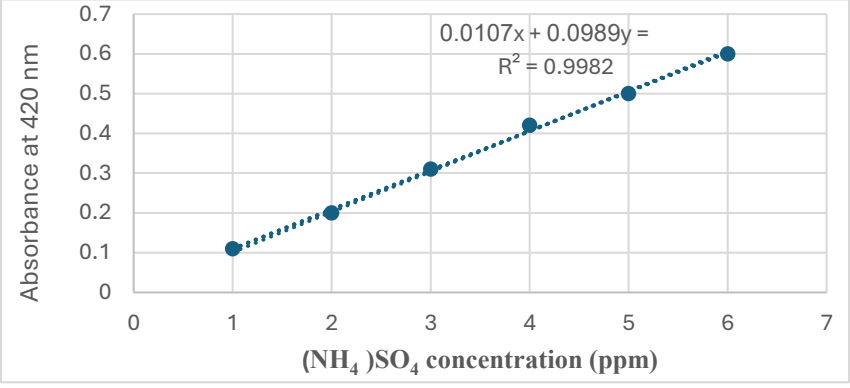

Figure 2. Standard calibration curve of ammonium sulfate for the determination of total nitrogen content.

Estimation of Crude Lipid Content*:*

The crude lipid percentage in the pomegranate samples was determined by the Randall submersion method using *n*-hexane as the extraction solvent, following the standard collaborative protocol. Briefly, the prepared samples were directly immersed into boiling hexane to initiate rapid lipid extraction. This submersion technique noticeable decreases the required extraction time while maintaining high efficiency. After the extraction phase, automated solvent evaporation and recovery were completed. The final crude lipid fractions were then calculated as a weight-percentage basis. (Thiex et al., 2003).

Estimation of Crude Fiber Content:

The fundamental principle for estimating crude fiber content relies on sequentially removing all non-fibrous organic and inorganic components, following the standard Weende method. First, the pre-defatted sample was boiled with a dilute sulfuric acid solution (1.25% w/v H_2SO_4) for 30 min to hydrolyze sugars and starches, followed by thorough washing with hot distilled water. Subsequently, the remaining residue was boiled with a dilute sodium hydroxide solution (1.25% w/v NaOH) for 30 min to remove proteins and some hemicellulose fractions, then filtered through a fine cloth mesh. The isolated residue was dried, weighed, and then incinerated in a muffle furnace at 550 °C to remove all remaining

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organic matter. Finally, the crude fiber content was quantified by calculating the weight loss percentage of the sample post-incineration (Ghamiaa et al., 2023).

Estimation of Moisture Content*:*

Moisture content was determined by removing water using a drying oven at 105 °C until a constant sample weight was achieved. The difference in weight was then calculated and expressed as a percentage relative to the initial sample (Oshah et al., 2019).

Estimation of Total Ash Content:

The total ash content was estimated on a dry weight basis by incinerating the samples in a muffle furnace at 555 °C (Oshah et al., 2019).

Estimation of Reducing Sugars Content*:*

The reducing sugar content in the pomegranate extract was determined spectrophotometrically according to the micro-Benedict method. Briefly, a 0.5 mL aliquot of the diluted pomegranate extract (10X dilution) was mixed with 1.0 mL of Benedict's reagent in a test tube, bringing the total reaction volume to 1.5 mL. The mixture was heated in a boiling water bath at 100 °C for 5 minutes, followed immediately by rapid cooling in an ice bath for 5 minutes to terminate the reaction. Subsequently, the solution was centrifuged to precipitate cuprous oxide Cu_2O, and the absorbance of the clear supernatant was measured at a wavelength of 740 nm using a UV-Vis spectrophotometer (Hernández-López et al., 2020).

The concentrations of reducing sugars in the samples were quantified by substituting the recorded absorbance values into the linear regression equation derived from the standard calibration curves. Specifically, the calibration curve plotted in Figure 3 was utilized for the pressed seed residue extracts, whereas the curve shown in Figure 4 was applied for the peel extracts:

$$\boldsymbol{y = ax + b}$$

Where:

$\boldsymbol{b}$ = The y-intercept of the regression line.

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a = The slope of the regression line.

$$C_{aliquot}(ppm) = (y - b) / a$$

C final = Final True Concentration on a Dry Weight Basis

$$C_{final}\left(\frac{mg}{g}\right) = \frac{(C_{aliquot} * DF * VL)}{Wg}$$

Where:

DF = Dilution Factor of the crude extract.

V= Total final volume of the crude aqueous extract in Liters (L).

W = Initial mass of the dry sample powder in grams (g).

$$\text{Percentage Yield (\% w/w)} = \left(\frac{C_{final}\left(\frac{mg}{g}\right)}{1000}\right) * 100$$

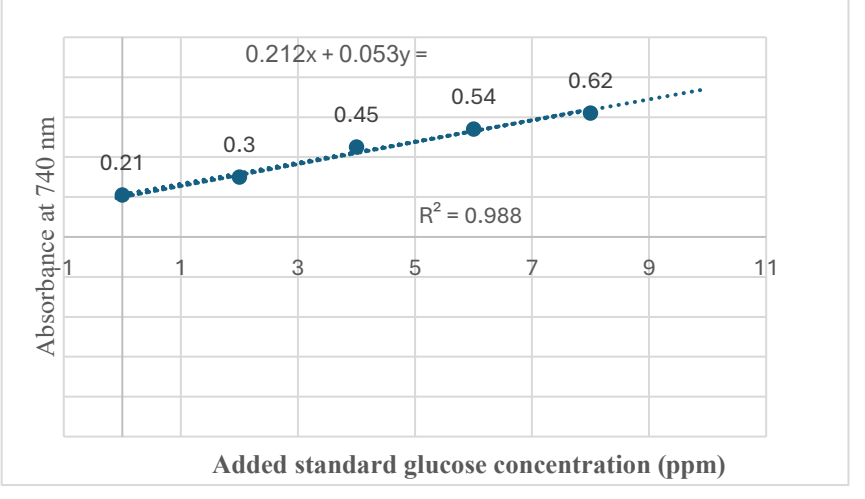

Figure 3. Standard calibration curve of the added glucose solution for the determination of reducing sugar content in pressed seed residue extracts.

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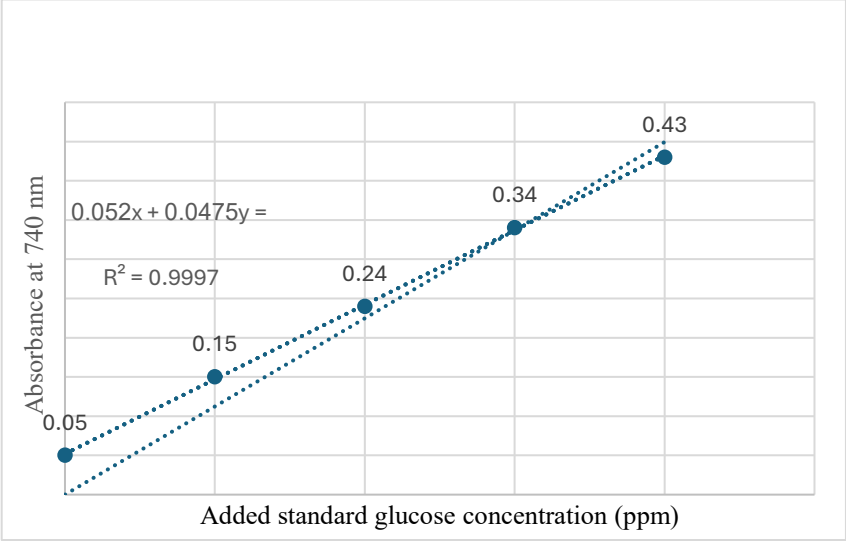

Figure4. Standard calibration curve of the added glucose solution for the determination of reducing sugar content in pomegranate peel extracts.

Data Analysis

All experimental measurements were performed in triplicate ($n = 3$), and the obtained data were expressed as *the mean ± standard deviation (SD)*. Characterization profiles and antibacterial activities of pomegranate peels and pressed seed residues were descriptively evaluated and compared based on these quantitative values. All descriptive statistical calculations and data plotting were performed using Microsoft Excel (version 2010).

Evaluation of the Antibacterial Activity of Pomegranate Extract Against Selected Pathogenic Bacteria:

Antibacterial Assay and Extract Preparation

Extract Preparation

The antibacterial efficacy of pomegranate aqueous extracts was evaluated against three pathogenic bacterial strains. For the assay, a high-concentration crude extract was prepared by dissolving 5 g of plant powder in 20 mL of distilled water, establishing a concentration of 25% (w/v), preheated to 30 °C. The mixture was incubated for 3 h, filtered, and dried. The resulting extract residue

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was accurately weighed and reconstituted to prepare standard working solutions of varying concentrations, as detailed in Table 3. This elevated concentration was methodologically necessary to maximize the density of bioactive compounds, such as phenolic compounds and tannins, enabling them to breach the physical barriers of the tested pathogens and ensuring an effective biological dose on the sterile paper discs.

Table 3. Preparation and concentration profiles of the pomegranate aqueous extract working solutions used for the antibacterial assay.

Extraction	Concentration of Dry Pomegranate Extract (mg/ml)	Concentration of Fresh Pomegranate Extract (mg/ml)
Pressed seed residues	38 mg/ml	18 mg/ml
Peels	26 mg/ml	18 mg/ml

The antimicrobial screening was systematically executed against the following designated pathogenic strains: *Escherichia coli*, a Gram-negative, rod-shaped intestinal bacterium utilized as a primary food and water contamination indicator capable of causing severe diarrhea and urinary tract infections (Naidoo & Zishiri, 2025); *Klebsiella* spp., a Gram-negative, rod-shaped opportunistic pathogen encapsulated by a thick polysaccharide mucous capsule responsible for pneumonia and nosocomial infections (Elramli et al., 2024); and *Streptococcus* spp., a Gram-positive, chain-forming spherical bacterium known to cause pharyngitis, skin infections, and respiratory tract diseases (Thacharod et al., 2024).

Antibacterial Assay:

The antibacterial efficacy of the prepared pomegranate fruit extracts (fresh peels, dry peels, fresh pressed seed residues, and dry pressed seed residues) was evaluated using a qualitative direct microbial inhibition assay based on standardized protocols (Jorgensen, 1997). Briefly, bacterial suspensions were prepared and adjusted to match the 0.5 McFarland turbidity standard. Equal aliquots of the bacterial inocula were introduced into tubes containing the respective extracts and incubated at 37 °C for 24 h. Following incubation, the presence

or absence of macroscopic bacterial proliferation (turbidity) was visually monitored. The results were qualitatively recorded as 'Growth' or 'No Growth' and comprehensively compiled in Table 4.

Results and Discussion:

The Qualitative Screening of Bioactive Chemical Compounds:

The qualitative analysis of dry pomegranate pressed seed residues and peel aqueous extracts revealed the presence of nine major bioactive groups, as detailed in **Table 1.**

Table 1.The qualitative analysis of dry pomegranate pressed seed residues and peel aqueous extracts

No.	Bioactive compounds	Screening Results (Pomegranate pressed seed residues)	Screening Results (Pomegranate peels)
1	Glycosides	++	+
2	Alkaloids	++	+
3	Resins	+	+
4	Terpenes	+	++
5	Steroids	+	++
6	Phenols	+++	++
7	Saponins	+	+
8	Flavonoids	++	+
9	Tannins	+	+

(+) Low presence, (++) Moderate presence, (+++) High presence, (-) Absence.

(-) indicates the absence of the bioactive compound in the pomegranate extract.

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The qualitative phytochemical screening profiles compiled in Table 1 demonstrate a clear tissue-specific alignment with established botanical literature. Notably, the comprehensive detection of glycosides, resins, terpenes, steroids, phenols, saponins, and flavonoids strictly mirrors the comparative trends documented by Campos et al. (2022). However, across all evaluated extraction solvents (0% to 75% ethanol) and cultivars (Acco, Big Full, and Wonderful), alkaloids (Alk) yielded a completely negative response (-) in their screening records, confirming their total absence in their extracted residues. This negative outcome represents a critical point of divergence from the current study; interestingly, the successful recovery and simultaneous presence of alkaloids in both the pressed seed residues and peels during our screening fully corroborates the qualitative data reported by Elfalleh et al. (2012) for the 'Gabsi' cultivar, where alkaloids were explicitly detected in both the pressed seed residues (+) and peel (++) matrices. These differences in alkaloid detectability among studies are likely driven by potential differences in extraction solvent polarities, genetic cultivar backgrounds, or post-harvest processing efficiency, validating the superior qualitative sensitivity achieved in the present investigation.

The Quantitative Determination of Primary Components:

The proximate chemical composition of dry pomegranate pressed seed residues and peels revealed noticeable differences between the two agro-industrial byproducts. These findings are comprehensively summarized and compared with previous studies in **Table 2,** while the direct comparative profile specifically between the peel powder and pressed seed residue extracts is illustrated in Figure 1.

Table 2.The proximate chemical composition of dry pomegranate pressed seed residues and peels

Contents		The Present Study	Previous Studies	References
Moisture	Pressed seed residues	14.50 % ±0.75	14.1-21.6%	(Campos et al., 2022)

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Contents		The Present Study	Previous Studies	References
	Peels	8.00±0.01 %	8.43–13.80 %	(Singh et al., 2023)
Ash	Pressed seed residues	3.60 ±0.25%	2.56 - 4.39%	(Campos et al., 2022)
	Peels	4.9± 0.07 %	3.35–6.07%	(Singh et al., 2023)
Fiber	Pressed seed residues	37.00±1.02 %	30–50%	(Talekar et al., 2018)
	Peels	21.00±0.77 %	16.6–45.6%	(Viuda-Martos et al., 2012)
Fat	Pressed seed residues	10.00 ±0.98 %	7.9 -16%	(Wang et al., 2024)
	Peels	5.00 ± 0.97%	6.63-19.3%	(Fadavi et al., 2006)
Protein	Pressed seed residues	8.76± 0.05%	8.719 ±0.1%	(Ullah et al., 2012)
	Peels	**6.14 ±0.09%**	4.9 %	(Middha et al., 2013)
Reducing Sugars	Pressed seed residues	20.00 ±0.35 %	13–24 %	(Peng et al., 2025)
	Peels	**7.00 ± 0.89%**	4. 34%	(Middha et al., 2013)

Values are expressed as the Mean ± Standard Deviation (SD) of triplicate determinations.

The proximate chemical composition of pomegranate pressed seed residues and peels generally aligns with published literature **Table 2.** However, notable discrepancies emerged within the peel matrix; specifically, crude protein (6.14% ± 0.09 compared with 4.9%) and

Chemical Characterization and Antibacterial Activity of Aqueous Extracts from Dried Pomegranate Peels and Pressed Seed Residues
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reducing sugars (7.00% ± 0.89 compared with 4.34%) were higher than the benchmarks documented by Middha et al. (2013). These compositional fluctuations, alongside minor deviations from baseline studies, are likely driven by distinct biological factors—such as cultivar traits, pesticide applications, and fruit maturity—and technical laboratory variables, confirming that the current findings remain highly compatible with global scientific benchmarks

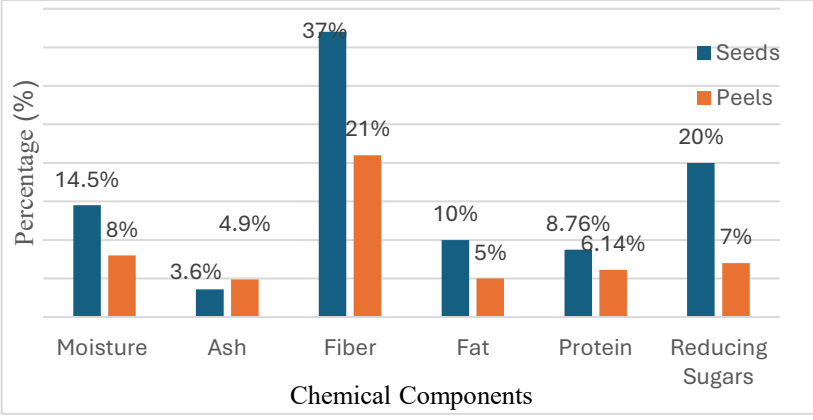

Figure 1. Presents the percentage of the Proximate Chemical Compositions Pomegranate fruit pressed seed residues and peels.

Figure 1 illustrates a distinct variation in chemical composition between the two investigated tissues. Pomegranate pressed seed residues exhibited noticeably higher levels of crude fiber, reducing sugars, moisture, crude fat and crude protein compared with the peels. Conversely, pomegranate peels demonstrated quantitative superiority only in total ash content relative to the pressed seed residues. This clear divergence reflects their specific biological functions, where the pressed seed residues prioritize dense structural, proteinaceous, and lipid reserves for embryo nourishment, while the peels primarily accumulate essential minerals and specialized non-proteinaceous compounds to serve as an outer protective barrier.

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The antibacterial activity of aqueous pomegranate extracts:

Table 4. Qualitative antibacterial effect of pomegranate peel and pressed seed residue extracts.

Bacterial species	Fresh peels	Dry peels	Fresh pressed seed residues	Dry pressed seed residues
E. Coli	No Growth	No Growth	No Growth	No Growth
Klebsiella	No Growth	**Growth**	No Growth	No Growth
Streptococcus	No Growth	No Growth	No Growth	No Growth

As presented in Table 4, the qualitative antibacterial screening demonstrated absolute efficacy for most pomegranate extracts in completely inhibiting the proliferation of *Escherichia coli* and *Streptococcus* spp. These findings corroborate the previous results (Mostafa et al., 2018; Nozohour et al., 2018), which indicated that pomegranate polyphenols and tannins effectively disrupt the cell membranes of Gram-negative intestinal rods and Gram-positive spherical chains. However, a selective resistance was observed in *Klebsiella* spp., which exhibited visual growth exclusively when treated with the dry peel extract. This specific survival supports the findings documented by Elramli et al. (2024). This resistance indicates that the structural mucous capsule of *Klebsiella* species, combined with processing variables, can physically obstruct bioactive absorption, resulting in localized survival.

Conclusion:

In conclusion, utilizing pomegranate peels and pressed seed residues as agro-industrial byproducts represents a highly sustainable strategy for functional food and pharmaceutical applications, owing to their valuable chemical profiles and natural antimicrobial properties. However, a key limitation of this study is its exclusive reliance on aqueous extraction and the lack of

quantitative determination of specific bioactive compounds. Consequently, future research should evaluate different organic solvent systems and employ advanced chromatographic techniques to precisely profile and quantify individual active constituents, thereby fully validating their therapeutic and industrial potential.

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