

Optimization and Comparative Analysis of Drying and Extraction Methods for Pomegranate Peel Powder with Antimicrobial Assessment

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Abstract

Pomegranate (*Punica granatum* L.) peel, typically considered a processing byproduct, is a rich source of bioactive compounds such as phenolics and flavonoids, which offer antioxidant and antimicrobial benefits. This study focused on optimizing and comparing two drying methods—sun drying and tray drying along with two extraction techniques—conventional solvent extraction and ultrasound-assisted extraction (UAE) to determine the most effective approach for maximizing these beneficial properties in pomegranate peel powder. Four different solvents (50% methanol, 50% ethanol, a 1:1 methanol-ethanol mixture, and distilled water) were used to extract the compounds. The extracts were then tested for total phenolic content (TPC), total flavonoid content (TFC), antioxidant activity (via 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay), and antimicrobial effects against *Escherichia coli*, *Staphylococcus aureus*, and yeast. The results showed that tray drying was more efficient in reducing moisture content, which is important for preserving the quality of the powder. UAE consistently outperformed conventional methods, particularly when used with methanol or the methanol-ethanol mixture, resulting in significantly higher levels of phenolic compounds and antioxidant activity. Among all combinations, tray drying followed by UAE using methanol produced the most potent antimicrobial effects. This study highlights the potential of repurposing pomegranate peel waste into functional ingredients for natural health products and food preservation. It also emphasizes the importance of selecting suitable drying and extraction techniques to ensure the recovery of high-value bioactive compounds, supporting both sustainability and innovation in food and nutraceutical industries.

Keywords

Pomegranate peel powders, Drying and extraction methods, Antimicrobial assessment

Introduction

Fruits are a crucial component of the human diet, supplying essential nutrients such as vitamins, minerals, antioxidants, and dietary fiber, etc. Among these, pomegranate (*P. granatum* L.) is known for its high nutritional and therapeutic value. The fruit is rich in polyphenols, flavonoids, and anthocyanins that contribute to its strong antioxidant and antimicrobial properties. However, during industrial processing, a significant amount of pomegranate peel, accounting for nearly 20 - 30% of the fruit's total weight, is discarded as waste [1].

Pomegranate peel is an underutilized source of potent bioactive compounds and has been reported to exhibit a wide range of pharmacological activities including antioxidants, antimicrobial, anti-inflammatory, and anticancer properties [2]. With increasing emphasis on sustainability and circular bioeconomy, such waste materials

are now being reconsidered for their potential in nutraceutical and functional food applications [3]. Efficient recovery of bioactive from fruit waste depends heavily on the choice of drying and extraction methods, both of which significantly influence the yield and activity of the resulting compounds.

Historically, pomegranates have been revered in various traditional medicinal systems. In Ayurveda, peels have been used for treating gastrointestinal issues, infections, and inflammation [4]. Early drying techniques like sun drying were common due to their simplicity and cost-efficiency, albeit with inconsistent quality and loss of heat-sensitive compounds. Mechanical drying methods such as tray drying and freeze-drying have since emerged, offering better preservation of bioactive [5].

Similarly, solvent extraction methods using ethanol, methanol, or aqueous solutions have been widely employed. However, these conventional techniques are time-consuming and may leave solvent residues. In recent years, UAE has gained prominence for its ability to enhance mass transfer and break down cell walls using acoustic cavitation, leading to higher yields of phytochemicals in a shorter time span [6].

Several studies have reported on the optimization of drying and extraction conditions for pomegranate peel. For example, Balasundram et al. [7] demonstrated that tray drying retained higher antioxidant properties than sun drying. UAE has been shown to extract significantly more polyphenols and flavonoids compared to traditional solvent extraction, as seen in the work of Tiwari et al. [8].

Pomegranate peel extracts have also shown significant antimicrobial effects against a range of bacteria and fungi. The effectiveness of these extracts, however, depends largely on the solvents used and the extraction method. Methanolic and ethanolic extracts generally exhibit better antimicrobial activity due to their polarity and extraction efficiency [9].

Despite extensive studies on pomegranate peel, comparative investigations incorporating multiple drying and extraction techniques under identical conditions are limited. This gap highlights the need for a comprehensive analysis to determine the optimal method for maximal bioactive recovery and functional potential.

This study aims to evaluate and compare two drying methods (sun drying and tray drying) and two extraction techniques (solvent extraction and UAE) on pomegranate peel powders. The objective is to identify the most efficient combination for extracting bioactive compounds with high antioxidants and antimicrobial properties.

After drying, the peels were processed into powder and subjected to extraction using four different solvents: methanol, ethanol, 1:1 methanol-ethanol mixture, and distilled water. The resulting extracts were assessed for TPC, TFC, antioxidant capacity (via DPPH assay), and antimicrobial activity against *E. coli*, *Staphylococcus aureus*, and yeast.

Materials and Methods

Materials

Fresh pomegranates were procured from the local markets

of Jaipur, Rajasthan. Pomegranates were washed under potable running tap water. Peels were manually separated, washed, and roughly divided into portions for different drying treatments.

Drying methods

Tray drying method where pomegranate peels were dried in a laboratory hot air oven at a fixed temperature of 50 °C for a period of 24 h [5], and sun drying method where pomegranate peels were sun-dried for 2 - 3 days under the direct sunlight in month of January at ambient temperatures (15 °C - 22 °C), peels were flipped regularly to ensure uniform drying [10].

Moisture content determination

Free water determination was done using oven-drying method [11]. A known weight of pomegranate peel sample was taken and placed in a preheated hot air oven maintained at a temperature of 105 °C. The samples were left in oven to dry until a constant weight was achieved.

$$\text{Moisture (\%)} = \left[\frac{(\text{Initial weight} - \text{Final weight})}{\text{Initial weight}} \right] \times 100$$

pH determination

The pH of the samples was determined following the Association of Official Analytical Chemists (AOAC) official Method 932.12 [11]. A digital pH meter was calibrated using standard buffer solutions at pH 4.00 and 7.00 was used for all measurements. For liquid samples, approximately 50 mL of the homogenized sample was placed in a clean beaker, and the pH electrode was immersed directly in the solution. For semi-solid or solid samples, a 1:1 (w/v) slurry was prepared using distilled water prior to measurement. The electrode was rinsed with distilled water and gently blotted dry between measurements to avoid cross-contamination. All measurements were performed at room temperature (22 ± 2 °C), and the pH was recorded to two decimal places after stabilization of the meter reading.

Determination of total soluble solids (TSS)

TSS content of the samples was determined in accordance with AOAC Official Method 942.15 [11], using a digital refractometer. The instrument was calibrated with distilled water prior to analysis. For clear liquid samples, a few drops were placed directly on the refractometer prism. In the case of pulpy or semi-solid samples, the juice was extracted by filtration or centrifugation to obtain a clear supernatant suitable for measurement. The TSS value was expressed as degrees Brix (°Brix), indicating the percentage (w/w) of soluble solids—primarily sugars—present in the sample. All measurements were carried out at 20 °C with automatic temperature compensation enabled. Each sample was analyzed in triplicate, and mean values were reported.

Determination of titratable acidity

Titratable acidity was determined according to AOAC Official Method 925.10 [11]. A known weight or volume of the sample (typically 10 g or 10 mL) was diluted with an equal volume of distilled water and thoroughly homogenized. The mixture was then titrated with standardized 0.1 N sodium hydroxide solution using phenolphthalein as an indicator. Ti-

tration was carried out until a persistent pale pink endpoint was observed, indicating neutralization of the organic acids present in the sample. The titratable acidity was calculated and expressed as a percentage of the predominant acid, depending on the sample type. Each sample was analyzed in triplicate, and the mean value was reported.

Peel powder preparation

Once the dried peels had cooled to room temperature, peels were grounded in a laboratory mixer and sieved (80-mesh) to obtain a fine powder and stored in airtight containers.

Extraction techniques

Solvent extraction

Four different types of solvents were used in this procedure. A correctly weighed 1 g of peel powder was mixed with 20 ml solvent, then the mixture incubated for 18 h at a set temperature of 25 °C and then centrifuged for 5 min at a speed of 4500 rpm and filtered out using Whatman filter paper no 1 [12]. Filtrate outcome was stored at freezing temperature of -18 °C in airtight plastic bags or containers until further analysis [13]. Same procedure needs to follow for all 4 solvents - methanol, ethanol, water and 1:1 methanol and ethanol.

UAE

This technique was performed using the same solvents as used in solvent extraction. A sample 1 g of peel powder was mixed with 20 ml solvent and bath sonicated at a set temperature of 45 °C for a timeframe of 30 min. This process was followed by centrifugation which was done at 4500 rpm for 15 min and the resultant supernatant was filtered out and stored at freezing temperature of -18 °C in airtight plastic bags or containers until further testing [8]. Same procedure was followed for extraction using all 4 solvents.

Analytical tests

To find out the best drying and extraction technique out of all performed techniques several parameters need to be addressed like TPC, TFC, DPPH assay and antimicrobial activity. All the analysis was performed in triplicate form.

TPC

TPC was measured by Folin–Ciocalteu method with some modifications and expressed as milligrams per 100 grams (mg/100 g) [14]. An accurately measured sample of 1 g was mixed with 20 ml of solvent. Then a quantity of 1 ml of reagent Folin–Ciocalteu was mixed to the mixture. After 6 min of time, 10 ml of 7% sodium carbonate solution was mixed to the mixture. To make up the volume to 25 ml distilled water was used. Then the mixture was placed in dark for 90 min of time period at room temperature for the development of color. After this the ultraviolet-visible (UV-Vis) spectrophotometer was used to record the absorbance of the mixture at wavelength of 750 nm. TPC was calculated using. [15].

$$TPC (mg/100 g) = \frac{c \times v}{M}$$

Where, c is concentration from the calibration curve (mg/mL), v is volume of extract (mL), and M is mass of dried extract (g).

TFC

TFC determination was done via aluminum chloride colorimetric method and expressed in milligrams per 100 grams (mg/100 g) [16]. A measured 0.5 ml extracted sample was taken and mixed with 0.3 ml volume of 5% sodium nitrite solution. After 5 mins of reaction time, 0.3 ml volume of 10% aluminum chloride solution was added to the mixture. Mixture was left to react for 6 min and 2 ml volume of sodium hydroxide of 1 normality was added to the mixture. The volume was made to 10 ml using the distilled water. After this the UV-Vis spectrophotometer was used to record the absorbance of the mixture at wavelength of 510 nm. TFC was calculated using.

$$TFC (mg/100 g) = \frac{c \times v}{M}$$

Where, c is concentration obtained from quercetin standard curve (mg/mL), v is total volume of extract (mL), and M is weight of the extract sample (g).

Antioxidant activity

Antioxidant activity was measured by DPPH radical scavenging assay [14]. 1 ml volume of extract sample was mixed with a volume of 3 ml of DPPH methanolic solution of 0.1 M and incubated in shady condition at room temperature for time frame of half an hour. Using the UV-Vis spectrophotometer, the absorbance of the final mixture was recorded against a blank wavelength of 517 nm.

$$Inhibition (\%) = \frac{A_0 - A_1}{A_0} \times 100$$

Where, A_0 is absorbance of control (DPPH without sample) and A_1 is absorbance of sample (DPPH with extract).

Antimicrobial activity

The antimicrobial activity of the extracts was evaluated using the 96-well microdilution method. *E. coli*, *S. aureus*, and yeast were used as test organisms. For *E. coli*, a microdilution assay was conducted in a 96-well microplate. Extracts were initially added to 100 µL of nutrient broth in the first row (A) to achieve a concentration of 10 mg/mL. Serial two-fold dilutions were performed down the rows to create a dilution range from 10 - 0.3 mg/mL.

An inoculum of *E. coli* (~10⁶ CFU/mL) was added to each well, and the initial optical density (OD) at 600 nm was recorded using a microplate reader. Plates were incubated at 37 °C for 24 h, after which final OD readings were taken. Fold change in bacterial growth was calculated by comparing OD values before and after incubation. All tests were conducted in triplicate.

Statistical analysis

All the experiments were carried out in triplicate, and two-way analysis of variance was performed to analyze the effects of moisture content in sun drying and tray drying peel powder of (Pomegranate). In contrast, students T-test was performed for pomegranate estimation experiments. The analysis included two independent variables: name of analysis (a row factor) and name of sample (a column factor), and their interaction. A percentage contribution of each factor as

a source of variation was calculated, and a significance level was checked. All statistical analyses were performed using GraphPad Prism, and the effect size was reported as mean \pm standard deviation of at least three biological replicates.

Results and Discussion

The drying technique had a clear impact on the moisture content of pomegranate peel powders. As reflected in **table 1**, tray drying proved to be more effective in reducing moisture than sun drying. Specifically, the moisture content in tray-dried pomegranate peels was recorded at $9.53 \pm 0.30\%$, whereas the sun-dried samples retained higher moisture at $12.27 \pm 0.45\%$. This reduction is significant, as lower moisture levels enhance the shelf life of peel powder and reduce the likelihood of microbial contamination during storage. The controlled temperature in tray drying likely contributed to a more uniform and efficient dehydration process compared to the variable conditions of sun drying (**Figure 1** and **figure 2**).

TFC, TPC, and DPPH

The levels of TPC, TFC, and DPPH in pomegranate peel extracts showed noticeable variation based on the drying process, extraction technique, and the solvent used. For sun-dried peels, 50% methanol under conventional extraction gave the highest phenolic content at 25.65 mg GAE/100 g and also showed strong antioxidant activity with 90.98% inhibition. In contrast, the flavonoid content peaked at 0.02363 mg QE/100 g when a 1:1 50% methanol was used. When UAE was applied to sun-dried samples, 50% methanol once again showed a relatively high TPC value of 11.98 mg GAE/100 g, while ethanol gave the highest antioxidant response, recording 94.29% inhibition. Ethanol also yielded the highest TFC value in this group at 0.0249 mg QE/100 g.

For tray-dried samples, UAE using methanol resulted in the most significant phenolic concentration at 79.40 mg GAE/100 g, along with a strong antioxidant activity of 91.29%. The highest flavonoid level in tray-dried samples was observed with 50% methanol under ultrasonication, reaching 0.077 mg QE/100 g. In general, traditional solvent extraction showed lower values for both phenolics and antioxidants in tray-dried peels, with 50% methanol producing 79.40 mg GAE/100 g of phenolics and an antioxidant inhibition rate of 91.29%. Distilled water and methanol-ethanol mixtures showed modest results across both drying and extraction methods, performing consistently lower in comparison to pure 50% methanol or 50% ethanol.

Antimicrobial activity

The antimicrobial effects of pomegranate peel extracts were studied against *E. coli*, *S. aureus*, and yeast with results are presented in **figure 3** to **figure 13**. The inhibitory activity was measured by comparing the fold change in OD at 600 nm over 18 h of incubation. A fold change of 1.0 indicates no impact on bacterial growth (neutral), while values below 1.0 suggest inhibition, and above 1.0 indicate bacterial stimulation.

Among all combinations of drying and extraction techniques, tray drying followed by UAE exhibited the strongest antimicrobial potential. In particular, extracts treated with

Table 1: Comparative evaluation of bioactive compounds and quality attributes in pomegranate powder produced using different drying techniques (sun drying and tray drying).

Parameter	Sun drying	Tray drying
pH	4.12 ± 0.02	3.88 ± 0.03
TSS (Brix)	1.40 ± 0.02	1.80 ± 0.03
TA (%)	2.41 ± 0.03	1.90 ± 0.02

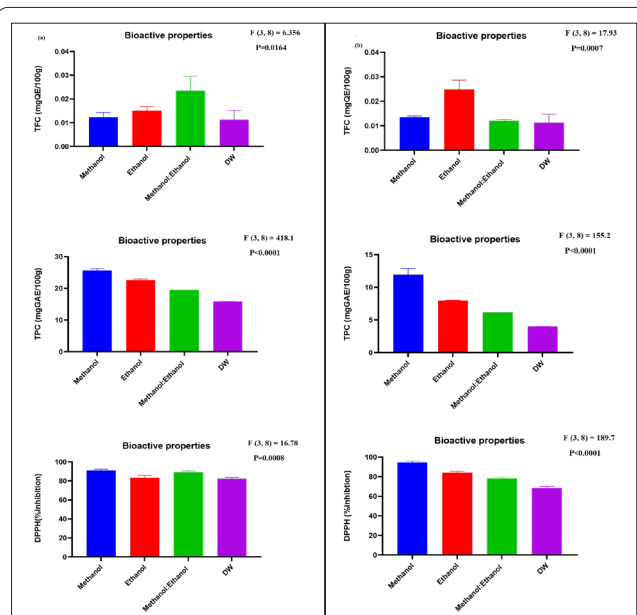


Figure 1: Bioactive properties of sun-dried pomegranate peel powder (a) solvents extraction and (b) ultrasonication.

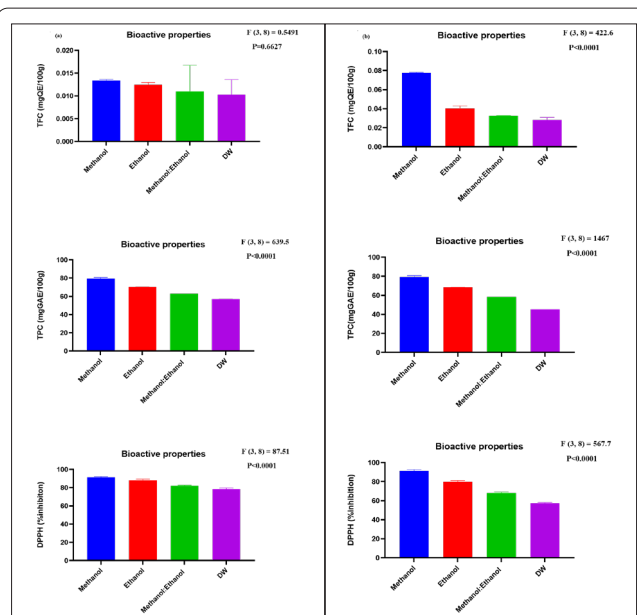


Figure 2: Bioactive properties of tray dried pomegranate peel (a) solvents extraction and (b) ultrasonication.

methanol and methanol-ethanol (1:1) solvents consistently showed the lowest fold change values, reflecting high inhibitory effects on both *E. coli*, *S. aureus* and yeast.

To categorize the samples, the following abbreviations were used throughout the analysis:

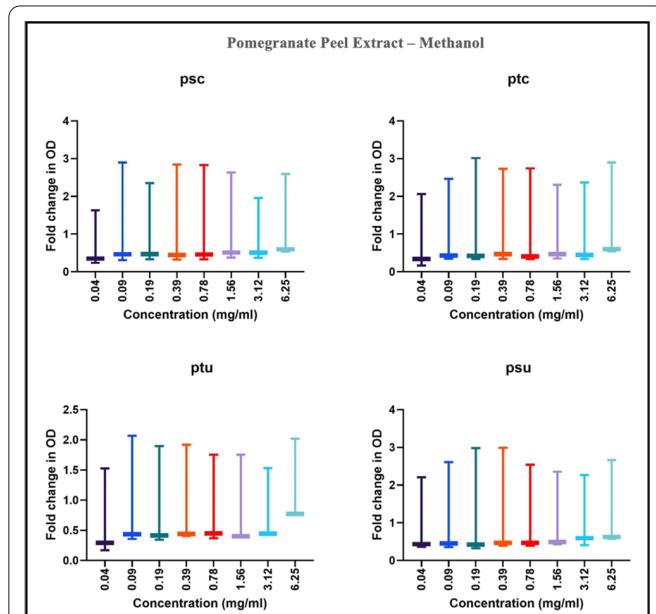


Figure 3: Effect of pomegranate peel 50% methanol extract on *E. coli* growth at varying concentrations. Fold change (OD_{18h}/OD_{0h}) is plotted for four drying and extraction conditions.

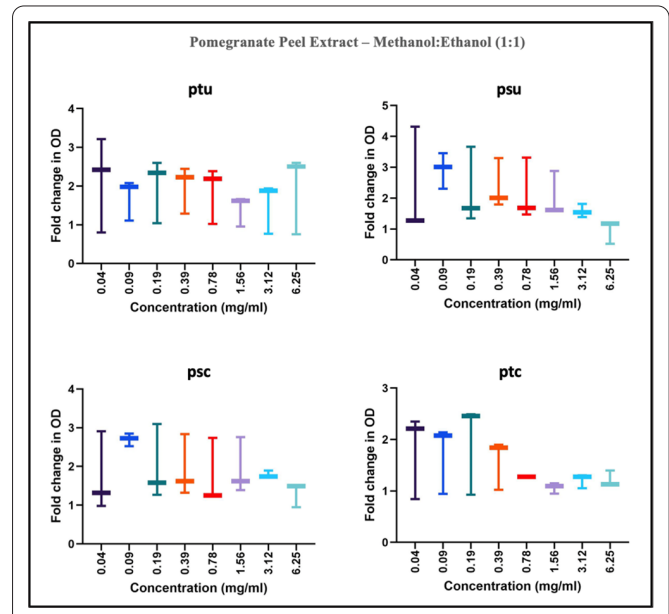


Figure 5: Effect of pomegranate peel methanol:ethanol (1:1) extract on *E. coli* growth at varying concentrations. Fold change (OD_{18h}/OD_{0h}) is plotted for four drying and extraction conditions.

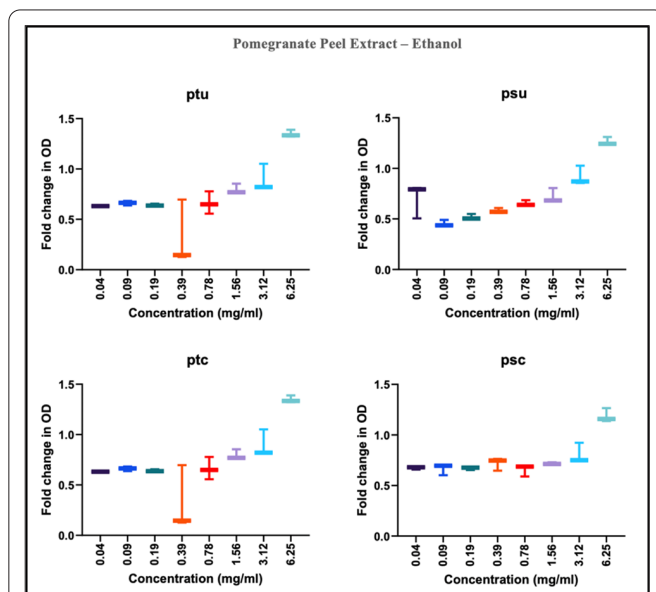


Figure 4: Effect of pomegranate peel 50% ethanol extract on *E. coli* growth at varying concentrations. Fold change (OD_{18h}/OD_{0h}) is plotted for four drying and extraction conditions.

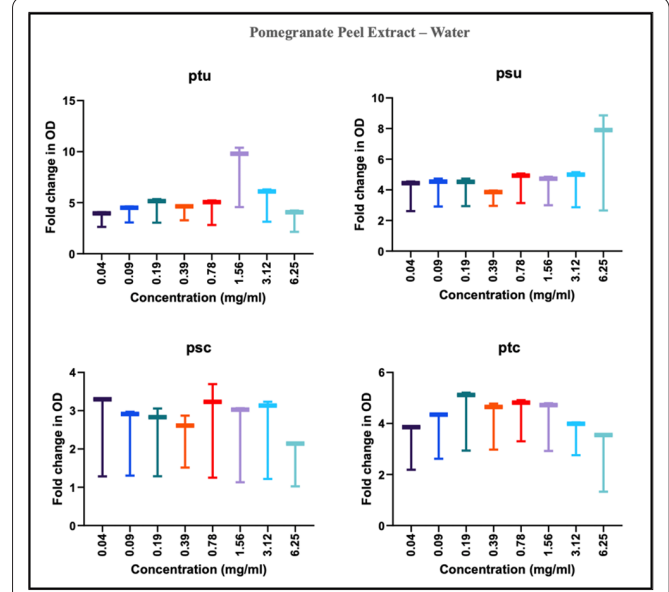


Figure 6: Effect of pomegranate peel water extract on *E. coli* growth at varying concentrations. Fold change (OD_{18h}/OD_{0h}) is plotted for four drying and extraction conditions.

- PSC = Pomegranate sun-dried + Conventional solvent extraction
- PSU = Pomegranate sun-dried + UAE
- PTC = Pomegranate tray-dried + Conventional solvent extraction
- PTU = Pomegranate tray-dried + UAE

Among these, PTU samples—especially those extracted with 50% methanol:ethanol and methanol-ethanol mixtures—showed the most significant reduction in microbial growth, with fold change values well below 1.0 for both target organisms. For *E. coli*, PTU-50% methanol:ethanol extract yielded the most notable inhibition. Similarly, *S. aureus* growth

was most effectively suppressed by PTU extracts using 50% ethanol and methanol-based solvents.

In contrast, PSC samples (sun drying + conventional extraction) generally showed higher fold change values, indicating reduced antimicrobial action—likely due to greater degradation of bioactive during sun exposure and less efficient extraction. These results confirm that tray drying, by preserving more bioactive compounds, coupled with ultrasonication, which enhances extraction efficiency, is the most effective method for preparing pomegranate peel extracts with potent antimicrobial properties. The combination of PTU and methanol or ethanol solvents demonstrates strong potential for use in natural antimicrobial formulations.

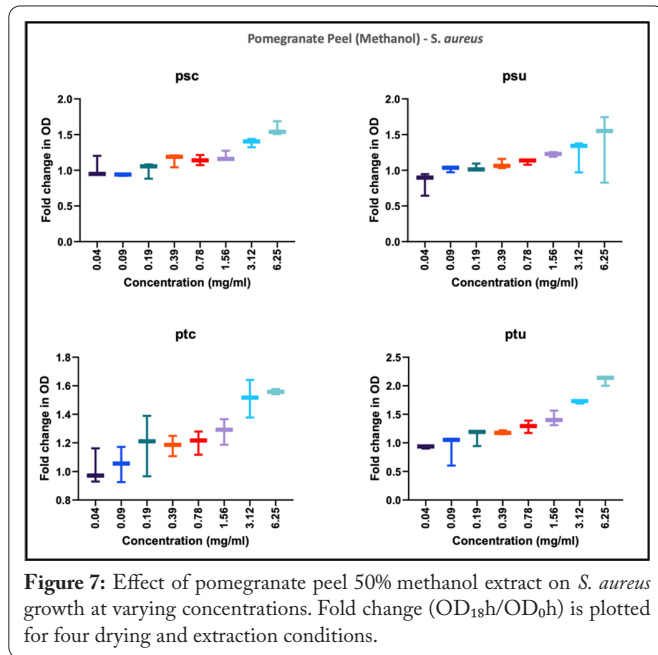


Figure 7: Effect of pomegranate peel 50% methanol extract on *S. aureus* growth at varying concentrations. Fold change (OD_{18h}/OD_{0h}) is plotted for four drying and extraction conditions.

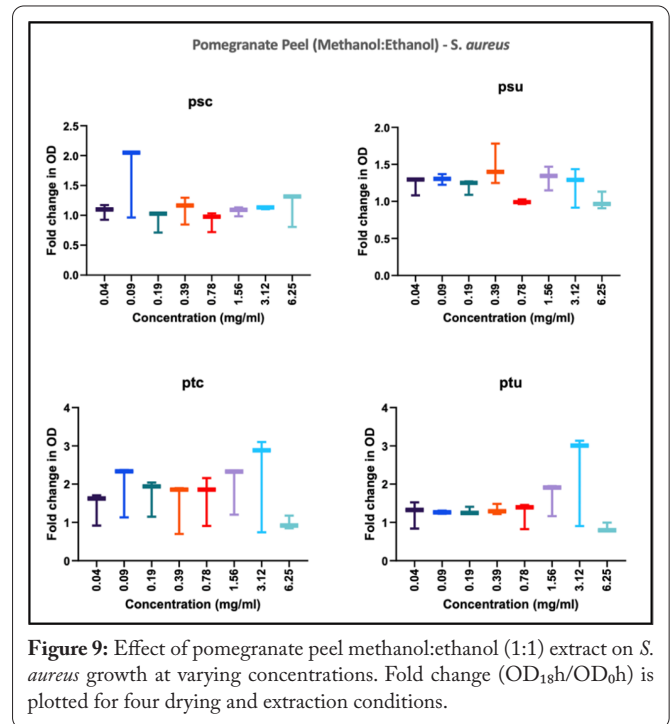


Figure 9: Effect of pomegranate peel methanol:ethanol (1:1) extract on *S. aureus* growth at varying concentrations. Fold change (OD_{18h}/OD_{0h}) is plotted for four drying and extraction conditions.

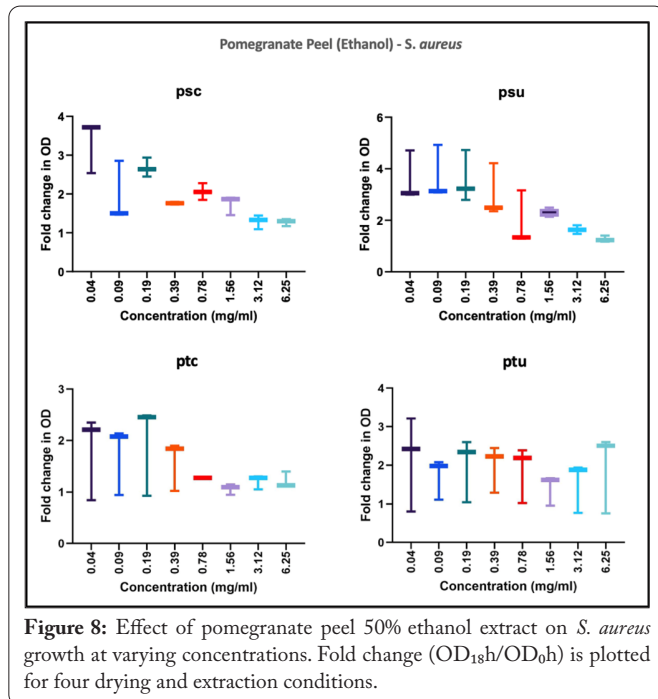


Figure 8: Effect of pomegranate peel 50% ethanol extract on *S. aureus* growth at varying concentrations. Fold change (OD_{18h}/OD_{0h}) is plotted for four drying and extraction conditions.

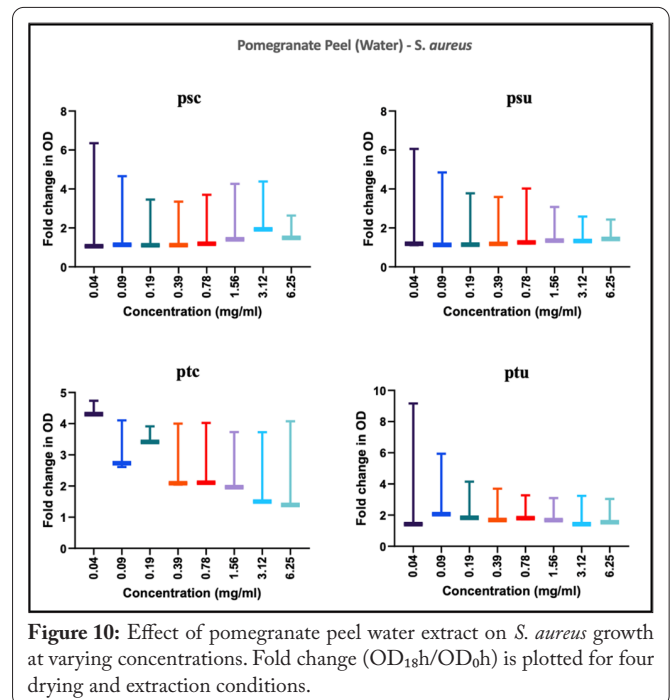


Figure 10: Effect of pomegranate peel water extract on *S. aureus* growth at varying concentrations. Fold change (OD_{18h}/OD_{0h}) is plotted for four drying and extraction conditions.

Conclusion

The study highlights the importance of selecting suitable drying and extraction techniques for maximizing the functional properties of pomegranate peel powder. Tray drying consistently outperformed sun drying in terms of moisture reduction, thereby supporting better storage potential and reducing spoilage risks. Among extraction methods, ultrasound-assisted techniques proved more effective in retaining and extracting bioactive compounds, particularly when paired with polar solvents like methanol and ethanol.

When assessing antimicrobial efficacy, extracts derived from tray-dried samples using ultrasonic extraction showed the most pronounced inhibition against both *E. coli*, *S. aureus*, and yeast. These results suggest that tray drying followed by

UAE, especially with 50% methanol or methanol-ethanol solvents, offers the most effective strategy for developing potent, natural antimicrobial agents from pomegranate peel waste. This approach not only supports waste valorization but also opens new possibilities for sustainable applications in food preservation and functional health products.

Acknowledgements

None.

Conflict of Interest

None.

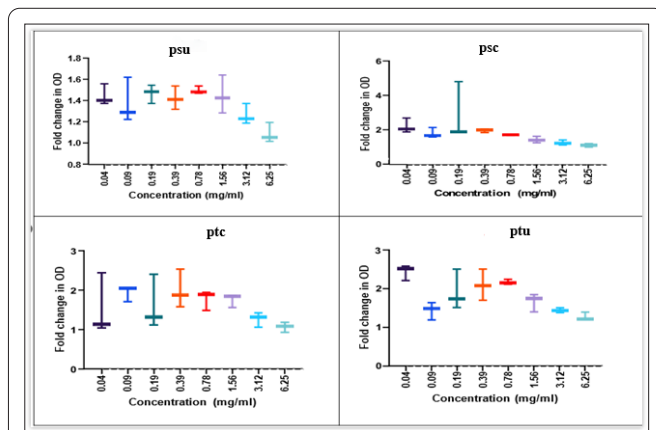


Figure 11: Effect of pomegranate peel 50% methanol extract on yeast growth at varying concentrations. Fold change (OD_{18h}/OD_{0h}) is plotted for four drying and extraction conditions.

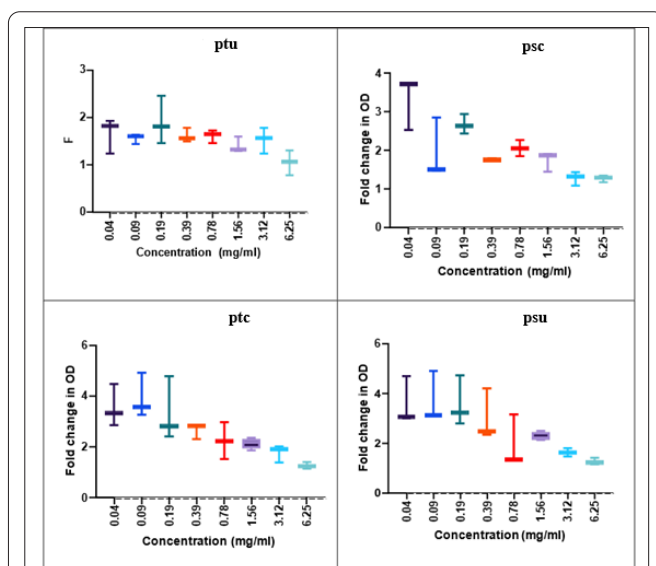


Figure 12: Effect of pomegranate peel 50% ethanol extract on yeast growth at varying concentrations. Fold change (OD_{18h}/OD_{0h}) is plotted for four drying and extraction conditions.

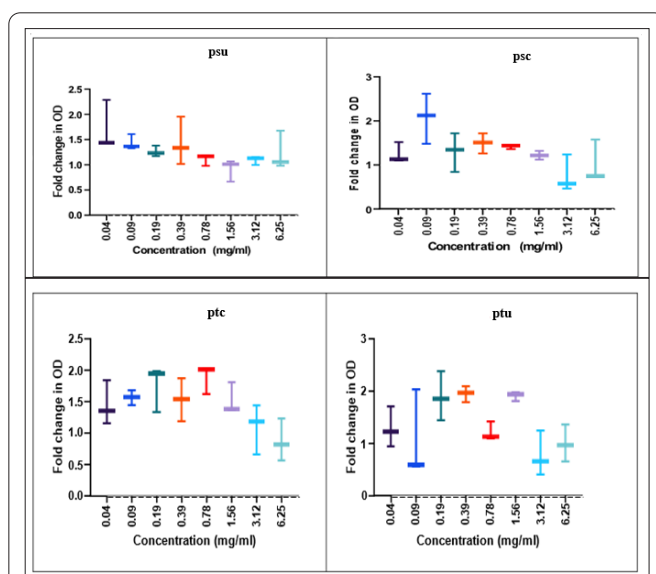


Figure 13: Effect of pomegranate peel methanol:ethanol extract on yeast growth at varying concentrations. Fold change (OD_{18h}/OD_{0h}) is plotted for four drying and extraction conditions.

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