



Evaluation of The Inhibitory Activity of Aqueous and Alcoholic (*Punica granatum*) Pomegranate Peel Extract on Some Bacterial species Isolated from Patients' beds at Al-Refai Teaching Hospital

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Abstract

Background: Pomegranate (*Punica granatum*) peel contains many active compounds such as tannins, phenols, and terpenes. Pomegranate has been used since ancient times in folk medicine to treat various stomach and intestinal diseases. People have also used it to treat epidermal and skin diseases, as well as to strengthen hair follicles. **Aim:** Evaluation of the inhibitory activity of alcoholic and aqueous extracts of pomegranate peel (*P. granatum*) on some antibiotic-resistant bacterial species in the patients' beds of the teaching hospital in Al-Rafai city. Which helps in improving the health and nursing care of hospital patients. **Methods:** Pomegranate peels were dried, ground, and extracted using methanol and water solvent. Bacteria were isolated from the patients' beds and two methods were used to test the sensitivity of microbes to the extracts, the first method was using disks and the second was using the density counting method. Nutrient broth was inoculated with single colonies of the isolated bacteria individually for each, then the medium was inoculated at 37°C for 24 h. **Results:** According to the study's findings, as it gave the lowest concentration of 2.5 mg/ml (minimum inhibition concentration (MIC) of the aqueous extract of the following readings (1.022, 1.186 0.698) at a wave length (595 nm) for each of *E.coli*, *Staph. aureus* and *Strep pyogens* respectively. **Conclusions:** This study found the efficacy of pomegranate peel in the inhibition of bacteria increases with increasing concentrations of the aqueous extract. It can be used to sterilize beds in hospitals, which helps improve health and nursing care for hospital patients.

Keywords: Antibacterial Effects; Al-Refai Teaching Hospital; Medical Plants; Pomegranate Peel; *Punica granatum*

Introduction

Medicinal plants are considered therapeutic materials for many diseases. Since ancient times and to this day, humans have used them to treat many diseases because they contain many bioactive compounds (Asad & Alhomoud, 2016). One of the most important of these medicinal plants is the pomegranate plant, which is of very high medical importance and is native to Southwest Asia and is widely spread. Also, it grown in India. Pomegranate seeds contain a high percentage of water, estimated at about 81% in addition to the presence of other substances such as Sugars, Proteins, Fats and some Vitamins. This is in addition to the presence of other various types of elements such as Iron, Phosphorus and Calcium (Cai *et al.*, 2011). Pomegranate Peels contain tannic acid, a substance whose powder is used as an antibacterial to treat cases of diarrhea (Guda *et al.*, 2022). A recent scientific study revealed that pomegranate peels fight local infections caused by a type of bacteria that is resistant to the same antibiotics, which may contribute to finding a new treatment for this type of infection. Researchers at Kingston University in the UK have succeeded in pointing out the role of pomegranate peels in combating local infections that may result from *Staphylococcus aureus* bacteria.

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According to some sources, this type of bacteria shows resistance to a number of antibiotics such as Penicillin, Amoxicillin, Methyleneaxine and others. This bacterium causes recurrent infections among hospital patients and healthcare users, such as kidney dialysis departments and elderly care departments (Alduhaidahaw *et al.*, 2019). Studies have shown that pomegranate peel has many benefits, including that it helps calm the stomach and treat various disorders such as diarrhea, dysentery, and cholera. Drinking tea made from pomegranate leaves may also help get rid of indigestion (Guda, 2023), and many studies have shown that pomegranate fruits have a lethal and inhibitory effect on the growth of Gram-negative and Gram-positive microbes and a large number of fungi (Kafil *et al.*, 2017). Hence, the study aims to test how well alcoholic and water extracts of pomegranate peel (*P. granatum*) can stop the growth of certain antibiotic-resistant bacteria found in the beds of patients at a teaching hospital in Al-Rafai city. It can be used to sterilize beds in hospitals, which helps improve health and nursing care for hospital patients.

Materials and Methods

The current study introduces a novel approach by utilizing water-based pomegranate peel extract as a natural antibacterial agent in hospital hygiene settings. While previous studies have focused on methanolic or ethanolic extracts, this work emphasizes the potential of aqueous extracts as a safe, cost-effective, and eco-friendly alternative for disinfecting hospital surfaces contaminated with pathogenic bacteria.

Study Design:

This was an experimental laboratory-based study conducted to evaluate the inhibitory activity of aqueous and alcoholic extracts of *Punica granatum* (pomegranate) peel on selected antibiotic-resistant bacterial species isolated from patient beds at Al-Rifai Teaching Hospital, Iraq. The study involved sample collection, preparation of plant extracts, microbial isolation, sensitivity testing, and statistical analysis.

Study Plant:

Pomegranate fruits were obtained from the local markets of Al-Rifai city, and the plant variety was verified at the College of Education, Department of Life Sciences, University of Basra.

Bacterial Isolates:

Bacterial species such as *Staphylococcus aureus*, *Streptococcus pyogenes* and *Escherichia coli* were isolated by taking swabs from the beds of patients lying in Al-Rifai Teaching Hospital in Al-Rifai city. The swabs were transferred using a nutrient broth medium to the laboratory of the College of Basic Education at Sumer University and were cultured in a special culture medium to isolate the isolated microbes. The isolated bacteria were diagnosed by conducting some biochemical tests in addition to some morphological characteristics according to (Chouhan *et al.*, 2017). The sensitivity test for antibiotics and extracts was conducted using a nutrient broth to test turbidity (Dakah & Maarrouf, 2019).

Microbial Isolation

- Swabs were taken from patient beds at Al-Rifai Teaching Hospital.
- Samples were transported using nutrient broth to the microbiology lab at Sumer University.
- The samples were cultured on selective media and incubated at 37°C for 24 hours.
- Pure colonies were identified based on morphological and biochemical tests following Chouhan *et al.* (2017).

Antimicrobial Testing

Two methods were used:

1. Disc Diffusion Method

- Nutrient broth was inoculated with individual bacterial colonies.

- Media were incubated at 37°C for 24 hours.
- Bacterial suspensions were spread on nutrient agar plates.
- Sterile discs impregnated with different concentrations (2.5–20 mg/ml) of extracts were placed on agar.
- Plates were incubated at 37°C for 24 hours.
- Zones of inhibition were measured in millimeters.

2. Turbidity Method

- Different concentrations of extracts (2.5–20 mg/ml) were added to nutrient broth tubes.
- Each tube was inoculated with 10 μ l of bacterial suspension (10⁶ cells/ml).
- Tubes were incubated at 37°C for 14–16 hours.
- Optical density (OD) was measured at 595 nm using a spectrophotometer.
- Minimum Inhibitory Concentration (MIC) was determined as the lowest concentration showing no visible growth compared to the control.

Inclusion Criteria

1. Bacterial isolates obtained from patient beds in Al-Rifai Teaching Hospital.
2. Isolates identified as *Staphylococcus aureus*, *Escherichia coli*, and *Streptococcus pyogenes*.
3. Availability of fresh, mature pomegranate fruits from local markets in Al-Rifai city.
4. Confirmation of extract purity and sterility before antimicrobial testing.

Exclusion Criteria

1. Contaminated or mixed cultures of bacteria were excluded.
2. Extracts that showed signs of microbial contamination after preparation were discarded.
3. Data from experiments not repeated at least three times were excluded from analysis.

Plant Material

Fresh *Punica granatum* fruits were collected from local markets in Al-Rifai city. The plant variety was authenticated at the Department of Life Sciences, College of Education, University of Basra.

Preparation for Aqueous Extract

Method:

- 40 g of finely ground pomegranate peels were mixed with 160 cm³ of distilled water (1:4 w/v).
- The mixture was stirred for 1 hour using a magnetic stirrer.
- It was left in the refrigerator for 24 hours for infusion.
- The mixture was filtered through layers of gauze to remove unground parts.
- The aqueous extract was freeze-dried under vacuum pressure using a lyophilizer (Edwards).
- The dried extract was stored in tightly sealed glass bottles under moisture-free and freezing conditions until use.

Preparation of Alcoholic Extract

Method:

- Pomegranate peels were crushed and extracted using methanol (1 g/5 ml).
- The mixture was shaken for 1–2 hours using a magnetic stirrer.
- It was refrigerated 24 hours before filtration.
- The solvent was evaporated using a rotary vacuum evaporator at $\leq 40^{\circ}\text{C}$.

The extract was freeze-dried and stored similarly to the aqueous extract (Guda *et al.*, 2020).

Sterilization of Aqueous and Alcoholic Extracts

The alcoholic extract was sterilized by pasteurization at 62°C for 10 minutes, thus obtaining the standard concentration, from which dilutions were prepared. The aqueous extract was sterilized by dissolving the dry aqueous extract with distilled water at a ratio of (19/5 ml) to obtain the standard concentration (200 mg/ml). Then the aqueous solution was passed through filter paper measuring (0.22) millipore, which prevents the passage of microbes. This concentration was adopted in preparing serial dilutions (Sabra & Al-Masoudi, 2014).

Testing The Antibacterial Activity of Extracts

Two methods were used to test the sensitivity of microbes to the extracts, the first method was using discs. According to (Husayn & Guda, 2023a) based on the method of (Sabra & Al-Masoudi, 2014). Nutrient broth was inoculated with single colonies of individually isolated bacteria for each, then the medium was inoculated at 37°C for 24 h. A series of dilutions were performed using normal saline solution and a concentration of 10 cells/ml was obtained by comparing tube No. (1) of McFarland standard tubes (Mcfarland No-1). One of the bacterial suspensions was spread on the surface of normal nutrient agar using a sterile glass diffuser and the plates were fixed on the surface of the agar plates and the plates were incubated at 37°C for 24 h. The inhibition zones were measured and compared with the standard antibiotic conamycin as a positive control sample (Husayn & Guda, 2023b). The second method for testing the antibacterial activity of extracts was using the turbidity method where (1) ml of the plant extract at different concentrations was added to tubes containing (9) ml of nutrient broth, then these tubes were inoculated with (10) ml of bacterial suspension at a concentration of 10 bacterial cells/ml, at a rate of five replicates for each concentration of extracts. The tubes were incubated at 37°C for (14-16) hours, after which the turbidity was measured using a spectrophotometer at a wavelength of (595) nm, to determine the effect of the extracts on bacterial growth. The results were compared with the control sample prepared by adding (10) ml of diluted bacterial suspension and (1) ml of solvent to (9) ml of nutrient broth. The minimum inhibitory concentration (MIC) was determined using the turbidity test. 1000 mg/ml of the extract was prepared and inoculated with bacteria. The highest dilution that prevented bacterial growth compared to the control sample was the minimum inhibitory concentration (MIC) (Ali *et al.*, 2023) according to (Silness & Loe, 1964).

Data Analysis

All experiments were performed in triplicate. Data were recorded as mean \pm standard deviation. Statistical significance between different concentrations was analyzed using one-way ANOVA followed by Tukey's post-hoc test using SPSS version 26. Graphs were plotted using Microsoft Excel.

Ethical Considerations

The study was approved by the Ethical Approval Committee of College of Basic Education, University of Sumer, Iraq vide ref number 12 dated 3/5/2023. All procedures involving biological materials followed biosafety protocols outlined by the World Health Organization (WHO, 2004). No human or animal subjects were directly involved in the study.

Result

Antibacterial Activity of Pomegranate Peel Aqueous Extract Against Some Species of Bacteria

A comparative analysis of the zone of inhibition (in mm) produced by varying concentrations (2.5–20 mg/ml) of aqueous pomegranate peel extract against three bacterial species isolated from patient beds at Al-Refai Teaching Hospital: *Staphylococcus aureus* (Gram-positive), *Escherichia coli* (Gram-negative), and *Streptococcus pyogenes* (Gram-positive) has been depicted in the table 1. The data were collected using the disc diffusion method.

Table 1 : Antibacterial activity of pomegranate peel aqueous extract against some species of bacteria

Species of Bacteria	Concentration of Aqueous Extract mg/ml					
	2.5	5	7.5	10	15	20
<i>Staph. aureus</i>	-	-	-	20	22	26
<i>E. coli</i>	-	-	6	8	10	13
<i>Strep. pyogenes</i>	-	-	10	14	16	20

The results were:

- At 20 mg/ml, *S. aureus* exhibited the largest inhibition zone (26 mm), indicating high sensitivity to the aqueous extract.
- *E. coli* showed moderate inhibition (13 mm) at the same concentration.
- *S. pyogenes* demonstrated a gradual increase in inhibition with increasing extract concentration, reaching 20 mm at 20 mg/ml.
- These results suggest that the aqueous extract has broad-spectrum antibacterial activity, particularly against Gram-positive bacteria, which may be due to differences in cell wall structure and permeability.

The results of the current study indicate the importance of pomegranate extracts and their effective role through their aqueous extracts in killing and inhibiting the growth of bacteria isolated from the beds of patients admitted to Al-Rafai Teaching Hospital as shown in Table (1). The highest effect was when diluted at a concentration of 20 mg/ml for the aqueous extract on *Staphylococcus aureus*. The diameter of the inhibition zone was 26 mm. This result is consistent with what was reached by (Guda, 2023). The same dilution showed a different inhibitory effect on *Escherichia coli* and *Streptococcus pyogenes*, as the diameter of the inhibition zone was 13 mm and 20 mm, respectively, as shown in Figures (1) and (2). This is what was indicated by (Guda & Ammar Semysim, 2022). The reason for inhibiting the growth of microbes may be due to the presence of one or more chemical compounds in pomegranate peels. The presence of tannin may affect the plasma membrane, leading to a change in its functional properties, and thus inhibiting the growth of microbes (Guda, 2023; Algelal *et al.*, 2024).

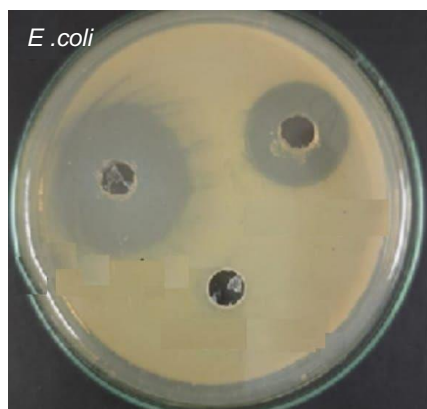


Figure 1: Inhibitory effect of pomegranate peel extracts on the growth of *E. coli* bacteria



Figure 2: Inhibitory effect of pomegranate peel extracts on the growth of *Strep. pyogenes* bacteria

Comparison of Antibacterial Activity Between Aqueous, Alcoholic Extracts, and Kanamycin

The table 2 compares the antibacterial efficacy of three treatments—aqueous extract, alcoholic extract, and kanamycin (a standard antibiotic—against the same bacterial species (*S. aureus*, *E. coli*, *S. pyogenes*) using the disc diffusion method.

- The aqueous extract outperformed both the alcoholic extract and kanamycin for *S. aureus*.
- For *E. coli*, the aqueous extract was slightly more effective than kanamycin, while the alcoholic extract had minimal effect.
- In the case of *S. pyogenes*, the aqueous extract showed comparable or slightly lower activity than kanamycin, but significantly better than the alcoholic extract.

- This comparison confirms that aqueous extraction preserves bioactive compounds such as tannins and polyphenols more effectively than alcohol, which may denature or reduce the solubility of these compounds.

Table 2: Antibacterial activity of pomegranate peel extracts against some species of bacteria compared to the antibiotic Kanamycin

Species of bacteria	Aqueous extract	Alcoholic extract	Kanamycin
<i>S. aureus</i>	20	-	11
<i>E. coli</i>	14	-	13
<i>Strep. Pyogens</i>	13	-	10

Table (2), showed inefficiency of the alcoholic extract at its different concentrations in inhibiting the growth of the bacteria isolated in the current study compared to the inhibitory effect of the aqueous extract and the antibiotic kanamycin, which represents the control sample. Perhaps the reason for this is due to the effect of alcoholic solvent on the chemical compounds of pomegranate peels, which leads to their loss of their ability to have an inhibitory effect against microbes. Therefore, the aqueous extract is very effective in killing and inhibiting microbes (Qazmooz *et al.*, 2020).

Results of Turbidity Test at 595 nm Wavelength

Table 3 illustrates the effect of different concentrations of aqueous pomegranate peel extract on bacterial growth, measured using the turbidity method at 595 nm wavelength. The optical density (OD) values indicate the degree of microbial growth inhibition. Interpretation:

- As the concentration of the extract increases, the OD decreases, indicating reduced bacterial growth.
- The Minimum Inhibitory Concentration (MIC) for all tested bacteria was found to be 2.5 mg/ml, where visible growth was inhibited compared to the control.
- Among the three species, *S. pyogenes* showed the most rapid decline in OD, suggesting higher susceptibility to the extract.
- These findings support the dose-dependent inhibitory activity of the aqueous extract, consistent with previous studies on plant-based antimicrobials.

Table 3: Results of turbidity test

Special bacteria	Aqueous extract concentration of pomegranate peel gm/ml					
	2.5	5	7.5	10	15	20
<i>Staph. aureus</i>	1.180	1.040	0.850	0.400	0.250	0.173
<i>E. coli</i>	1.022	0.644	0.555	0.300	0.350	0.320
<i>Strep. pyogens</i>	0.698	0.300	0.183	0.140	0.130	0.150

Table (3) indicates the turbidity results for different dilutions of the aqueous extract of pomegranate peels, which inhibit the growth of Gram negative and Gram-positive bacteria under study. It is clear that the inhibition of bacteria increases with increasing concentrations of the aqueous extract as indicated by the results, as it gave the lowest concentration of 2.5 mg/ml (minimum inhibition concentration (MIC) of the aqueous extract of the following readings (1.022, 1.186 0.698) at a wavelength (595 nn) for each of *E-coli*, *staph. aureus* and *strep Pyogens* respectively.

Discussion

The plant extract of both studied plants contains active compounds including free radicals such as halo compounds as well as alkanes, ketones and organic acids, all of which are toxic to bacteria and cause damage to the bacterial cell membrane and contribute to changing the properties of the bacterial cell membrane. This change causes the leakage of vital materials from the cell and the death of bacteria and its interaction with proteins present in bacteria, which affects their vital functions and disrupts cellular processes and hinders cellular respiration processes in bacteria, which leads to a decrease in the energy level within the cell (Parthipan *et al.*, 2021).

The produced samples contribute to the inhibition of bacteria, with different values. These particles, with their small size, help to penetrate the cell membrane easily, thus disrupting metabolism and other functions (Kumaresan *et al.*, 2018). It can be seen from the results presented in Figures (1) and (2) that the inhibition was achieved in all bacteria. This may be due to the release of antibacterial metal ions from the particle surface and the antimicrobial activity associated with the physical properties of the

extracts, which are related to cell wall penetration or membrane damage (Seil & Webster, 2012). Pomegranate peel extracts are effective, and their free radical scavenging ability is 85%. Another reason for the existing aggregation is the small size of the nanoparticles causes an increase in the surface area, i.e. they are larger in relation to the volume. The high surface area of Pomegranate peel extracts contributes to an increase in the charge on the surface, which leads to an increase in the effect of the nanoparticles on bacteria due to electrostatic attraction (Alias & Abd–Alsada, 2021). The results of researchers (Yedurkar, Maurya & Mahanwar, 2016) confirmed that the small size and spherical shape of Pomegranate peel extracts have antibacterial activity. The results were in agreement with (Hamad, Rahi & Guda, 2023; Rahi *et al.*, 2009).

Limitations of the Study

Sample size is limited; only three bacterial species were tested. Future studies should include a broader range of pathogens. Findings are limited to laboratory conditions (*in vitro* in nature) *in vivo* validation is needed. Variability in extract composition due to seasonal or geographical factors was not assessed.

Future Scope

Further analysis of the active compounds (chemical characterization) in the extract is recommended.

Conclusion

These results highly demonstrate the effect of aqueous extract of Pomegranate peels in inhibiting or killing both Gram-negative and Gram-positive bacteria. Therefore, the current study recommends using the Pomegranate as an antibiotic to kill many types of pathogenic bacteria. The study also recommends intensifying the study and conducting Various Scientific research in the field of plants with medicinal effect. The effect of aqueous extract of Pomegranate Peels at different concentrations on some species of bacteria isolated from Patient's beds has been observed. It can be used to sterilize beds in hospitals, which helps improve health and nursing care for hospital patients.

Conflict of Interest

The authors have no conflicts of interest.

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