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Antimicrobial Activity of Pomegranate *Punica Granatum* Fruit on Bacteria Isolated from Health Centers

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A B S T R A C T

This study explores the antibacterial potential of pomegranate (Punica granatum) peel and arils extracts against common pathogenic bacteria, including Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, and Staphylococcus aureus. With the rise of antibiotic-resistant bacteria posing a significant health threat, plant-based antimicrobial agents are gaining attention as viable alternatives to conventional antibiotics. In this research, ethanol and acetone extracts of pomegranate peel and arils were tested using the agar-well diffusion method. The antibacterial effectiveness was further assessed through Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) determinations. Results showed varying inhibition among bacterial strains, with both extracts displaying antibacterial activity. The ethanol extract generally produced larger inhibition zones, except for E. coli, where the acetone extract of the peel was more effective, exhibiting an MIC of 6.25 mg/ml. Meanwhile, the ethanol extract of pomegranate peel and arils showed superior activity against P. aeruginosa, with an MIC of 6.25 mg/ml. These findings suggest that pomegranate peel and aril extracts possess significant antimicrobial properties and could serve as promising natural alternatives to combat bacterial infections, particularly in the context of rising antibiotic resistance.

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INTRODUCTION

Punica granatum, commonly known as pomegranate, is a deciduous shrub or small tree belonging to the Lythraceae family. This plant is renowned for its health-promoting properties, particularly for its antimicrobial and antioxidant effects. The fruit is composed of arils (edible seeds) surrounded by a thick rind, and both parts have been found to exhibit a range of medicinal benefits, including antibacterial properties against a variety of pathogens [1]. The pomegranate is a small, deciduous shrub or tree, typically growing between 5 and 10 meters tall, and it produces large, round, reddish fruits that contain numerous seeds, called arils, which are surrounded by a tough outer rind. Its widespread cultivation across the Mediterranean region, India, and Persia has made it a significant crop in various parts of the world. Its scientific and commercial relevance has grown, especially due to its potential health benefits [2,3].

Today, it is widely cultivated in subtropical and arid climates, with major production areas in regions like California, Spain, India, and Turkey [4]. It thrives in warm climates, and it was introduced into the United States by Spanish settlers in the 16th century, where it is now predominantly grown in California and Arizona [5]. Pomegranates require a warm, dry climate and are tolerant of drought. They are grown in well-drained soils, and they can survive with minimal water, although irrigation improves the yield and quality of the fruit. The tree is propagated both by seeds and through vegetative methods, such as cuttings or grafting [6]. Pomegranate trees are relatively hardy, able to withstand mild frost, but the optimal temperature for growth ranges between 20-30°C. The fruit ripens in late summer or early fall and is harvested when the rind turns bright red [7].

Many studies focusing on the phytochemical analysis showed how rich the plant is in various bioactive compounds, including polyphenols, tannins, anthocyanins, and flavonoids. The peel and arils are particularly high in ellagic acid, anthocyanins, and punicalagins, which contribute to the fruit's antioxidant, anti-inflammatory, and antimicrobial effects [8]. Recent studies have also highlighted the antimicrobial potential of these phytochemicals, particularly in inhibiting bacterial growth and biofilm formation [9,10]. These compounds are largely responsible for the medicinal

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properties of the fruit, making it a subject of interest in the search for natural alternatives to conventional antimicrobial agents. On the other hand, many studies concerning with medicinal effects of pomegranate fruits have explored the antibacterial effects of pomegranate extracts against a range of pathogenic bacteria, including *E. coli*, *S. aureus*, and *P. aeruginosa*.

These pathogens are responsible for a wide array of infections, from gastrointestinal diseases to skin and respiratory infections. Research has shown that pomegranate extracts, particularly those derived from the peel, possess potent antibacterial properties. For example, *E. coli* and *S. aureus* growth can be significantly inhibited by both ethanolic and aqueous pomegranate peel extracts [11,12]. Studies indicate that the minimum inhibitory concentrations (MIC) of these extracts range between 50 and 100 mg/mL for the tested bacteria [13]. Moreover, pomegranate peel and aril extracts are effective against *P. aeruginosa*, a multidrug-resistant pathogen associated with severe infections in immunocompromised patients [14]. The antibacterial effects are believed to be attributed to the high concentration of ellagic acid and tannins, which can disrupt bacterial cell membranes, thereby preventing bacterial proliferation and ultimately leading to cell death [15] [16].

The antibacterial action of pomegranate extracts is multifaceted, involving several antibacterial mechanisms against various bacterial species. These extracts also inhibit bacterial enzymes crucial for cell wall synthesis and protein function, thereby reducing bacterial metabolism and growth [17]. Additionally, pomegranate extracts can prevent or disrupt biofilm formation, which helps enhance their antimicrobial efficacy, as biofilms provide bacteria with resistance to antibiotics [18]. Furthermore, pomegranate compounds, particularly ellagic acid, induce oxidative stress by generating reactive oxygen species (ROS), which damage key cellular structures like lipids, proteins, and DNA, resulting in bacterial cell death [19,20]. This study explores the antibacterial potential of pomegranate (Punica granatum) peel and arils extracts against common pathogenic bacteria, including Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, and Staphylococcus aureus.

MATERIALS AND METHODS

Preparation of Pomegranate Fruit Peel and Aril Powders

Fresh pomegranate fruits (*Punica granatum*) were purchased from local markets in AlZawia, Libya, and provided by Dr. Ahlam Alaraf. The fruits were washed thoroughly, peeled, and the arils separated. Both parts were sun-dried for 3–5 days and ground into fine powders using an electric grinder. The powders were stored in airtight containers at room temperature, protected from moisture and light.

Extraction Procedure

To obtain the extracts, 100 g of dried pomegranate peel powder was separately macerated in 500 ml of acetone and 500 mL of 95% ethanol. Each mixture was shaken for 72 hours and then concentrated using rotary evaporation at the Biotechnology Research Centre (BTRC), Tripoli. The final extracts were weighed and stored in airtight containers at 4°C until use.

Bacterial Strains and Culture Conditions

Four bacterial strains were used: *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* (Gram-negative), and *Staphylococcus aureus* (Gram-positive), obtained from BTRC. Cultures were revived in Mueller-Hinton broth and incubated at 37°C for 24 hours.

Culture Media

S. aureus was grown on Nutrient Agar and Mueller-Hinton Agar, while *E. coli*, *K. pneumoniae*, and *P. aeruginosa* were cultured on Nutrient Agar, MacConkey Agar, and Mueller-Hinton Agar. All strains were incubated at 37°C for 24–48 hours.

Antibacterial Assay

Extracts were prepared by dissolving 200 mg of dried extract in 2 mL of DMSO. The agar well diffusion method was used: 100 µl of bacterial suspension was spread on Mueller-Hinton Agar, and 8 mm wells were filled with 100 µl of extract. Controls included DMSO and standard antibiotics (Amikacin 30 µg, Ceftriaxone 30 µg, Cefepime 30 µg). Plates were incubated at 37°C for 18–24 hours, and inhibition zones were measured.

MIC and MBC Determination

The bacterial suspensions were adjusted to match a 0.5 McFarland standard. MIC was assessed using microdilution in Mueller-Hinton broth with serial extract concentrations (0.4–200 mg/ml) inoculated with bacteria (1.5×10^8 CFU/ml). After 24-hour incubation at 37°C, MIC was defined as the lowest concentration with no visible growth. MBC was determined by sub-culturing MIC wells onto Nutrient Agar to find the lowest concentration showing no colony growth.

Statistical Analysis

All experiments were performed in triplicate. Data were expressed as mean ± standard deviation (SD). Statistical analysis was conducted using One-way ANOVA, and significances were accepted at p- value < 0.05.

RESULTS

Agar Well Diffusion Method

The antibacterial activity of acetone and ethanol pomegranate fruit peel extracts was evaluated using the agar well diffusion method. Inhibition zones were observed around the wells containing the extracts, with positive control antibiotics (Amikacin, Ceftriaxone, and Cefepime) showing significant zones of inhibition. No inhibition zones were

observed around the DMSO negative control, confirming that the solvent did not contribute to bacterial growth inhibition. Both acetone and ethanol extracts exhibited antibacterial activity against all tested bacterial strains (*Klebsiella pneumoniae*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*). There was no significant difference ($P > 0.05$) in the activity of acetone pomegranate extract and ethanol pomegranate extract on all the bacterial stains tested.

The ethanol extract demonstrated larger inhibition zones compared to the acetone extract, particularly against *S. aureus* and *K. pneumoniae*, with the largest zone of inhibition (40 mm) observed against *S. aureus*. Notably, the acetone extract exhibited less antibacterial efficacy, especially against *P. aeruginosa*, where the zone of inhibition was smaller (15 mm for acetone extract versus 23 mm for ethanol extract).

The measured inhibition zones are summarized in Table 1, showing that both extracts performed similarly to the antibiotics in some cases, though the ethanol extract generally showed higher activity against most bacterial strains.

Table 1. Zone of inhibition diameter (mm). Means and standard deviation for n=3.

Bacteria Strains	Acetone Pomegranate Extract (mm)	Ethanol Pomegranate Extract (mm)	Antibiotic Drugs (mm)	CEF	CTX	AMK
Klebsiella pneumoniae	29 ± 4	32 ± 2	35	26	16	2
E. coli	30 ± 2	27± 2	22	22	16	-
Pseudomonas aeruginosa	15± 2	23± 2	32	-	16	-
Staphylococcus aureus	39± 2	40± 2	27	25	20	-

(-) Sensitive to antibiotics

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The MIC and MBC of both acetone and ethanol pomegranate peel extracts were determined by the broth microdilution method (Table 2). The MIC values varied depending on the bacterial strain and the solvent used for extraction. For *E. coli* (G177), the acetone extract exhibited a MIC of 6.25 mg/mL, while the ethanol extract showed a MIC of 12.5 mg/mL. Both extracts demonstrated similar MIC values for *K. pneumoniae* (12.5 mg/mL) and *S. aureus* (25 mg/mL), while the MBC values for both extracts were slightly higher, indicating the potential for both bacteriostatic and bactericidal effects. The ethanol extract was more effective against *P. aeruginosa*, showing a MIC of 6.25 mg/mL compared to 25 mg/mL for the acetone extract. The MBC values were consistently higher for both extracts, with the highest values observed against *P. aeruginosa*, possibly due to its more robust outer membrane and virulence factors. The results of the broth dilution assay confirm the antimicrobial potency of pomegranate peel and aril extracts, with the ethanol extract showing slightly greater antibacterial efficacy.

Table 2: MIC and MBC of Two Pomegranate Extracts (Peels and Arils) Determined by Broth Dilution Method

Bacteria Strains	Acetone Pomegranate Extract (mg/ml)		Ethanol Pomegranate Extract (mg/ml)	
	MIC	MBC	MIC	MBC
Klebsiella pneumoniae	12.5	25	12.5	25
E. coli	6.25	12.5	12.5	25
Pseudomonas aeruginosa	25	50	6.25	12.5
Staphylococcus aureus	25	50	25	50

DISCUSSION

Three Gram-negative bacteria (*Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*) and Gram-positive *Staphylococcus aureus* strains were tested for their sensitivity to acetone and ethanol pomegranate fruit peel and aril extracts. The antibacterial properties of the extracts were tested using the agar diffusion method. As observed in Table 1, all pomegranate extracts from peels of fruits and arils displayed inhibition areas **(**clear zones around wells) against the tested bacteria, showing their antibacterial activity. In contrast, the DMSO control did not show inhibition. The selection showed different levels of effectiveness, as some produced greater inhibition areas than others, as shown in Table 1. The highest inhibition zones were observed in ethanol extracts, except for *E. coli* (27 mm inhibition zones). On the other hand, the antibacterial activity of the acetone extract of peels had a lower effect on bacterial strains compared to ethanol extracts. Interestingly, *P. aeruginosa* showed larger inhibition zones on ethanol extracts (23 mm) compared to acetone extracts (15 mm).

In general, pomegranate extracts had less effect on *P. aeruginosa*. This may be due to the presence of the capsule, a virulence factor of the bacteria, which increases their resistance to antimicrobial agents [16]. In study conducted by Alnees *et al* in (2023) reported the same outcome that both acetone and ethanol pomegranate fruit peel and aril extracts contained high levels of phenolics and exhibited antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* [13]. They also found that Gram-positive bacteria were generally more sensitive to the extracts than Gram-negative ones, which aligns with the present study's findings. However, contrary to their results, this study found that all test bacteria showed sensitivity against

pomegranate extracts, and *Staphylococcus aureus* was more sensitive than Gram-negative bacteria. Also, our study showed high efficacy of the plant extract compared with the effect amikacin and ceftriaxone which matched the same findings of some previous studies [17]. A quantitative evaluation of antimicrobial activity was carried out by the broth dilution technique. The MIC (in mg/mL) values of all extracts are presented in Table 2.

The acetone extract of the pomegranate extracts was the most effective against *E. coli*, with an MIC of 6.25 mg/ml and this agreed with Darmawan et al (2022). However, the ethanol extract of the pomegranate extracts was also effective against *P. aeruginosa*, with an MIC of 6.25 mg/ml. *Klebsiella pneumoniae* had an MIC of 12.5 mg/mL with both pomegranate extracts, while *Staphylococcus aureus* had the same MIC value for both extracts. This may relate to some components in the extract that dissolved better in ethanol or acetone but did not diffuse well through the agar. In contrast, Farhan Heiss et al. (2022) Heiss et al (2007) showed that less polar solvents like acetone extracted more phenolic compounds compared to ethanol, which could explain the differences observed in this study [19][20].

CONCLUSION

The results of this study confirm the antimicrobial potential of pomegranate fruit peel and aril extracts, especially their activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Ethanol extracts demonstrated superior antibacterial efficacy compared to acetone extracts. The findings suggest that pomegranate extracts, due to their bioactive polyphenolic compounds, could serve as potential alternatives to conventional antibiotics, particularly against multidrug-resistant bacteria. Future studies should focus on isolating specific compounds responsible for the antimicrobial activity and further exploring their mechanisms of action.

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