



Antimicrobial activity of pomegranate peel extract against various type of microorganism

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Abstract

Background: Multidrug-resistant bacteria pose a significant threat, necessitating the development of new antimicrobial agents. Medicinal plants have been explored for their antimicrobial potential, with pomegranate peel being a rich source of bioactive compounds. Studies in Palestine and elsewhere have highlighted the antimicrobial properties of pomegranate peel extract (PPE) against various pathogens.

Objective: It was to evaluate the antimicrobial activity of aqueous, ethanolic and methanolic extract of Pomegranate peel on some species of Gram-positive and Gram-negative pathogenic bacteria and *Candida albicans*. **Methods:** Aqueous, ethanolic, and methanolic extracts of pomegranate peel were prepared and tested against Gram-positive and Gram-negative bacteria, including clinical isolates, and *Candida albicans*. Antimicrobial activity was assessed using well diffusion, minimum inhibitory concentration (MIC), and minimum bactericidal concentration (MBC) methods. **Results:** PPE exhibited good antibacterial activity against Gram-positive bacteria, including *S.*

aureus and MRSA, with the methanolic extract being the most effective. However, VRSA remained resistant to all extracts. Variable results were observed for Gram-negative bacteria, with *P. aeruginosa* being the most susceptible. *P. vulgaris* showed sensitivity to the ethanolic extract. Notably, all extract types demonstrated antimicrobial activity against *Candida albicans*, with the methanolic extract being the most potent. **Conclusion:** Pomegranate peel extract possesses antimicrobial activity against a range of pathogens, with variations based on the type of extract and the target microorganism. Methanolic PPE, in particular, showed strong activity against *Candida albicans*. These findings suggest the potential use of PPE as an alternative antimicrobial agent in combating infectious diseases, particularly those caused by Gram-positive bacteria and *Candida albicans*.

Keywords: Pomegranate peel extract. Antimicrobial agent. Aqueous extract. Ethanol extract. Methanol extract. *Candida albicans*. Gram positive bacteria. Gram negative bacteria.

Introduction

Due to the emergence of multidrug-resistant bacteria, there is an urgent need to develop a new antimicrobial agent to treat infectious diseases [1]. In medicinal Plant, different extracts and oils from plants are used as a therapeutic agents. Many plant extracts have antimicrobial activity as it contains powerful bioactive agents as hydrocarbons and oxygenated compounds [2]. In Palestine, the biological activities of many plants have been studied [3]. The pomegranate tree is a common tree that has been cultivated in the Mediterranean region many years ago. It is belonging to Punicaceae family. Pomegranate (*Punica granatum* L. fruit) contains many seeds separated by white membrane. Pomegranate has many therapeutic effects, it is a potent antioxidant, antimicrobial agent, and it is also used for the treatment of dysentery and respiratory diseases [4].

The peel of pomegranate comprised approximately 50% of pomegranate whole fruit, which is usually discarded and not used [5]. Pomegranate peel contains a high amount of bioactive compounds like phenolic acid, flavonoid and tannins [6]. Several in vivo and in vitro studies showed the antibacterial, antiviral, anti-inflammatory, and anticancer activity of pomegranate products and extracts [7]. Pomegranate Peel Extract (PPE) has an inhibitory effect against *Staphylococcus aureus* (*S.aureus*) and *Escherichia coli* (*E.coli*) [8], in addition to its antimicrobial effect against the oral pathogen as *Streptococcus mutans* (*S. mutans*) and dental disease [4,9].

A study carried out by Malviya et al. (2014), PPE was tested against four types strains of bacteria: *Staphylococcus aureus*, *Enterobacter aerogenes*, *Salmonella typhi* and *Klebsiella pneumoniae*. Pomegranate extract showed a noticeable effect against all four bacterial strains that were mentioned [10]. Several studies confirm the antibacterial activity of pomegranate extracts against Gram-positive bacteria (*S. aureus*, *Bacillus subtilis*, *Bacillus cereus*) and Gram-negative bacteria (*E. coli*, *Enterobacter aerogenes*, *Pseudomonas aeruginosa*) [11,12]. Another study proved that aqueous extracts of pomegranate peel could effectively control the growth of *Salmonella enterica* [13].

Also, in vivo study conducted on rats to evaluate the effectiveness of PPE against oral candidiasis, the study recommended the use of PPE as an alternative agent to treat oral candidiasis [14]. In the same theme, another study illustrates the anticoccidial and the antihelminthic activities of PPE [15]. A study conducted by El-Kady et al. (2021) illustrates the anti-parasitic activity of ethanolic PPE against *Giardia lamblia* in infected rats, was higher than metronidazole activity in the control rat group [16].

Thus, the aim of this study was to evaluate the antimicrobial activity of aqueous, ethanolic and methanolic extract of Pomegranate peel on some species of Gram-positive and Gram-negative pathogenic bacteria and *Candida albicans*.

Materials and Methods

Preparation of peel extract for microbiological study

Fresh large pomegranate fruits with red peel were bought, washed and peeled. The peels were microwave dried and then ground to powder using a blender. An aqueous extract of pomegranate peel powder was prepared in which 10 grams of the powder were soaked in 100 mL sterile distilled water with continuous agitation in the shaker for 3 days. Then the extracted solution was centrifuged at 4500 rpm for 10 minutes and the supernatant was filtered and lyophilized in the rotary evaporator. The lyophilized powder was solubilized in 5% dimethyl sulfoxide (DMSO) to reach a final concentration equal to 100mg/mL. Similarly, an ethanol extract and methanol extract of pomegranate peel were prepared, this time the 10 grams of the powder were soaked in each 100 ml of 70% ethanol and 70% methanol with frequent agitation for 3 days. Then the extracted solutions were centrifuged at 4500rpm for 10 minutes. The resulting supernatants were filtered and lyophilized in the rotary evaporator. The lyophilized powders were solubilized in 5% dimethyl sulfoxide (DMSO) to reach a final concentration equal to 100 mg/ml.

Bacterial isolate

The antibacterial activity of aqueous, ethanol and methanol PPE were evaluated against American type culture collection isolates (ATCC) which are *S. aureus* (ATCC#6538P), *E.coli* (ATCC#25922), *Klebsiella pneumonia* (*K.pneumonia*) (ATCC#13883), *Proteus vulgaris* (*P.vulgaris*) (ATCC#8427), *Pseudomonas aeruginosa* (*P.aeruginosa*) (ATCC#9027), *Candida albicans* (ATCC# 90028), and clinical bacterial isolates, that were obtained from local hospitals (An Najah hospital and Rafidia hospital, Nablus- Palestine). The clinical isolates were Methicillin-resistant *Staph aureus* (MRSA), vancomycin-resistant *Staph aureus* (VRSA), and the identity of the clinical isolates was confirmed by several biochemical tests which are done at the microbiology research lab at Najah National University. Each tested microbe was cultured on nutrient agar and incubated for 24h at 37°C. In the next day, microbial suspension for each microbe was prepared by inoculating 1 or 2 colonies into a sterile normal saline tube until the turbidity of each inoculating tube was equal to McFarland 0.5 (1.5×10^8 CFU/mL).

Antibacterial activity by well diffusion method

A sterile swab was immersed in the nutrient broth in which the tested bacteria were grown, and then the swab was squeezed into the inner wall of the tube before inoculating the surface of the entire nutrient agar plate. The plate was streaked two more times after rotating it at approximately 60°C to ensure the even distribution of the bacteria onto the agar surface. The rim of the agar was also swabbed. After 10 minutes of streaking, 5 wells were bored in the nutrient agar about 7mm in diameter and at a suitable distance from each other. Three wells were pipetted with 50µl of one of the three types of peel extracts. Distilled water and 40% DMSO were pipetted to the last two well as negative and positive control respectively. Gentamicin antibiotic disk (10mg) was also placed onto the surface of the agar for comparison purposes and also as a positive control. The plates were left at room temperature for 10 minutes, to allow diffusion of the well contents through the agar, before being incubated at 35°C for 24 hours. In the next day, the inhibition zone diameter (IZD) was measured to determine the potency of the peel extract as an antibacterial agent.

Minimum inhibitory concentration (MIC) by micro broth dilution method

Each extract was twofold serially diluted in sterile nutrient broth in micro titer plate (100, 50, 25, 12.5, 6.25, 3.125, 1.5625, 0.781, 0.390, 0.159mg/mL). A duplicate was made for each dilution. Then the bacterial suspension that was prepared equivocally to 0.5 McFarland, was diluted 1:3 by Muller Hinton Broth. 1.0 µl of diluted bacterial broth was inoculated into each microtiter well except for the last two wells in each row. As one of them was left only with the Muller Hinton broth and the extract to consider it as a negative control, while the last well was left with bacterial suspension only to consider it as a positive control.

The microtiter plates were incubated at 35°C for 24 hours. The minimum inhibitory concentration (MIC) is the lowest extract concentration that inhibits bacterial growth in it. This was determined by measuring the absorbance of the microtiter plates by Enzyme-linked immunosorbent assay (ELISA) reader at a wavelength of 650 nm and considering the well with zero absorbance and lowest concentration as MIC well.

Determination of minimum bactericidal concentration (MBC)

This was achieved by subculturing a few microliters from each microplate well which showed no bacterial growth after the incubation period onto the surface of Muller Hinton Agar (MHA) and incubating them at 35°C for 24 hr. The plate with no bacterial growth and lowest

concentration was considered as minimal bactericidal concentration.

Results

The result of the agar well diffusion method (Table 1) shows a good antibacterial activity for aqueous, ethanol, and methanol extracts against the studied gram-positive bacteria (ATCC *S.aureus*, MRSA, from the clinical sample), while VRSA was resistant to all types of extracts. Variable results were obtained in Gram-negative bacteria. For *P. aeruginosa*, the three types of extracts have antimicrobial activity against it with the highest effect recorded for methanolic extract. In the case of *K. pneumonia*, both ethanolic and methanolic extracts exhibit the same antibacterial activity while the aqueous extract has no effect against it at all. For *P. vulgaris*, the ethanolic extract has antibacterial activity against it with no antibacterial effect for aqueous or methanolic extract. Regarding *E.coli*, the three extracts have no antibacterial activity against it. On the other hand, all extracts types have antimicrobial activity against ATCC *Candida albicans* with the highest activity recorded for methanolic extract.

Table 1. Inhibition diameter zone (IDZ) for aqueous, ethanol and methanol PPE for tested bacteria by agar well diffusion method.

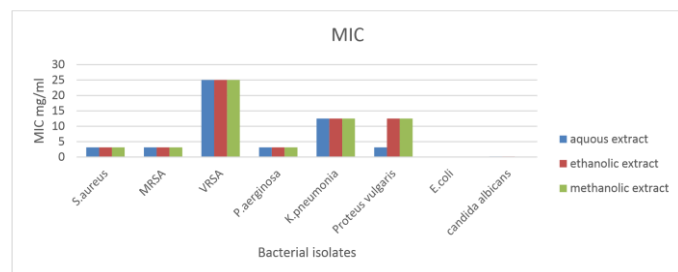
	Microbial isolate	Inhibition Diameter Zone (IDZ)		
		Aqueous extract	Ethanol extract	Methanol extract
Gram-positive bacteria	<i>S.aureus</i>	24	22	23
	VRSA	0	0	0
	MRSA	24	24	22
Gram-negative bacteria	<i>P.aeruginosa</i>	11	15	17
	<i>K.pneumonia</i>	0	11	11
	<i>E.coli</i>	0	0	0
	<i>P.vulgaris</i>	0	10	0
Unicellular fungi	<i>Candida albicans</i>	13	15	17

Source: Own Authorship.

Quantitative assessment for an antimicrobial activity for the extracts was determined by measuring the MIC concentration for each type of extract against the studied microbes. Figure 1 illustrated that the MIC value for *S.aureus* was the same whether the extract was aqueous or ethanol or methanol (MIC=3.125). The same thing for MRSA (MIC= 3.125), VRSA (MIC= 25), *P.aeruginosa* (MIC= 3.125), *K.pneumonia* (MIC=12.5). For *P. vulgaris*, the aqueous extract (MIC= 3.125) was more effective than ethanolic and methanolic extract (MIC=12.5) in inhibiting its growth. For ATCC *Candida albicans*, methanolic extract (MIC=0.097) was more

effective than aqueous and ethanolic extract (MIC= 0.195) in inhibiting its growth. No effect for any type of extract on *E.coli*.

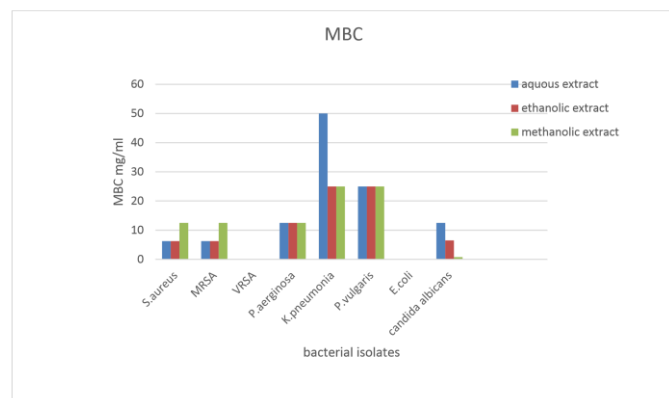
Figure 1. Minimum inhibitory concentration of aqueous, ethanol and methanol PPE for the studied microbes.



Source: Own Authorship.

Regarding MBC for the extracts, the results shown in Figure 2 illustrated that in addition to the strong antifungal activity for the three types of extracts, methanolic extract was the strongest in killing *Candida albicans* (MBC= 0.781 mg/mL). However lower concentration of aqueous and ethanolic extract (MBC= 6.25) was able to kill ATCC *S.aureus* and MRSA from clinical samples than methanolic extract. For *K.pneumonia*, ethanolic and methanolic extract are more potent in killing it (MBC= 25mg/mL) than aqueous extract (MBC=50mg/mL). For *P. aeruginosa*, it seems that the three types of the extract have the same antibacterial activity against it (MBC=12.5 mg/mL) and this concentration is much less than the extract concentration that was able to kill *P. vulgaris* (MBC-25mg/mL). Unfortunately, the extracts have no bactericidal effect against *E.coli* and VRSA.

Figure 2. Minimum bactericidal concentration of aqueous, ethanol and methanol PPE for the studied microbes.



Source: Own Authorship.

Discussion

The excessive use of antibiotics results in the development of antimicrobial-resistant microbes. The

emergence of such resistant microbes is a life-threatening problem. There is an urgent need to find a choice to face this dilemma. In Palestine, Folk medicine is greatly based on natural plants for the treatment of many diseases including infectious diseases. In this research, the antimicrobial study of PPE that has been prepared in three different solvents (aqueous, ethanol and methanol), suggest a promising future for using PPE as an antimicrobial agent, particularly for *Candida albicans* infection. The result showed that very low MIC (0.097) and MBC (0.781) for methanol PPE were able to inhibit and kill *Candida albicans*, respectively. Moreover, in the agar well diffusion method, methanol PPE exhibited the highest inhibition zone diameter (17mm).

Regarding the studied Gram-positive bacteria, *S.aureus*, MRSA, and VRSA, a relatively, high concentration of the extract were needed to inhibit VRSA (MIC=25mg/mL), but the extracts were unable to kill it even at high concentrations. So consequently, the extract is considered ineffective against VRSA. The aqueous and ethanolic extracts were able to kill *S.aureus* and MRSA at low MBC (6.25mg/mL) compared to methanolic extract which killed them at MBC of 12.5mg/mL.

For Gram-negative bacteria, the extracts were able to inhibit and kill *P.aeruginosa*, *K.pneumonia* and *P.vulgaris* with some degree of variations in the extract concentrations in both MIC and MBC. The three extract types recorded the same MIC value against *P.aeruginosa* (MIC= 3.125 mg/mL) and *K. pneumonia* (MIC- 12.5 mg/mL) but in case of *P.vulgaris* the aqueous extract was the one with highest inhibitory action (MIC= 3.125 mg/mL) compared to ethanolic and methanolic extracts (MIC= 12.5mg/mL).

On the other hand The three extract types recorded the same MBC value against *P.aeruginosa* (MBC=12.5 mg/mL), and *P.vulgaris* (MBC=25mg/mL), but in case of *K. pneumonia*, ethanol and methanol extracts were able to kill it at lower MBC concentration (25mg/mL) than aqueous extract (MBC- 50mg/mL). It is clear from these results that PPE have antibacterial activity against most of the studies bacteria in this article with a strong effect on Gram-positive bacteria than Gram-negative bacteria. Among Gram-positive bacteria, VRSA was not influenced by the extracts, it seems that this species of bacteria develops many mutations and resistant mechanisms that render it highly resistant not only to several antibiotics but also to plant extracts of pomegranate. The results of this work are consistent with results from a previous study that reported the antimicrobial activity of PPE against a variety of Gram-positive and Gram-negative bacteria including *S. aureus*, *Bacillus subtilis*, *E.coli*, and *P.vulgaris* [17].

In general, this study agrees with a study that

found that the pomegranate extracts are more effective as an antimicrobial agent against Gram-positive bacteria than Gram-negative bacteria [18]. In the same path, Olaniyi et al, 2012, reported that methanolic PPE exhibit a board antimicrobial activity against Gram-positive and Gram-negative bacteria with a minimum inhibitory concentration ranging between 0.2-0.78 mg/dL [19].

Regarding the anti-candida activity of PPE, this study confirmed the high potency of methanolic, ethanolic and aqueous extracts in inhibiting and killing *Candida albicans*. A study in Egypt that investigated the anticandidal activity of PPE supported our results, as it found that aqueous, ethanol and methanol extract of PPE have the most anticandidal activity against *Candida albicans* strains compared with standard fungisides [20].

Conclusion

PPE has antimicrobial activity against *S. aureus*, MRSA, *P.aeruginosa*, *K. pneumonia*, *P.vulgaris*, and *Candida albicans*. The extract activity varies according to the method of its preparation whether it is in aqueous, ethanolic or methanolic solvents and also its activity depends on the tested microbes. The highest activity was for methanolic extract against *Candida albicans*.

Recommendations

It is recommended to use Pomegranate Peel powder as an alternative medicine to treat some microbes such as those studied in this research. Further studies should be carried out to isolate the bioactive substance from PPE.

Ethical Approval and Consent to Participate

The current study has been conducted under the relevant guidelines; safety and ethical consideration.

Consent For Publication

Not applicable

Availability of Data and Material

The data are documented directly in tables and figures, and the samples have been disposed of according to the most appropriate safety measure.

Competing Interests

The author(s) declared that they have no potential conflicts of interest concerning the research, authorship and publication of this article.

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Authors' contributions

All authors listed have contributed and approved the work for publication. The authors worked in an orderly manner. Dr. Lubna Kharraz designed the study and supervised the work, and write the manuscript. Dr Wafaa Menawi reviewed and approved the manuscript. Miss, Nour Dalu, Ghofran Kilani, and Rozan Ammar, worked at the same pace, taking turns collecting samples, working in the lab, observing the results, and documenting the investigations.

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