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Effect of *Punica granatum* L. peel extract on phytopathogenic bacteria

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Pathogens, animals and weeds, among the biotic factors, cause yield losses between 20 to 40% in agricultural production. Therefore, much research has focused on minimizing these product losses and improving product yield. Mainly, synthetic agents and antibiotics have been used for a long time against plant pathogenic bacteria. But there is a search for alternative natural compounds because of undesirable effects on the environment. For this purpose, in this study, the pomegranate peels extract was prepared in different concentrations (600-100 µg/mL) and evaluated against plant pathogenic bacteria (*Pectobacterium atrosepticum*, *Pectobacterium carotovorum* subsp. *carotovorum*, *Pseudomonas corrugate*, *Pseudomonas mediterranea*, *Rhizobium radiobacter*, and *Xanthomonas campestris*). According to the results, the antibacterial effect of pomegranate peel extract was observed for *P. mediterranea*, *P. carotovorum* subsp. *carotovorum*, and *R. radiobacter*. The largest zone diameter (10.4 mm) and lowest MIC value (100 µg/mL) were observed against *P. mediterranea*. Therefore, it is possible to conclude that pomegranate peel extracts is effective against some phytopathogenic bacteria. Also, according to the study results, it is deduced that waste materials can be used as an alternative to synthetic drugs and thus, prevent toxic substance pollution with completely organic waste.

Keywords: Antibacterial activity, Plant pathogenic bacteria, Pomegranate peel extract, *Punica granatum.*

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Introduction

Pomegranate (*Punica granatum* L.), belonging to the family of Punicaceae, is a fruit grown from Northern India to Iran[1]. Since ancient times, it has been used to prevent and treat diseases such as diabetes, diarrhoea, and cancer. In addition, it is used as a fruit to eat and produce wine, fruit juice, pomegranate syrup, etc[2,3]. After processing the beverages such as fruit juice, a remarkable amount of pomegranate peel (fifty per cent of pomegranate) is released as waste and causes environmental pollution[4]. There is a big necessity to adopt suitable methods for producing valuable products from fruit processing wastes[5,6]. The recovery of fruit peels will be very beneficial to improving fruit processing units' economics and reducing environmental pollution in the long term. Therefore, many studies have focused on the utilization of fruit peel. According to the literature, pomegranate peels have significant bioactivity and have been used for centuries for human health due to their rich active constituents[7,8]. The parts obtained from the peels of sweet, sour-sweet, and sour pomegranate cultivars may differ in their chemical composition and antimicrobial capacity. Especially pomegranate peels from different geographical areas have significant antimicrobial properties[1]. Besides, pomegranate peels have been recognized for wound-healing properties, antiatherosclerotic, and antioxidative capacities[9,10]. Plant extracts are generally considered to be safer than synthetic materials. Therefore, pomegranate peel extract can be used for alternative synthetic hazardous products[1,11-15].

Additionally, pomegranate peel extract with antibiotic combinations might have a synergistic effect on antimicrobial therapy[16]. Although the antimicrobial activity of pomegranate peel has been widely studied, there is no study to report its impact on plant pathogenic bacteria. About 80,000 plant diseases have been reported throughout the World[17]. The species of most pathogenic bacteria belong to the genera of *Erwinia*, *Pectobacterium*, *Pantoea*, *Agrobacterium, Pseudomonas*, *Ralstonia*, *Burkholderia*, *Acidovorax*, *Xanthomonas*, *Clavibacter*, *Streptomyces*, *Xylella*, *Spiroplasma*, and Phytoplasma. One of the gravest consequences caused by plant diseases in history is the late blight of potatoes in Ireland (1845–1860); a million

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people died of hunger, and more than a million evacuated[18]. The plant diseases reduce the quality, marketability, and productivity of the agricultural products and increase the costs of agricultural products[19]. Synthetic substances or antibiotics have been widely used to overcome crop loss caused by plant pathogenic bacteria. But synthetic substances and antibiotics have undesirable effects on the environment, and there is an increase in the number of antibiotic-resistant pathogens. Therefore, researchers have searched for alternative harmless substances against plant pathogenic bacteria[20]. This study evaluated the antibacterial effects of pomegranate peel extract against some of the plant pathogenic bacteria.

Materials and Methods

Extraction of *P. granatum* L. peels

The pomegranate fruits used in the study were obtained from Antalya. The peels of the fruits were dried in the shade and then grinded. About 100 g of the grounded peels was extracted in 1000 mL ethanol by the Soxhlet extraction technique for 10 h. The extract was filtered, and the ethanol was completely evaporated at 40 ºC in a rotary evaporator. Then, the extract was prepared in different concentrations (100, 200,300, 400, 500, 600 µg/mL) with 96% ethanol.

Bacterial strains

The studied plant pathogenic bacteria were obtained from the culture collection of Erzurum Technical University, Molecular Biology and Genetics Laboratory. The bacteria were cultured aerobically on Nutrient Agar at 27 °C for 24 h.

Antibacterial studies

The prepared extracts were evaluated against six plant pathogenic bacteria isolated from different sources by disc diffusion assay and microdilution method described by Gormez *et al.*[21]. For the disc diffusion assay, 100 µL of bacterial culture (10^8 CFU/mL) was spread on MHA with a swab. The discs (6 mm in diameter) were individually saturated with ten µL of the prepared concentrations of *P. granatum*'s peel extract and placed on the same medium. Negative control was the discs (6 mm in diameter) saturated with ten µL of ethanol (96%). Positive controls were the antibiotics defined as ofloxacin (OFX) (10 µg/disc), netilmicin (NET30) (30 µg/disc), sulbactam (SCF) (30 µg/disc). Then, the assay setups were incubated at 27 °C for 48 h, and the antibacterial potentials of the studied extract were evaluated by measuring the inhibition zone diameter. The MIC values are defined as the lowest concentration of the extract to inhibit the growth of bacteria. Each experiment was performed in triplicate.

Results and Discussion

The bacterial strains used in this study and their isolation source are given in Table 1. The antibacterial effect of pomegranate peel extract against plant pathogenic bacteria was explored by disc diffusion and microdilution methods. Commercial antibiotic discs (ofloxacin, netilmicin, sulbactam) were used as a positive control. The results are summarized in Table 2. The results showed that the tested antibiotics were more effective than pomegranate extract on plant pathogenic bacteria. The highest antibacterial

Table 1 — The studied bacteria and isolation source

Bacteria	Isolation source
Pectobacterium atrosepticum	Potato
Pectobacterium carotovorum subsp. *carotovorum*	Potato
Pseudomonas corrugata	Tomato
Pseudomonas mediterranea	Tomato
Rhizobium radiobacter	Apricot
Xanthomonas campestris	Tomato

Table 2 — Antibacterial activity of the ethanol extract of *Punica granatum* L.

Bacteria	Disc diffusion test* Concentrations (µg/mL)						MIC**	Negative control	Standard antibiotic discs***		
	100	200	300	400	500	600		Ethanol	OFX	NET30	CFS
Pectobacterium atrosepticum	-	-	-	-	-	-	-	-	14.6	11.6	11.2
Pectobacterium carotovorum subsp. *carotovorum*	-	-	-	-	7.3	8.4	500	-	13	12.6	13
Pseudomonas corrugate	-	-	-	-	-	-	-	-	11.6	9.8	10
Pseudomonas mediterranea	8.2	8.2	8.5	8.4	8.6	10.4	100		10	9.4	-
Rhizobium radiobacter	-	-	6.4	7.9	8.3	9.3	300	-	13.5	13.8	12.4
Xanthomonas campestris	-	-	-	-	-	-	-	-	10.4	10	10.2

*Diameter of the inhibitory zone [mm] for different concentrations, **MIC: Minimum inhibitory concentrations, ***OFX: ofloxacin (10 µg/disc) NET-30: Netilmycin (30 µg/disc), CFS: Cefsulodin (30 µg/disc) was used as positive reference standard antibiotic discs (Oxoid)

effect of pomegranate peel extract against plant pathogenic bacteria was obtained for *P. mediterranea.* The inhibition diameter was 10.4 mm at a 600 µg/mL concentration. It can also be observed that pomegranate peel extract had different antibacterial effects on other species of the same genus. Although *P. mediterranea* was followed with inhibition zones ranging from 8.2 to 10.4 mm, the pomegranate peel extract was ineffective against *P. corrugata.* At the same time, the pomegranate peel extract showed an inhibition zone ranging from 6.4 to 9.3 mm at a 300-600 µg/mL concentration against *R. radiobacter*, 7.3 to 8.4 against *P. carotovorum* subsp. *carotovorum*. It is recorded that pomegranate peels extract had no antimicrobial effect for some species (*P. atrosepticum*, *P. corrugata*, *X. campestris*).

Although there are many studies about the antimicrobial activity of pomegranate peel extracts, there has been only one study that evaluated the antibacterial potential of pomegranate peel extract against plant pathogenic bacteria yet[22]. This study demonstrated that *P. granatum* ethyl acetate peel extracts showed significant antibacterial activity against *Xanthomonas gardneri*, *Ralstonia solanacearum*, *P. carotovorum* subsp. *carotovorum.* The present study clearly showed that pomegranate peel extract has antibacterial potential against some plant pathogens and the results differed according to the pomegranate peel extract concentration value. According to the literature, the main reason for the antimicrobial effects of the pomegranate peel extract is the high content of tannins such as anthocyanins and polyphenolic compounds (especially punicalagin), ellagitannin (especially ellagic acid) in pomegranate[11]. Tannins disrupt the metabolic activity of bacterial enzymes and membranes by forming stable complexes with proteins, starch, and physiological metals[12]. The chemical composition of *P. granatum* L extracts was determined in different world regions in literature[23-25]. It is known that the chemical compositions of the plants can differ according to the climate and soil chemistry of the plant growth area and the extraction method. Therefore, the chemical composition of the peel extract has to be identified by GC-MS analyses to reveal the exact reason for the antibacterial mechanism of the extract. It can be concluded that more effective antibacterial activity can be obtained by increasing the extract concentration or combining the extract with different active ingredients.

Conclusion

In conclusion, it has been shown that the extract of pomegranate peels was active against *P. mediterranea*, *P. carotovorum* subsp. *carotovorum*, and *R. radiobacter*. Among these bacteria, *P. mediterranea* was more sensitive to the extract than other bacteria. According to the results presented in this study, it is suggested that the pomegranate peel extract can be used to manage some plant diseases due to its antibacterial activity and non-cytotoxic potential as an alternative to the use of synthetic drugs.

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Conflict of interest

The authors declare that there is no conflict of interest.

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