Journal of Education for Pure Science
Vol.15, No.1 (2025)
DOI: https://doi.org/10.32792/jeps.v15i1.557
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Inhibitory effect of extracts of fruit peels in the growth of some bacteria positive and negative dye Cram

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Received 28 /09 /2024, Accepted 02/ 02 /2025, Published 1/03 /2025

Abstract:

Fruit peels are often regarded as agricultural waste and are typically discarded instead of being utilized for their antimicrobial potential. Studies indicate that these peels harbor valuable compounds that could be beneficial for medical applications. This study assessed the antibacterial properties of ethanol and aqueous extracts from the peels of kiwi (Actinidia deliciosa), pomegranate (Punica granatum), and orange (Citrus sinensis) against four bacterial pathogens: Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, and Bacillus cereus. It was collected the fruits from local markets in the city of Nasiriyah. Then, it was removed the peels, washed, dried, and ground them, preparing both aqueous and alcoholic extracts of these fruits to study their effectiveness in inhibition. The findings revealed that the pomegranate fat extract demonstrated the highest antibacterial activity, with an inhibition zone of 39.66±1.60 mm against Staphylococcus aureus, followed by 34.00±1.20 mm against Pseudomonas aeruginosa. The ethanol extract of pomegranate also showed significant activity, with 32.33±1.60 mm against Pseudomonas aeruginosa, while the lowest inhibition was recorded at 14.66±1.80 mm against Bacillus cereus. The kiwi and orange extracts exhibited inhibition zones ranging from 11.66±1.20 mm to 6.33±1.00 mm. The present study shows that the examined fruit peel residues have potential therapeutic uses against multidrug-resistant pathogenic bacteria. This approach can also reduce waste by promoting recycling in an economically and environmentally friendly way.

Keywords: Pomegranate Fat, Kiwi, Growth Inhibitor, Lowest Concentration of Inhibitor

1-Introduction

The rise of multidrug-resistant bacteria is becoming a significant global issue. There is an urgent demand for new antimicrobial alternatives, leading to increased interest in this area. Over time, herbal medicines have contributed significantly to the evolution of medical pharmacology and have been fundamental to traditional medicine practices [1]. Herbal remedies are believed to be utilized by 80% of the population for various ailments, attributed to their wide accessibility, affordability, minimal side effects, and their effectiveness as anti-inflammatory agents and antimicrobials against harmful pathogens [2]. As a result, there is ongoing research focused on extracting phytochemicals from secondary plant metabolism, including phenolic compounds, alkaloids, anthraquinones, flavonoids, saponins, tannins, glycosides, and reducing sugars [3]. Fruit peels are categorized as agricultural waste, and rather than being used for their potential as antimicrobial agents, they are often thrown away. Research has shown that these peels contain important compounds that could be valuable for pharmacological or medical uses [4,1]. The kiwi fruit, Actinidia deliciosa, belongs to the family Actinidiaceae and the genus Actinidia [5]. This tropical fruit is recognized for its therapeutic and medicinal benefits, which may help treat various conditions, including diabetes, cancer, digestive issues, kidney disorders, and cardiovascular diseases. Kiwi fruit is particularly rich in vitamin C and contains a significant amount of essential minerals [6]. Kiwi fruit has antioxidant properties and exhibits antibacterial activity. In addition to its antioxidant benefits, it has shown effectiveness against specific harmful bacteria, including Escherichia coli, Listeria monocytogenes, and Staphylococcus aureus [7]. The recognized antibacterial, anti-inflammatory, and antioxidant properties of kiwi fruit extract aid in alleviating inflammation as well as the symptoms and signs associated with inflammatory diseases[8,9]. Pomegranate (Punica granatum) is a fruit belonging to the Punicaceae family [10]. Originally native to India and Iran, it is now cultivated across the Mediterranean region and South-Western America. Pomegranate peel is an unusable byproduct from the production of pomegranate juice and from eating the fruit directly. It is high in tannins, flavonoids, and various phenolic compounds [11]. Pomegranate peels have long been utilized in traditional medicine across America, Asia, Africa, and Europe to treat various health issues, serving as an antiparasitic agent, a "blood tonic," and for healing aphthae and ulcers [1,10] . Oranges are among the most widely consumed fruit globally and contain beneficial compounds that support health [12]. Citrus fruits feature a tough outer peel and a spongy white layer surrounding the juicy segments. The juice contains a significant amount of citric acid, contributing to its distinct sour taste. Citrus fruits also contain sugars, organic acids, lipids, polysaccharides, vitamins, minerals, flavonoids, carotenoids, and aromatic compounds. They are particularly rich in vitamin C and flavonoids. The levels of phytochemicals vary based on the species, variety, and cultivation methods. Among the flavonoids, there are several types of flavanones and flavones [13]. Citrus fruit products are known for their ability to fight bacteria and fungi. The peels of citrus fruits are rich in flavanones and polymethoxylated flavones, compounds that are rare in many other plants. These substances play an important role in promoting human health and benefiting the environment, while also being valuable in various economic applications in the food and pharmaceutical industries. Additionally, citrus peel oils are known for their strong antioxidant and antibacterial effects [14,15]. The aim of this study was to assess the antimicrobial activity of peels different fruits (kiwi, Pomegranate and orange).

2- Methods

2-1 Samples collection and Preparation of fruit peel powders and extracts

The preparation of fruit peel powders involved collecting fresh kiwis, pomegranates, and oranges from the market in Al-Nasiriyah city. Oranges were gathered in April, while kiwis were collected in May, and pomegranates were harvested in October. After peeling the fruits, the peels were thoroughly washed with tap water followed by

distilled water. The cleaned peels were then chopped into small pieces and dried in an oven at 50 °C for 48 hours. Once dried, they were ground into a fine powder using an electric blender and stored in the refrigerator at 4 °C until needed. Ten grams of powdered peels (from kiwi, pomegranate and orange) were immersed in 250 ml of a solvent mixture (99% ethanol and distilled water) and shaken at room temperature for 24 hours. The resulting clear extracts were filtered using Whatman paper no. 1. A rotary evaporator was then utilized to concentrate the filtrates by removing the solvent. The extracts were subsequently sterilized with a 0.45 µm filter and stored in bottles in a refrigerator at 4°C until needed.

2-2 Determining antibacterial activity: involved using specific stock cultures, including (Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, and Bacillus cereus) to prepare fresh bacterial cultures at a 0.5 McFarland standard. These cultures were then sub-cultured onto Muller Hinton agar plates. Using sterile forceps, extract disks were placed evenly on the agar surface. Vancomycin antibiotic discs (20 µg) served as positive controls, while DMSO acted as a negative control. The plates were incubated at 35 ± 0.5°C. The inhibition zones were measured from the center of the extract disk to the edge of the area where no bacterial growth occurred, using a clean ruler.

2-3 Statistical Analysis: SPSS was used to examine the collected data. Descriptive statistics (Mean value and SD) along with comparison in mean zone of inhibition between the extracts of kiwi, pomegranate and orange peels were used to perform One way analysis of variance (ANOVA).

3- Results

The current study examined the antimicrobial properties of extracts from three types of fruit peels: kiwi, pomegranate, and orange, as well as pomegranate fat. The antimicrobial effects of these plant extracts were tested against specific pathogenic strains, including Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, and Bacillus cereus, which are common bacterial species. All fruit extracts demonstrated strong antibacterial effects against both gram-positive and gram-negative bacteria. Notably, the ethanolic extracts from all fruit peels exhibited significantly greater antibacterial activity compared to the water extracts.

Pomegranate water and ethanol extracts in addition to ethanol extract of Pomegranate Fats were the more effective fruit extract against all strains and especially Staphylococcus aureus (39.66±1.60 mm for ethanol extract Pomegranate fat) then Pseudomonas aeruginosa (34.00±1.20 for ethanol extract Pomegranate fat ; 32.00±1.40 for water ; 31.33±1.60mm for ethanol), then Escherichia coli (28.00±1.80 for ethanol ;25.00±1.40mm for water) ,Staphylococcus aureus (24.33±1.40 for ethanol; 20.00±1.80 mm for water) and the lowest with Bacillus cereus(11.00±1.00 for water; 14.66±1.80 for ethanol) Table (2) and figure (2)

Kiwi extracts come after pomegranate extracts in terms of effectiveness ,where the highest inhibition zone of kiwi peels extract with Staphylococcus aureus (20.33±1.20 mm for ethanol) and the lowest inhibition zone with Bacillus cereus (11.66±1.20mm for water) Table (1) and figure (1).

Orange peel extracts showed significant antibacterial activity against all pathogenic bacteria, but less than pomegranate and kiwi extracts, and the activity was higher with Staphylococcus aureus (18.00±0.80 mm for ethanol) and the lowest inhibition zone with Pseudomonas aeruginosa (6.33±1.00 mm for water) Table (3) and figure (3).

Table1: Antimicrobial Activity Of Kiwi Peel (Water And Methanol) Extracts Against Pathogenic Bacteria

Fruit Peel / Pathogenic bacteria	kiwi	
	Water extracts Mean ± SD	Ethanol extracts Mean ± SD
Escherichia coli	18.00 ± 1.30	18.66±1.20
Staphylococcus aureus	19.33±0.60	20.33±1.20
Pseudomonas aeruginosa	18.33±1.40	19.66±1.80
Bacillus cereus	11.66±1.20	19.66±1.00

The experimental values in each row show significant differences ($p < 0.05$) based on the One-way ANOVA test.

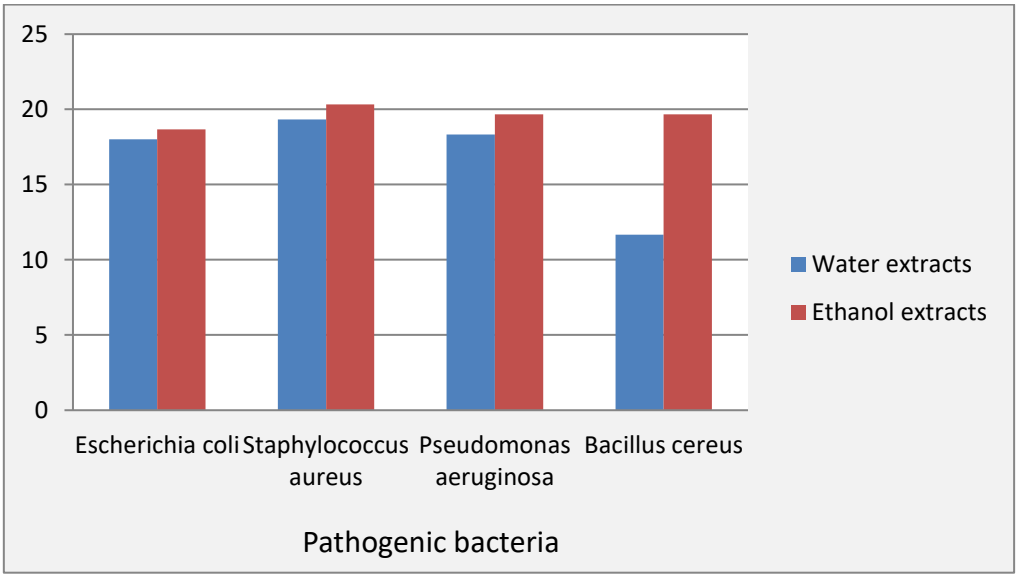

Figure 1: Antimicrobial Activity of Kiwi Peel (Water And Methanol) Extracts Against Pathogenic Bacteria

Table 2: Antimicrobial Activity Of Pomegranates Peel (Water And Methanol) Extracts And Pomegranate Fat Against Pathogenic Bacteria

Fruit Peel / Pathogenic bacteria	pomegranates		Pomegranate fat
	Water extracts Mean ± SD	Ethanol extracts Mean ± SD	
Escherichia coli	25.00±1.40	28.00±1.80	29.66±1.00
Staphylococcus aureus	20.00±1.80	24.33±1.40	39.66±1.60

Pseudomonas aeruginosa	31.00±1.40	32.33±1.60	34.00±1.20
Bacillus cereus	11.00±1.00	14.66±1.80	29.66±1.60

The experimental values in each row that show significant differences ($p < 0.05$) based on the One-way ANOVA test.

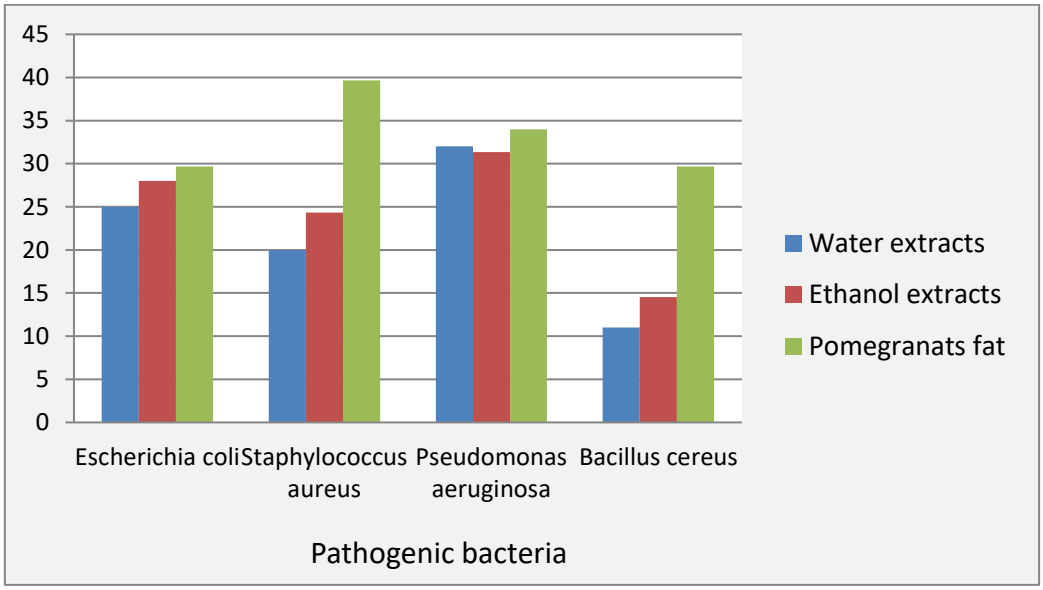

Figure 2: Antimicrobial Activity Of Pomegranates Peel (Water And Methanol)

Extracts Against Pathogenic Bacteria

Table3: Antimicrobial activity of orange peel water and methanol extracts against pathogenic bacteria

Fruit Peel / Pathogenic bacteria	oranges	
	Water extracts Mean ± SD	**Ethanol extracts Mean ± SD**
Escherichia coli	9.33±1.00	11.00±0.40
Staphylococcus aureus	11.00±0.80	18.00±0.80
Pseudomonas aeruginosa	6.33±1.00	14.33±1.60
Bacillus cereus	6.66±0.60	10.66±0.80

The experimental values in each row that show significant differences ($p < 0.05$) based on the One-way ANOVA test.

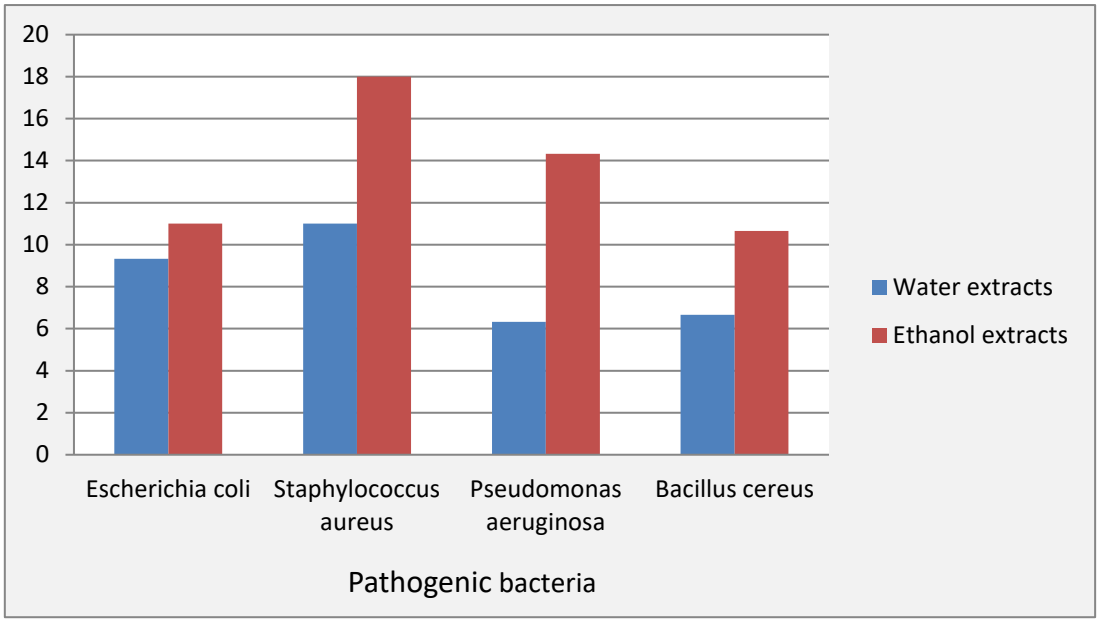

Figure3: Antimicrobial Activity of Orange Peel Water and Methanol Extracts Against Pathogenic Bacteria

4- Discussion

Kiwi peel is recognized for its antibacterial properties, largely due to its abundant bioactive compounds such as phenolic acids, flavonoids, and antioxidants. These substances exhibit antimicrobial effects against a range of bacterial strains, helping to suppress bacterial proliferation and reduce the risk of infections.Recent research indicates that kiwi peel extracts can successfully hinder the growth of harmful bacteria. This effect may arise from their capacity to interfere with bacterial cell membranes, obstruct the function of virulence factors, and impede biofilm development. Furthermore, the antioxidant characteristics of these compounds likely enhance their antimicrobial effectiveness by neutralizing the reactive oxygen species produced by bacteria [16, 17]. Pomegranate is the most potent extract, demonstrating both bactericidal and bacteriostatic effects against highly susceptible pathogenic bacteria strains. This effectiveness may be attributed to the presence of a variety of secondary metabolites in pomegranate peel, including polyphenols, tannins, flavonoids, and anthocyanins (such as cyanidins and delphinidins), all of which have antibacterial properties . Pomegranate extract exhibited a higher phenolic content compared to extracts from kiwi and orange peels. The action mechanisms of phenolic compounds on bacterial cells are thought to include damage to the bacterial membrane, inhibition of virulence factors like enzymes and toxins, and the reduction of bacterial biofilm formation [18, 19]. Extracts from orange peels demonstrate notable antibacterial effects against various pathogenic bacteria, albeit with less inhibition when compared to extracts from pomegranate and kiwi peels. This efficacy may stem from the presence of various chemical compounds in orange peels, including alkaloids, flavonoids, glycosides, saponins, resins, oleoresin, sesquiterpenes, phenolic compounds, fats, and oils. The peel is noted for containing a higher concentration of organic compounds relative to other fruit parts. Specifically, orange peel extract exhibits the strongest antibacterial activity against Staphylococcus aureus, while showing minimal effectiveness against Pseudomonas aeruginosa. Additionally, orange extracts possess antimicrobial properties against microorganisms responsible for causing acute diarrheal infections, such as Salmonella spp. and Escherichia coli [20].

5- Conclusion

This study investigated the antibacterial properties of water and ethanol extracts from kiwi, pomegranate, pomegranate seed, and orange peel. The findings revealed that both ethanol and water extracts, particularly the ethanol extracts, were effective against pathogenic bacteria such as Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, and Bacillus cereus. These extracts could potentially be beneficial in addressing infections caused by these microorganisms

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