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Antibacterial Activity of Fruit Peels Extracts Against Pathogenic Bacteria

M. R. Zaki[1], T. E. Farrag[2], A. H. Mohamedin[3], M.I. El-Bana[4], W. I. A. Saber[5]
[1]Dakahlia central lab for drinking water, Mansoura, Egypt.
[2]Chemical Engineering Department, Faculty of Engineering, Port Said University, Port Said, Egypt.
[3]Department of Botany, Faculty of Science, Mansoura University, Mansoura, Egypt.
[4]Department of Botany, Faculty of Sciences, Port Said University, Port Said, Egypt.
[5]Microbial Activity Unit, Department of Microbiology, Soils, Water and Environment Research Institute, Agricultural Research Center (ID: 60019332), Giza (P.N. 12619), Egypt.
[*]**Corresponding author:** alfad2004@gmail.com

ABSTRACT

Fruit peels are a major byproduct of processing food and are not currently used commercially, but they can be a potential antimicrobial agent. As microorganism resistance to marketed antibiotics is a prime concern nowadays, the current study evaluated the antibacterial activity of ethanol and aqueous extracts of peels of banana (Musa acuminate), pomegranate (Punica granatum), and orange (Citrus sinensis) against four pathogens *Escherichia coli*, *Salmonella enterica*, *Staphylococcus aureus*, and *Enterococcus faecalis*. Results showed that pomegranate extract was the most effective against four pathogenic bacteria were Highest zone of inhibition (25.2 ±1.2mm) with *Staphylococcus aureus*, then (21.5 ±1) with *Salmonella enterica* then (18.3 ±1.6mm) with *Escherichia coli* and the lowest was (15.3 ±1mm) with *Enterococcus faecalis*. Banana and orange extract inhibition zone were ranged between (9 ±0.9 and 19 ±1.4). The minimum inhibitory concentration (MIC) value was found the least was pomegranate extract (3.125 mg/mL) with *Staphylococcus aureus*, and the highest MIC was (25 mg/mL) of banana and orange with *Escherichia coli* and *Enterococcus faecalis*.

The current investigation demonstrates that the fruit peel residues studied can be used therapeutically to treat multidrug-resistant pathogenic bacteria. This will also help to decrease trash and reuse it in a cost-effective and ecologically beneficial manner.

Key Words:
pomegranate, banana, orange, extract, antibacterial.

1. INTRODUCTION

Multidrug-resistant bacteria are becoming a very large problem throughout the world [1]. Antimicrobial antibiotic resistance is thought to be responsible for 700,000 fatalities each year. Antimicrobial resistance is expected to cause 10,000,000 deaths per year by 2050 if it continues to rise. [2]. So, the urgent need to discover new antimicrobial substitutes has been of great interest. Herbal medicines have developed the foundations of medical pharmacology over time and formed the basis of traditional medicine systems. [3]. Herbal treatments are thought to be used by 80 % of the population to treat a range of disorders due to their broad availability, cost savings, and minimal side effects, their anti-inflammatory activities, and antimicrobials against pathogenic microorganisms, their anti-inflammatory activities, and antimicrobials against pathogenic microorganisms [4]. Consequently, research for extraction of phytochemicals of the secondary plant metabolism e.g., phenolic compounds, alkaloids, anthraquinones, flavonoids, saponins, tannins, and glycosides, as well as reducing sugars are currently

significant [5]. Fruit peels are classified agro-waste, and instead of being employed as a source of antimicrobial agents, they are discarded into the environment [6]. Several investigations on peels have indicated the existence of essential elements that can be exploited in pharmacological or medicinal applications. [7].

Pomegranate (*Punica granatum*) is a fruit from the *Punicaceae* family, which originated in India and Iran, is now grown throughout the Mediterranean and South-Western America. [4]. The entire global pomegranate output in 2017 is predicted to be 3.8 million metric tons [8]. Pomegranate peel is an inedible byproduct of pomegranate juice manufacturing and direct fruit consumption. It is a rich source of tannins, flavonoids, and other phenolic compounds [9]. Pomegranate peels have been used in traditional medicine in America, Asia, Africa, and Europe to cure a variety of maladies as an antiparasitic agent, a "blood tonic," and to repair aphthae and ulcers. [4].

Banana (*Musa acuminate, Musaceae* family) is a tropical fruit that is harvested all year. It is growing in about 122 countries and has an annual production of more than 165 million tons [10]. Its peel represents the main industry by-product processing, accounting for 35-38% of the entire fresh bulk of ripe fruit [11]. However, it is not further involved in remarkable industrial applications, although it contains many beneficial compounds, such as carotenoids, alkaloids, steroids, saponin, flavonoids phenolics, glycosides, amines, vitamins, fibers, minerals and carbohydrates [12]. Banana peels were used as traditional medicine in diarrhea, dysentery, enteric infections, diabetes, promote wound healing, primarily from burns, and aid in the treatment or prevention of a wide range of illnesses [13].

Orange (*Citrus sinensis,* family *Rutaceae*) is recognized as one of the most significant fruit crops, with a total global output of 120 million tones, of which about 20% is utilized as drinks in addition to sauces, dressings, and remaining peels are classified as waste. [14]. Orange peel extracts have been reported pharmaceutical effects against colic, upset stomach, cancer, diuretic, carminative, immune-enhancing, stomachic, tonic to the digestive system, immune system, and skin [15]. It is also used to treat the flu, colds, scurvy, and vitamin deficiencies, and it has antibacterial activity against both viral and bacterial infections [16].

Considering the importance of fruit peels as good sources for secondary metabolites and antimicrobial agents [17]. The aim of the current study is to reduce the environmental impact of agricultural waste as a source of pollution and to use the extracts of pomegranate peel (*Punica granatum*), banana (*Musa acuminate*) and orange (*Citrus sinensis*) as a new and good source to antimicrobial agents in order to reduce the use or as an alternative source of conventional antibiotics, and to control antibiotic-resistant bacteria, through the use of these extracts against common pathogenic bacteria (*Staphylococcus aureus, Salmonella enterica, Escherichia coli* and *Enterococcus faecalis*).

2. MATERIALS AND METHODS

2.1. Preparation of fruit peel powders and extracts: Fresh fruits (pomegranate, banana and orange) were collected from the wholesale market for vegetables and fruits in Mansoura city, bananas and oranges were collected in January and February 2020 and the pomegranate in August of the same year**.** The fruit was peeled and the peel was washed with tap water and then with distilled water. The collected peels were cut into small pieces and dried for 48 hours at 50 °C in the oven and grounded into a fine powder using an electric blender. 10 g of peels powder (pomegranate, banana, and orange) were soaked in 250 ml of solvent (methanol 99%, distilled water), then shaken at room temperature for 24hours. Whatman paper no. 1 was used to filter the clear extracts. Using a rotary evaporator, the filtrates were concentrated by evaporating solvent., then the extracts were sterilized using a filter (0.45 μm). The extracts were kept in bottles, in a refrigerator at 4°C until they were used.

2.2. Tested pathogenic bacteria and culture: In order to test the most common pathogenic bacteria, two Gram-negative bacteria (*Salmonella enterica* ATCC 14028 / NCTC 12023, *Escherichia coli* ATCC 8739), and two Gram-positive bacteria (*Enterococcus faecalis* ATCC 29212 / NCTC 12697, and *Staphylococcus aureus* ATCC 25923 / NCTC 12981) from today laboratories (Bucharest, ROMANIA),

were used for examining the antibacterial activity of the three targeted fruit peels. Bacterial cultures from solid media were sub-cultured in tryptic soy broth and incubated at 35°C for 24 hours.

2.3. Determination of antibacterial activity: The selected stock cultures (*Salmonella enterica, Staphylococcus aureus, Escherichia coli and Enterococcus faecalis*) were used for preparation of 0.5 MacFarland standard fresh bacterial cultures, which sub-cultivated in Muller Hinton agar plates. By sterile forceps, extract disks were distributed on the surface of the plate, and Positive controls were Vancomycin Antibiotic discs (20 g), while negative controls were DMSO, then incubated at 35 ± 0.5°C. The clear zone was measured by a clean ruler from the center of the extract disk to the edge of the area with zero growth.

2.4. Minimum Inhibitory Concentration (MIC): based on the agar well diffusion method and defined as the lowest concentration of the peels extracts that showed inhibition of visible growth against tested pathogenic strains. 6 mm filter papers were added to different concentrations of tested extract that was prepared by adding 1 ml of fruit extract to 1ml of sterile distilled water. Then, serial dilutions of 50 mg/ml, 25 mg/ml, 12.5 mg/ml, 6.25 mg/ml, 3.125 mg/ml, and 1.56 mg/ml were obtained using a micropipette. Such diluted solutions were added on the surface of Muller Hinton agar plates that were inoculated by tested bacteria and then incubated at 35 ± 0.5°C for 24 h. When the inhibition clear zones without growth of tested bacteria were found in the medium containing the lowest extracts concentration, the MIC was recorded at this point of dilution.

2.5. Statistical Analysis: Microsoft Excel was used to examine the collected data (office 2019). Descriptive statistics (Mean value and SD) along with comparison in mean zone of inhibition between the extracts of pomegranate, banana, and orange peels were used to perform One way analysis of variance (ANOVA).

3. RESULTS AND DISCUSSION

The present study evaluated the antimicrobial activity of extracts obtained from three types of fruit peels pomegranate, banana, and orange. Antimicrobial activity of plant extracts was detected against selected pathogenic strains: *Escherichia coli, Salmonella enterica, Staphylococcus aurous,* and *Enterococcus faecalis* species of widely distributed bacteria these results agreed with [18, 4, 19, 20, 21].

All fruit extracts showed good effective antibacterial against gram-positive and gram-negative bacterial. However, methanol extracts of all fruit peels have significantly higher antibacterial activity than water extracts. This result agreed with [22] reported that methanol extract of peels was a higher inhibition zone (20mm) than water peels extract (12mm).

Table1: Antimicrobial activity of fruit peel water and methanol extracts against pathogenic bacteria.

Species / Strain	Pomegranate		Banana		Orange		Vancomycin
	W	M	W	M	W	M	
Escherichia coli	18.3 ±1.6	25.7 ±1.8	12.5 ±1	20 ±1.4	10.5 ±1.4	17.3 ±0.8	17.5 ±0.8
Salmonella enterica	21.5 ±1	27.3 ±0.8	15.5 ±1.4	18.2 ±1.2	19 ±1.4	22.7 ±1.4	
Staphylococcus aureus	25.2 ±1.2	31.8 ±1.2	16.3 ±1.2	24.7 ±0.8	15.7 ±1.6	17.8 ±0.8	23.8 ±1.5
Enterococcus faecalis	15.3 ±1	19.2 ±1.6	10 ±0.9	10.7 ±0.8	9 ±0.9	12.2 ±1	19.5 ±1.4

*W= Water extract – M =Methanol extract
* Mean value ± SD, n = 6
*The experimental values within each row that have significantly different ($p < 0.05$) according to One-way ANOVA test

Pomegranate water and methanol extracts were the more effective fruit extract against all strains and especially *Staphylococcus. aureus* (31.8 ±1.2 for methanol ;25.2 ±1.2mm for water), then *S. enterica* (27.3 ±0.8 for methanol; 21.5 ±1mm for water), *Escherichia coli* (27.3 ±0.8 for methanol; 25.7 ±1.8mm for water), and the lowest with *E. faecalis* (19.2 ±1.6 for methanol; 15.3 ±1.8mm for water) Table (1).

These results are consistent with [18] who showed that pomegranate peel extract had stronger activity than banana and orange extract against gram-positive and gram-negative bacteria except for *Salmonella typhimurium*. Against *Aspergillus niger* and *Aspergillus flavus*, pomegranate and orange extracts had activity between 65–100% more than the positive control, and [21] The growth of *Staphylococcus aureus* and *Escherichia coli* was not inhibited by the orange peel extract, however the pomegranate peel extract demonstrated levels of inhibition against all of the foodborne pathogens tested. [20].

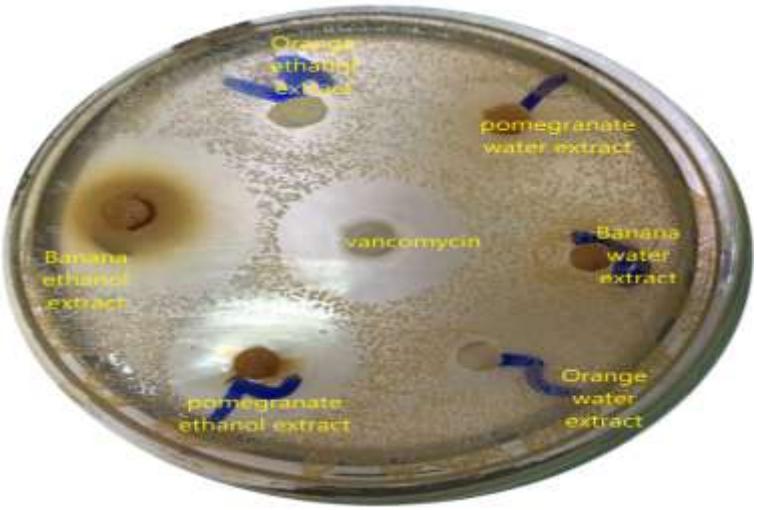

Figure 1: Antibacterial activity of pomegranate banana and orange water and ethanol extracts fruit peels.

The antimicrobial activity of all extracts, except for the ethanol extract of pomegranate, depended on the concentration of the extract to which the bacteria were exposed during the experiments, and [23] reported pomegranate was the most effective extract that showed bactericidal and bacteriostatic activities against the highly susceptible strains of pathogenic bacteria. This may be due to pomegranate peel having a wide spectrum of secondary metabolites as polyphenols, tannins, flavonoids, and anthocyanins (Cyanidins, delphinidins) as bioactive compounds which have an antibacterial activity that was reported with [**24, 25, 26**], and [**8, 4**) showed the highest antimicrobial activities against different pathogenic bacteria, also (**18,19]** reported the pomegranate extract had the highest phenolic content than banana and orange peels extract. The mechanisms of action of phenolic compounds on bacterial cells have been partially attributed to damage to the bacterial membrane, inhibition of virulence factors such as enzymes and toxins, and suppression of bacterial biofilm formation [27,28].

However, the results of the banana and orange extracts were very close to each other with most pathogenic bacteria, where the highest inhibition zone of banana peels extract with *Staphylococcus aureus* (24.7 ±0.8 for methanol and 16.3 ±1.2 for water) and the lowest inhibition zone with *E. faecalis* (10.0 ±0.9 for water extract and 10.7 ±0.8 mm for methanol extracts) Table (1), that were near the standard antibiotic vancomycin. This activity may be due to compounds isolated from the banana peel as total phenolic (chyrsin, qurectein and catchin), flavonoid, steroids, saponin, terpenoids, anthraquinones and tannin, malic acid, succinic acid, ß-

sitosterol, glycoside and monosaccharide components that have high antimicrobial activities The results are in agreement with the results published by [11, 17, and 28] that has been reported The chemical contents of the banana peels extract were determined by GC-MS and have strong antibacterial activity was the fatty acids stearic, oleicpalmitic, and linoleic acids and their methyl esters as well as 2,3- dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one, dibutyl phthalate,cyclododecane, 5-(hydroxymethyl)-2-furancarboxyaldehyde, b-sitosterol, sesamin and epi-sesamin. The results were reported by [19], Banana extracts can be used to treat *Salmonella typhi, Escherichia coli, Klebsiella pneumoniae, and Staphylococcus aureus* infections. Opportunistic diseases caused by *Micrococcus Spp*. and Pseudomonas aeruginosa, such as bronchopneumonia, bacterial endocarditis, and meningitis, will also be treated using extracts of this medicinal peel, [**29]** banana extract shown exceptional in-vitro broad-spectrum antibacterial activity that may be utilised to combat infections caused by *Escherichia*. *coli, Salmonella typhi, Staphylococcus aureus*, and *Klebsiella pneumoniae*, also it was in agreement with [30, 31**].** who revealed that banana peel extract has the best antimicrobial activity and preservatives of banana peel extract are believed to be associated with phytochemical components of it, like phenolic and tannins as reported by [17]. However, the result was not the same with [13] reported that banana peel extract was not effective against all strains of Gram-positive bacteria. Also, **[32]** reported that banana peel extract is only effective against *Agrobacterium tumefaciens*, Other bacterial strains, on the other hand, demonstrated resistance to the extract-treated wood.

Orange peel extracts have a notable antibacterial activity with all pathogenic bacteria and the activity was higher with *Salmonella enterica* (22.7 ±0.8 for methanol and 19 ±1.4 for water) Table (1) which has bacterial activity close to the bacterial activity of pomegranate extract and higher than the antibiotic vancomycin (16.5 ±0.5), and the lowest inhibition zone with *Enterococcus. faecalis* (9 ±0.9 for water extract and 12.2 ±1 mm for methanol extracts). This may be due to orange peels extracts having many chemical compounds such as alkalis, flavonoids, glycosides, saponins, resins, oleoresin, sesquiterpene, phenolic compounds, fats, and oils have been reported in orange fruit. The peel contains the largest number of organic compounds compared to other parts of the fruit. **[16, 25, 33, 34, 35].** This result was agreed with **[15]** Orange peel extract was found to have the maximum antibacterial activity against *Staphylococcus aureus*, *Escherichia coli*, with the least impact against *Pseudomonas aeruginosa.* The juice and peel were phytochemically screened, and the presence of alkaloids, terpenoids, flavonoids, tannins, saponins, reducing sugar, and amino acids was discovered., and [**36]** reported that orange extract has higher antibacterial activity that inhibits all four pathogenic bacterial strains (*Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, and Klebsiella pneumoniae*), and [**36]** orange extract has antimicrobial activity against microorganisms that causes acute diarrhea Infections (*Salmonella spp., and Escherichia coli*).

Table2: MIC of fruit peel water and methanol extracts against tested pathogenic bacteria

Species / Strain	Pomegranate		Banana		Orange		Vancomycin
	W	M	W	M	W	M	
Escherichia coli	6.25	6.25	25	25	25	12.5	3.125
Salmonella enterica	6.25	1.56	12.5	6.25	6.25	1.56	6.25
Staphylococcus aureus	3.125	3.125	12.5	12.5	12.5	6.25	3.125
Enterococcus faecalis	12.5	6.25	25	6.25	25	12.5	3.125

The minimum inhibitory concentration (MIC): The MIC was also examined to get quantitative data on the efficacy of the researched extracts and to validate earlier findings. The susceptibility of microorganisms to antimicrobial agents varies greatly. A high MIC number suggests a low level of activity, and vice versa. The MIC for each extract varies depending on the type of bacteria strain as the highest MIC with *Escherichia coli* was banana (water and methanol extracts) and orange (water extracts) 25 mg/ml, the lowest with pomegranate (water and methanol extracts) 6.25 mg/ml (Table 2).

The highest MIC with *Salmonella enterica* was of banana peels water extract (12.5mg/ml), the lowest with pomegranate and orange methanol extracts (1.56 mg/ml). The lowest MIC for *Staphylococcus aurous* was pomegranate water and methanol extracts (3.125 mg/ml), and the highest was banana (water and methanol) and orange water extracts (12.5mg/ml). While *Enterococcus faecalis* the least MIC was (6.25mg/ml) with pomegranate and banana peels methanol extracts and the highest was (12.5mg/ml) for pomegranate peels water extracts, and orange peels methanol extract. On the other hand, Briefly, these findings suggested a predominance of pomegranate peel extract (water and methanol) with all bacteria strains over banana and orange extract. where the lowest MIC value was pomegranate with *Salmonella enterica* (1.56 mg/ml)**.** Except for pomegranate and orange methanolic extracts with *Salmonella enterica*, the MIC value of the standard antibiotic (vancomycin) is smaller than that of all examined extracts utilized in the study **(Table1)**. This conclusion was supported by [18], who observed that MIC values for orange peel and banana extracts were greater than MIC values for pomegranate peel extracts against all tested microorganisms, and [19], who reported that the MIC value of the standard antibiotic (amoxicillin) is lower than the MIC value of all studied extracts used in the study. But also, the extract fruit peels have high antibacterial activity and low MIC with some pathogenic bacteria, [20] showed low MIC values have been reported for plant extracts for Gram-positive bacteria and comparatively high values for Gram-negative bacteria that agreed with [22, 35, 39, 40]. According to the findings of this study, certain Gram-negative bacteria were more impacted than Gram-positive bacteria, this might be related to differences in the structure of the cell wall as well as the nature of the extract and its components, as confirmed by [41, 42; 43]. The higher antimicrobial activity against gram-positive bacteria was because the gram-positive bacteria have a less stable cell wall which allows the permeation of some antimicrobial agents. The lowest antimicrobial activity against gram-negative bacteria was due to the presence of the outer cell membrane of bacterium composed of phospholipids bilayer and proteins, avoiding the permeation of antimicrobial agents inside the cell wall. [19, 41, 22] revealed that Gram-positive bacteria were more sensitive than Gram-negative bacteria, which might be related to variations in these bacteria's cell wall architectures. Gram-negative bacteria contain numerous layers of thick peptidoglycan cell walls that function as a barrier to different environmental chemicals, including natural and manufactured antibiotics. Gram-positive bacteria, on the other hand, have a limited tolerance to somatic disturbances due to a weak cell wall construction. However, several authors [21; 44] reported the difference in inhibition zone of peel extracts against tested pathogenic bacteria that may be due to the different extraction methods followed, the freshness of fruits peel used and the variations in the region and season of growth.

4. CONCLUSION

Recycling fruit waste is one of the most important ways to benefit from it in several creative ways to produce new products in the pharmaceutical industry and meet the requirements of basic products required in human, animal and plant nutrition. This work determined the antibacterial activity against the tested organisms by water and methanol extracts of pomegranate banana, and orange peel. The results of this study clearly showed that all methanol and water extracts (especially methanol extract) were effective against pathogenic bacteria (*Salmonella enterica, Staphylococcus aureus, Escherichia coli and Enterococcus faecalis*) thus may be useful in treating pathological conditions caused by these microorganisms.

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