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Antimicrobial Activity of Pomegranate (Punica granatum) Peel Extract: A Potential Natural Preservative

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Abstract

Pomegranate (*Punica granatum*) peel is rich in bioactive compounds with strong antimicrobial properties, making it a potential natural alternative to synthetic preservatives. The present study evaluates antimicrobial efficacy of pomegranate peel extract (PPE) against major bacterial and fungal pathogens.

Methods: Pomegranate peels were dried, powdered, and extracted using water, methanol, and hexane. The antimicrobial activity was tested using the agar well diffusion method against seven bacterial pathogens (*S. aureus, B. subtilis, E. coli, E. aerogenes, K. pneumoniae, P. aeruginosa,* and *S. typhi*) and three fungal pathogens (*A. niger, C. albicans,* and *M. furfur*).

Results: The methanol extract showed the highest antimicrobial activity, with 22 mm zone of inhibition against *B. subtilis* and *P. aeruginosa*. The hexane extract and the water extract demonstrated moderate antimicrobial activity with moderate zone of inhibition of 15mm and 12mm respectively. The methanol extract also exhibited a strong antifungal effect, particularly against *C. albicans* (21 mm) and *A. niger* (20.6 mm) respectively.

Conclusion: PPE, especially with methanol extract, confirmed to exhibit a promising antimicrobial potency against several pathogens, supporting its use as a natural preservative.

Keywords: Pomegranate peel extract, antimicrobial activity, natural preservative, foodborne pathogens, food safety.

1. Introduction

The scientific name of pomegranate is *Punica granatum* belongs to the family *Lythraceae*, subfamily *Punicoideae*. Pomegranate peel extract (PPE) is derived from the outer layer of the *Punica granatum* fruit, which is typically discarded during juice and food processing. However, research has shown that this byproduct is rich in bioactive compounds. Traditionally, pomegranate has been used in herbal medicine for its antioxidant, antimicrobial, and anti-inflammatory properties, but in recent years, its peel extract has gained attention in the food industry as a natural preservative and functional ingredient. Given the growing demand for clean-label and chemical-free food preservation methods, PPE is now being explored for its ability to enhance food safety and extend shelf life without the use of synthetic additives. (Belgacem I. *et.al.,* 2021) About 30–40% of pomegranate (Punica granatum L.) fruit is made up of peel. The peel comprises around 30% of the total anthocyanins in the pomegranate fruit. The amount of anthocyanin in pomegranate peel varies depending on the cultivar, stage of fruit growth, and the color.

Pomegranate (*Punica granatum*) is also confirmed as an excellent natural food colorant due to its vibrant red pigments, primarily anthocyanins. These pigments are extracted from the pomegranate peel can be used to impart a rich red color to various food products. (Kanemozhi1 S. *et al.,* 2020)

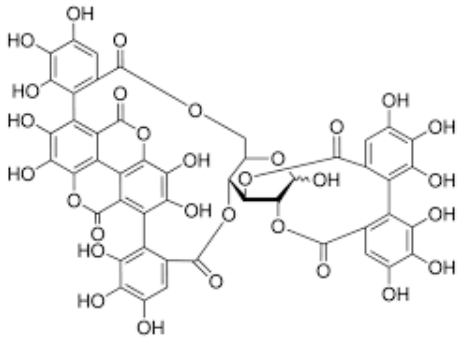
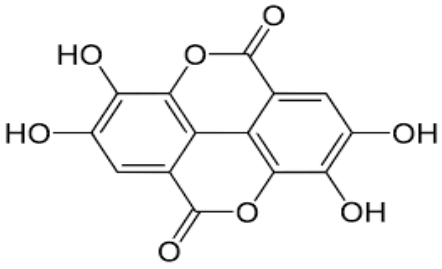
Studies have shown that PPE can effectively inhibit the growth of several pathogens such as *Escherichia coli*, *Staphylococcus aureus*, and *Salmonella spp.*, and etc. making it an excellent natural alternative to synthetic preservatives. It has been incorporated into edible coatings, biodegradable packaging, and processed foods such as dairy, meat, and baked goods to improve shelf stability. With its eco-friendly and health-promoting

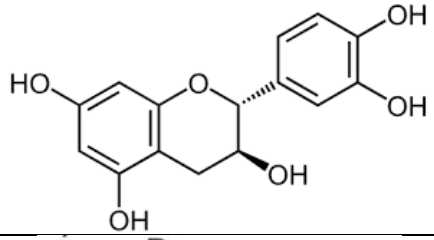
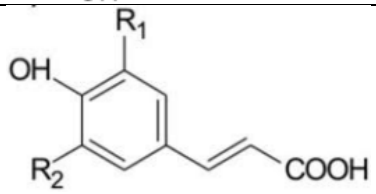
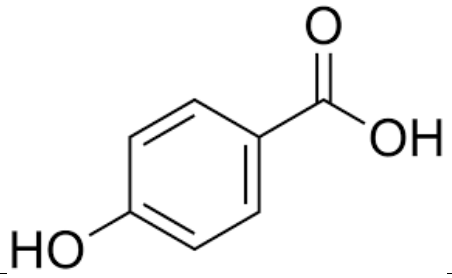
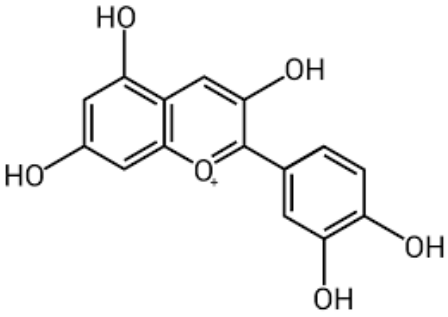
attributes, PPE is emerging as a sustainable ingredient that aligns with modern consumer preferences for natural and safe preservation solutions. (Saeed M. *et al.,* 2018)

Bioactive Compounds

Pomegranate peels have been considered as a source of beneficial active compounds in traditional medicine since ancient times. Several earlier studies also confirmed the concentration of bioactive compounds in the peels is higher as compare with the edible parts of the fruit. (Biesalski H. K. et al, 2009 and Padayachee A. et al, 2017) Several bioactive components as illustrated in below table no. 1 may play a significant role more precisely that in the complex bio mixture of all active compounds found in pomegranate peel. Their combined effects can lead to a range of physiological activities. (Guil-Guerrero J.L. et al, 2016)
Pomegranate pill extract has been shown to contain a high concentration of bioactive components, specifically phenolic acids, flavonoids, alkaloids and hydrolysable tannins. The main phenolic acids found in pomegranate pill extracts include ellagic acid, gallic acid, caffeic acid, chlorogenic acid, syringic acid, ferulic acid, vanillic acid, p-coumaric acid, and cinnamic acid. Alkaloids in pomegranate peel include acetyl grenadine, 2-(2-hydroxypropyl)-Δ′-piperidine, sedridine, and N-acetyl sedridine. Pomegranate Pill Extracts are high in tannins. The reported tannins include ellagitannins, punicalagin, granatins, punicalin, pedunculagin, castalagin, corilagin, gallagyldilactone, and tellimagrandin. There were nearly 49 compounds, with the majority being flavonoids, phenolic acids, and tannins Ellagitannins, especially punicalagin, are the main tannins in pomegranate peel that have antimicrobial properties that attribute to its preservation capability. (Jing C. *et al.,* 2020)

Table No 1: Major Bioactive compounds in pomegranate peels and their chemical structures

Bioactive compound	Chemical structure
Pinicalagin is a Ellagitannin: Broad-spectrum antimicrobial, inhibits bacteria and fungi	
Ellagic Acid is a Ellagitannin: antimicrobial, inhibits bacteria and fungi	

Catechins: Flavonoid: Antibacterial, antifungal, enhances preservation	
Hydroxycinnamic acid is a Phenolic acid: Protects against oxidative stress and Antibacterial	
Hydroxybezoic acid is a Phenolic acid: Antioxidant and antimicrobial	
Anthocyanin is a Flavonoid used as a natural food dye and in other food products, Anthocyanins are associated with antioxidant, anti-inflammatory, and antimicrobial properties	

As a valuable by-product, pomegranate peel is rich in bioactive compounds, particularly phenolics like tannins, flavonoids, and polyphenols, which display significant biological activity. Pomegranate peel extracts (PPEs) show promising potential in improving specific disease biomarkers. PPEs have demonstrated antioxidant properties by reducing oxidative biomarkers such as TBARS, OxLDL, and lipid peroxidation in healthy individuals. Compounds like punicalagin and ellagic acid in pomegranate peel have chemopreventive effects against cancers, including prostate, breast, and colon cancer, partly due to the ellagic acid-derived metabolite, urolithin. Additionally, the bioactive substances in pomegranate peels contribute to anti-inflammatory and antibacterial effects, as well as improvements in cardiovascular health, anti-infection properties, and wound healing, playing a key role in modulation and intervention. (Haslam E., 1996, Mo Y et al, 2022)

Equivalent to its antioxidant properties, the antimicrobial activity of pomegranate peel can be attributed to its phenolic compounds, which have the potential to prevent or treat pathogenic microorganisms. These compounds work by precipitating membrane proteins and inhibiting enzyme activity, ultimately leading to

bacterial cell death and producing antibacterial effects. (Naz S et al, 2007; Vasconcelos L.C. et al, 2003; Braga LC et al, 2005)

Present research study was conducted to evaluate the antimicrobial potential of pomegranate peel extract in different solvents and to undertake qualitative assessment of most promising pomegranate peel extract to ascertain major bioactive components accountable for its antimicrobial potency.

2. Materials and Method

Materials Pomegranates Fruits were procured from the local market of Mumbai, Maharashtra, India

2.1. Extraction of peel Powder

Peels from pomegranates were separated, cleaned with tap water, and dried in a vacuum oven set at 50 °C until completely dry while under a 700 mm Hg vacuum. Using a pestle and mortar, the dried peels were ground into a coarse powder about 1 mm in size, and they were then store in an incubator at 4 °C until further use. (Shalini M., *et.al.,* 2014)

2.1.1 Method of Extraction

In separate conical flasks, 20 g of powdered pomegranate peel was soaked in 100 ml of methanol, hexane, and water to create the samples. For 24 hours, the samples were incubated at room temperature in a rotatory shaker set to 200 rpm. (Shalini M., *et.,* 2014) In this step the bioactive substances in the peels were able to dissolve into the solvents. The samples were filtered using Whatman no. 1 filter paper after a 24-hour. Using a rotary evaporator at lower pressure, the filtrates which has the dissolved bioactive compounds were concentrated. This procedure was carried out at temperatures lower than the solvents boiling points which prevent bioactive compounds from degrading. and the resulted extracts were stored in sterile glass vials at 4°C for future use. (Uswa S. *et.al.,* 2024)

2.1.2 Test Microorganisms

pomegranate peel powder was investigated for anti-microbial activity against Foodborne pathogen
Bacterial Pathogens: *Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Enterobacter aerogenes, Klebsiella pneumoniae, Pseudomonas aeruginosa,* and *Salmonella typhi,*
Fungal Pathogens: *Aspergillus Niger, Candida albicans* and *M. Furfur*

2.1.3 Antibacterial Activity Testing (Agar Well Diffusion Method)

Prepare 100ml of Muller-Hinton Agar (MHA) and sterilize it in an autoclave (121°C, 15 min). Pour 10 ml of sterilized agar into sterile Petri dishes and allow it to solidify. Spread 100 µl of each bacterial culture onto separate agar plates. Create wells (8 mm in diameter) in the agar plates using a sterile cork borer. Load 10 µl of each extract into separate wells. Use deionized water as a negative control. Incubate plates at 35°C for 24 hours. After 24 hours, measure the zone of inhibition (mm) around each well. Record the inhibition zones and compare between different extracts. (Lubna F.A.N. *et.al.,* 2023)

2.2. Qualitative Phytochemical Analysis

Phytochemical screening of the most promising pomegranate peel extract was performed as per the methods described in previous research studies. Presence or absence of alkaloids was observed using dragendroff's reagent, terpenoids were tested using Salkowski test, for flavonoids evaluation, Shinoda's test was carried out (Rojas et al, 2006), and tannins with ferric chloride test (Sayed M.A.D, *et.al.,* 2020). The preliminary qualitative test of pomegranate peel extract confirmed the presence of notable active secondary metabolites, paving the way for further evaluation of specific bioactive components.

3. Results

3.1 Antibacterial activity of pomegranate peel powder

The antimicrobial activity of pomegranate peel extract varied across different pathogens and solvents, demonstrating its potential as a natural antimicrobial agent.

Table No 2: Zone of Inhibition of Bacterial and fungal strains

Bacterial Pathogens	**Zone of Inhibition**		
	Water Extract	**Methanol extract**	**Hexane Extract**
S. aureus	12mm	15mm	18mm
Bacillus subtilis	9mm	22mm	15mm
E. coli	18mm	20mm	15mm
Enterobacter aerogenes	18mm	18mm	16mm
Klebsiella pneumoniae	12mm	13mm	9mm
Pseudomonas aeruginosa	18mm	22mm	13mm
Salmonella typhi	18mm	12mm	15mm
Fungal Pathogens	**Zone of Inhibition**		
	Water Extract	**Methanol extract**	**Hexane Extract**
Aspergillus Niger	18.5mm	20.6mm	17.60mm
Candida albicans	20mm	21mm	20mm
M. Furfur	18mm	20mm	12mm

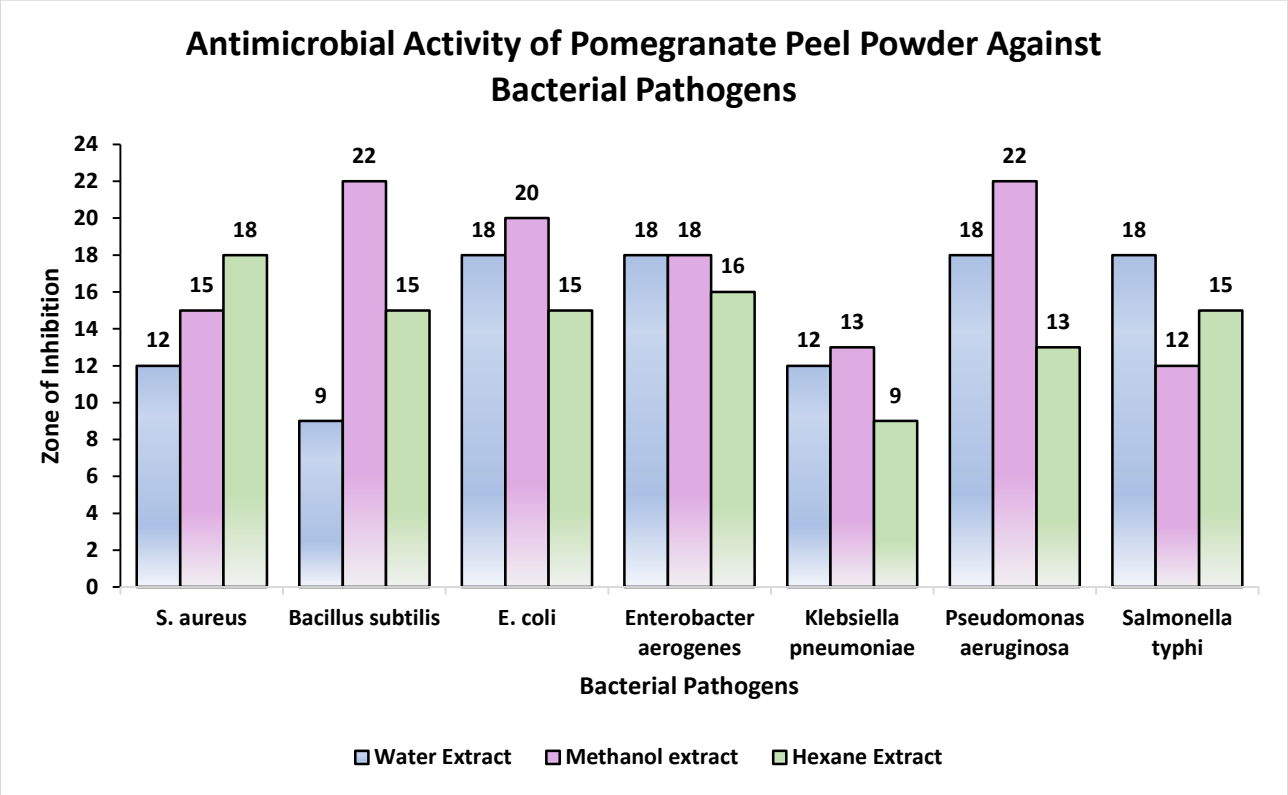


Figure No. 2 Antimicrobial Activity of Pomegranate Peel Powder Against Bacterial Pathogens

The antimicrobial potential of the extract of pomegranate peel was tested against Gram positive bacteria *Staphylococcus aureus* and *Bacillus subtilis* and Gram-negative bacteria *Escherichia coli, Enterobacter aerogenes, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Salmonella typhi* using water, methanol and hexane extracts. The greatest antibacterial activity was associated with the methanol extract, with the highest inhibition zones of 22 mm against *Bacillus subtilis* and *Pseudomonas aeruginosa.* The hexane extract exhibited moderate inhibition, with the highest activity observed against *Staphylococcus aureus* (18 mm) and *Enterobacter aerogenes* (16 mm). The water extract showing inhibition on all tested bacteria with the strongest activity observed on the four recognized pathogenic bacteria, *K. pneumonia, E. coli, and E. aerogenes, P. aeruginosa,* and *Salmonella typhi* (18 mm each). Among all bacterial strains tested, Klebsiella pneumoniae showed the lowest susceptibility mainly against the hexane extract (9 mm).

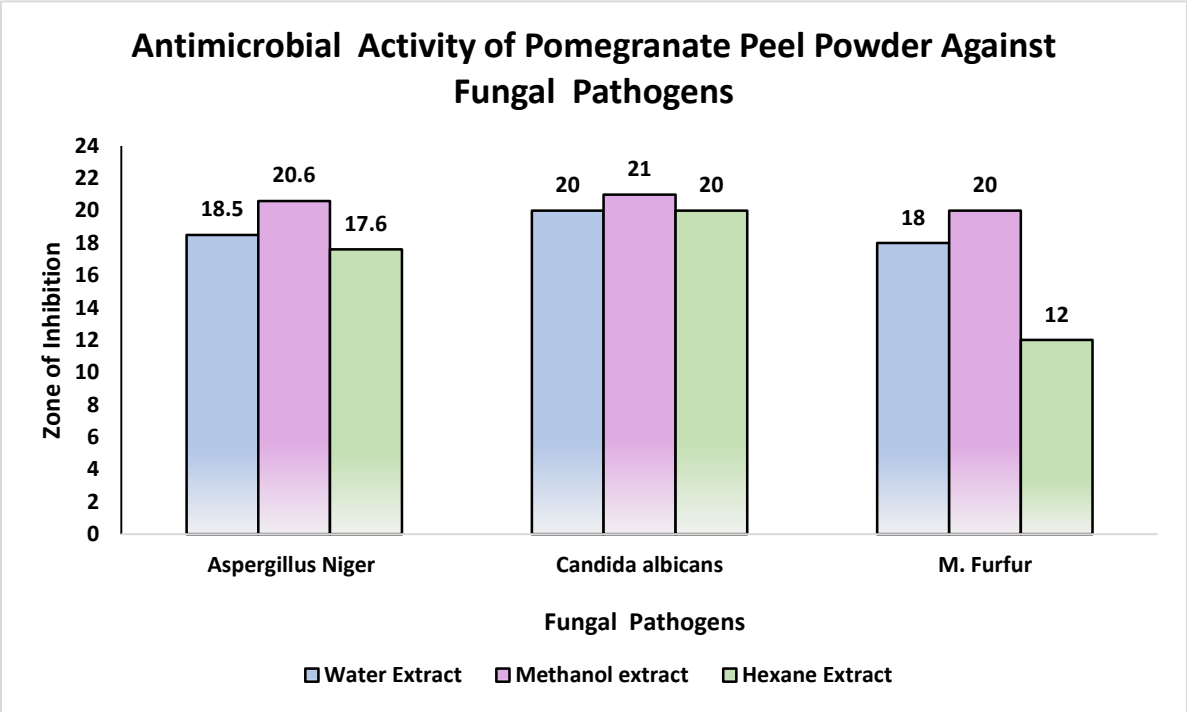


Figure No. 3 Antimicrobial Activity of Pomegranate Peel Powder Against Fungal Pathogens

In vitro antifungal activity of pomegranate peel extract against *Aspergillus niger, Candida albicans,* and *Malassezia furfur.* The highest antifungal activity was shown by the methanol extract, with clear zones of inhibition of 20.6 mm against *Aspergillus niger*, 21 mm against *Candida albicans*, and 20 mm against *Malassezia furfur*. The water extract had strong antifungal activity against *C. albicans* (20 mm) and *A. niger* (18.5 mm). The hexane extract exhibited the lowest antifungal activity, with a notable reduction in inhibition against *Malassezia furfur* (12 mm). Overall, the methanol extract proved to be the most effective against both bacterial and fungal pathogens, indicating its strong antimicrobial potential.

3.2 Qualitative test for Tannins

Upon adding ferric chloride ($FeCl_3$) solution to the sample, a blue-green coloration was observed. The positive result indicates the presence of tannins in the tested sample. The results confirm that the methanol extract of pomegranate peel contains a substantial amount of tannin, as a bioactive compound known for its strong antioxidant and antimicrobial properties. These findings highlight the potential of pomegranate peel as a rich natural source of bioactive component, which could be valuable natural source as a food preservative, nutraceutical and pharmaceutical applications.

4. Conclusion

These results demonstrate a promising antibacterial and antifungal activity of pomegranate peel extract against a wide range of pathogenic microorganisms. Methanol extract showed the highest antibacterial and antifungal potential especially on *Bacillus subtilis, Pseudomonas aeruginosa, Aspergillus niger*, and *Candida albicans.*

These findings confirmed the potential of pomegranate peel extract as a natural preservative in variety of industrial products, offering a safer and more sustainable solution to preservation methods. This is more relevant in view of growing demand for natural alternatives to synthetic antimicrobials.

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