Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.53555/jptcp.v31i8.7434

EVALUATING THE ANTIMICROBIAL EFFICACY OF VARIOUS SOLVENT EXTRACTS FROM POMEGRANATES PEEL AGAINST PATHOGENIC MICROORGANISMS

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Abstract

Pomegranates *(Punica granatum L.)* have been celebrated for their medicinal properties for centuries, prominently in traditional medicine systems such as Ayurveda and Traditional Chinese Medicine. This study evaluates the antimicrobial efficacy of solvent extracts from pomegranate peel against pathogenic microorganisms. Extracts were obtained using methanol, ethanol, chloroform, and n-hexane. The antimicrobial activity was assessed against Escherichia coli, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Aspergillus niger*, *Trichoderma viridens*, and *Candida albicans*. The disc diffusion method was employed to measure inhibition zones. Results demonstrate significant antimicrobial activity across all extracts, with methanol and ethanol showing the highest efficacy. Chloroform and n-hexane extracts also displayed notable activity against certain pathogens. Notably, methanol extract showed inhibitory effects on *E. coli* (P=0.0378, F=3.861), *B. subtilis* (P=0.0001, F=20.56), *P. aeruginosa* (P=0.0402, F=3.775), *A. niger* (P=0.0031, F=8.353), *Trichoderma viridens* (P=0.0001, F=59.72), and *Candida albicans* (P=0.0001, F=34.23). Ethanol extract exhibited significant inhibition on *E. coli* (P=0.0001, F=71.48), *B. subtilis* (P=0.0003, F=14.76), *P. aeruginosa* (P=0.0001, F=26.02), *A. niger* (P=0.0001, F=19.21), *Trichoderma viridens* (P=0.0001, F=22.44), and *Candida albicans* (P=0.0002, F=16.60). Chloroform extract showed significant inhibition on *E. coli* (P=0.0005, F=13.45), *B. subtilis* (P=0.0456, F=3.602), *P. aeruginosa* (P=0.0027, F=8.673), *A. niger* (P=0.0001, F=95.34), *and Candida albicans* (P=0.0001, F=35.09). Furthermore, n-hexane extract exhibited significant inhibition on *E. coli* (P=0.0001, F=57.23), *B. subtilis* (P=0.0120, F=5.662), *P. aeruginosa* (P=0.0072, F=6.599), *A. niger* (P=0.0001, F=69.44), and *Candida albicans* (P=0.0001, F=35.09). These findings underscore the potential of pomegranate peel extracts as natural antimicrobial agents and provide valuable insights for their future application in pharmaceuticals and other industries.

Keyword: antimicrobial, pomegranates, methanol, ethanol, chloroform, and n-hexane

1. INTERODUCTION

In recent years, the rise of antibiotic resistance has emerged as a formidable challenge in healthcare, urging the exploration of alternative antimicrobial agents. Natural compounds derived from plants have garnered attention for their potential therapeutic properties, including antimicrobial activity (Balaban, Koç, Sar, & Akbas, 2022; Farooq et al., 2023; Yassin, Mostafa, & Al Askar, 2021). Among these, pomegranate (*Punica granatum*) stands out as a rich source of bioactive constituents with diverse pharmacological effects, including antimicrobial properties. Pomegranate, a fruit native to the Middle East and cultivated worldwide, has been historically esteemed for its medicinal value in various traditional systems of medicine (Daoutidou, Plessas, Alexopoulos, & Mantzourani, 2021; Kharchoufi et al., 2018). While the arils (juicy seed sacs) of the pomegranate fruit are commonly consumed for their nutritional benefits, the peel has often been overlooked despite its rich content of bioactive compounds (Kupnik, Primožič, Vasić, Knez, & Leitgeb, 2021; Mitsagga, Petrotos, & Giavasis, 2021). The peel constitutes about 50% of the total fruit weight and is abundant in polyphenols, flavonoids, tannins, and other phytochemicals, which contribute to its antioxidant, anti-inflammatory, and antimicrobial activities (Hanafy, Abd El-Shafea, Saleh, & Fathy, 2021; Mitsagga et al., 2021). The antimicrobial potential of pomegranate peel extracts has been a subject of growing interest in recent research endeavors. Various studies have highlighted the inhibitory effects of pomegranate peel extracts against a wide spectrum of pathogenic microorganisms, including bacteria, fungi, and viruses (Manapure, Naik, Satbhai, & Mohite, 2015; Tanveer et al., 2015). However, the specific mechanisms underlying this antimicrobial activity and the comparative efficacy of different solvent extracts from pomegranate peel remain areas warranting further investigation (Al-Zoreky, 2009; Alexandre et al., 2019). Solvent extraction is a widely employed method for isolating bioactive compounds from plant materials. Different solvents possess varying polarities, which influence their ability to extract specific classes of phytochemicals. Ethanol, methanol, acetone, and water are among the commonly used solvents for extracting bioactive compounds from pomegranate peel (El Khetabi et al., 2020; Malviya, Arvind, Jha, & Hettiarachchy, 2014). Each solvent extract may yield a distinct profile of phytochemicals, thereby impacting its antimicrobial potency against pathogenic microorganisms. The pathogenic microorganisms selected for evaluation encompass both Gram-positive and Gram-negative bacteria, as well as fungal strains known for their clinical significance (Benslimane, Rebai, Djibaoui, & Arabi, 2020; Gullon, Pintado, Pérez-Álvarez, & Viuda-Martos, 2016). These include but are not limited to *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Candida albicans,* and *Aspergillus fumigatus*. These organisms represent a diverse array of infectious agents implicated in a wide range of human infections, from superficial skin infections to life-threatening systemic diseases (Ismail et al., 2016; Jaisinghani, Makhwana, & Kanojia, 2018). The significance of this research lies in its potential to contribute to the development of alternative antimicrobial agents amidst the global challenge of antibiotic resistance. By harnessing the antimicrobial properties of pomegranate peel extracts, we aim to explore sustainable and eco-friendly solutions for combating infectious diseases. Furthermore, elucidating the optimal extraction conditions and solvent selection for maximizing antimicrobial efficacy could pave the way for future applications in pharmaceuticals, food preservation, and other industries (Scaglione et al., 2024). This study aims to evaluate the antimicrobial efficacy of various solvent extracts obtained from pomegranate peel against clinically relevant pathogenic microorganisms. By employing a range of solvents differing in polarity, we seek to assess the differential extraction of bioactive compounds and their subsequent impact on antimicrobial activity. Understanding the comparative efficacy of solvent extracts will not only shed light on the potential mechanisms of antimicrobial action but also inform the development of novel antimicrobial agents derived from natural sources.

2. MATERIAL AND METHOD

2.1. Pomegranate Peel Collection

Pomegranates (*Punica granatum L.*) were sourced from a local market, washed thoroughly to remove contaminants, and manually separated from the arils and seeds. The peels were cut into small pieces, air-dried in a well-ventilated area away from direct sunlight, and then ground into a fine powder. This powder was sieved for uniform particle size and stored in airtight containers at room temperature. Analytical grade solvents (methanol, ethanol, chloroform, and n-hexane) were obtained from a reputable supplier. These solvents were handled following safety guidelines in a well-ventilated lab and stored properly to maintain their purity(Ahmad, 2022; Munir et al., 2023).

2.2. Preparation of Pomegranate Peel Extracts

Pomegranate peel extracts were prepared by soaking 100 grams of the powdered peel in 500 ml of each solvent (methanol, ethanol, chloroform, and n-hexane) in separate conical flasks (Ahmad, 2021; Robina et al., 2021). These flasks were sealed and placed on a rotary shaker, set at a constant speed, for 72 hours at room temperature. This process allowed the bioactive compounds in the peels to dissolve into the solvents. After 72 hours, the mixtures were filtered through Whatman No. 1 filter paper to remove solid residues. The filtrates, which contained the dissolved bioactive compounds, were then concentrated using a rotary evaporator under reduced pressure. This step was performed at temperatures below the boiling points of the solvents to prevent degradation of the bioactive compounds. The resulting crude extracts were collected and stored in sterile glass vials at 4°C until further use(Abdullah, 2022).

2.3. Microorganisms and Culture Conditions

The antimicrobial efficacy of the extracts was tested against six microorganisms: two Gram-negative bacteria (*Escherichia coli, Pseudomonas aeruginosa*), one Gram-positive bacterium (*Bacillus subtilis*), and three fungi (*Aspergillus niger, Trichoderma viridens, Candida albicans*). These microorganisms were obtained from a microbial culture collection and maintained on nutrient agar slants for bacteria and Sabouraud dextrose agar slants for fungi.

2.4. Antimicrobial Susceptibility Testing

The antimicrobial activity of the pomegranate peel extracts was determined using the disc diffusion method. Sterile discs of 6 mm diameter were impregnated with 50 µl of each extract at concentrations of 50 µg/ml, 100 µg/ml, 150 µg/ml, and 200 µg/ml. The impregnated discs were placed on agar plates inoculated with the test microorganisms, which were spread uniformly using a sterile cotton swab. The plates were incubated at 37°C for 24 hours for bacteria and at 30°C for 48 hours for fungi (Ahmad, 2021).

2.5. Measurement of Inhibition Zones

After the incubation period, the diameter of the inhibition zones around the discs was measured in millimeters using a vernier caliper. Each test was performed in triplicate to ensure accuracy and reproducibility. The mean and standard deviation of the inhibition zones were calculated.

2.6. Statistical Analysis

The data were analyzed using one-way ANOVA to determine the significance of the differences in antimicrobial activity among the various extracts and concentrations. P values less than 0.05 were considered statistically significant. The F values were calculated to assess the variance between the groups. All statistical analyses were performed using SPSS software.

3. RESULTS

3.1. Antibacterial activity of different fruit peels extract

Evaluation of the antibacterial activity of fruits peels and crown leave extract at different

concentrations (50mg/ml, 60mg/ml, 70mg/ml and 80mg/ml) was done. The methanol, ethanol, chloroform and n-hexane extract of the peel of pomegranate, sweet lime, banana, corn, custard apple and crown leaves of pineapple, were tested against the gram negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*) bacteria and gram Positive bacteria (*Bacillus subtilis*), and the antibiotic gentamicin was used as a positive control. In the pomegranate peel extract, the concentration of 80mg/ml was found to be the most effective against *Escherichia coli, Pseudomonas aeruginosa* and *Bacillus subtilis*. The pomegranate peel extract exhibited the maximum inhibition activity of methanol ±16mm, *Escherichia coli* compared to other extract like ethanol ±15 mm, chloroform ±15mm and hexane ±15mm, in case of *Escherichia coli*, and *Pseudomonas aeruginosa*.

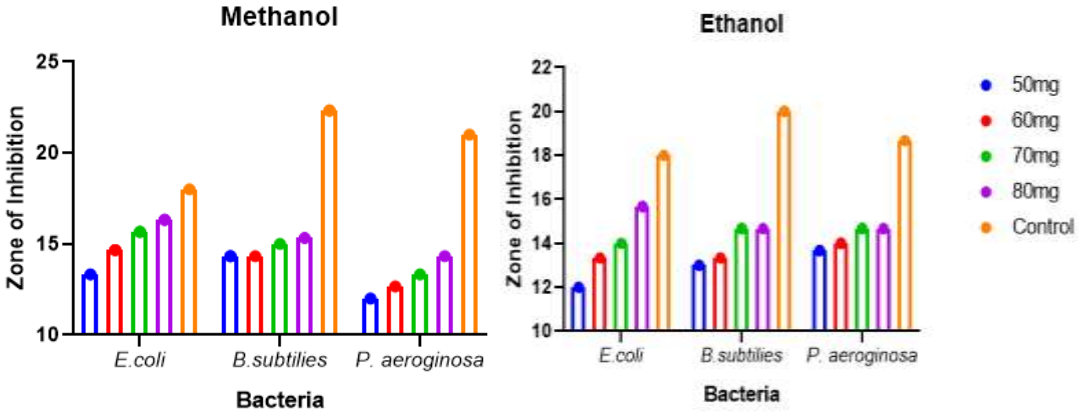

Figure 1:-Antibacterial activity of pomegranate peel extracts of methanol and ethanol.

The antibacterial potency of pomegranate peel extract was determined by the agar well diffusion method as shown (figures 1, 2, 3 and.4) and the maximum antibacterial activity was observed in the methanolic and ethanolic extract. The comparative study was done for methanol, ethanol, chloroform and n-hexane extracts of pomegranate peels extract against gram positive and gram negative bacteria respectively at four different concentrations (50mg/ml, 60mg/ml, 70mg/ml and 80mg/ml). The control used was gentamicin used as control. The methanolic extract 80mg/ml was found to be most effective against *Escherichia coli*, in comparison to the other two bacterial species *Bacillus subtilis, Pseudomonasaeruginosa.* Ethanolic extract, 80 mg/ml concentration exhibited the maximum zone of inhibition in *Escherichia coli* as compared to *Pseudomonas aeruginosa* and *Bacillus subtilis* (graph 1 &.2). Chloroform extract, 80mg/ml showed the same zone of inhibition against *Escherichia coli, Bacillus subtilis* and *Pseudomonas aeruginosa.* In case of n-hexane extracts, effective zone of inhibition was observed in *Escherichia coli* and *Bacillus subtilis* at 80mg/ml concentration.

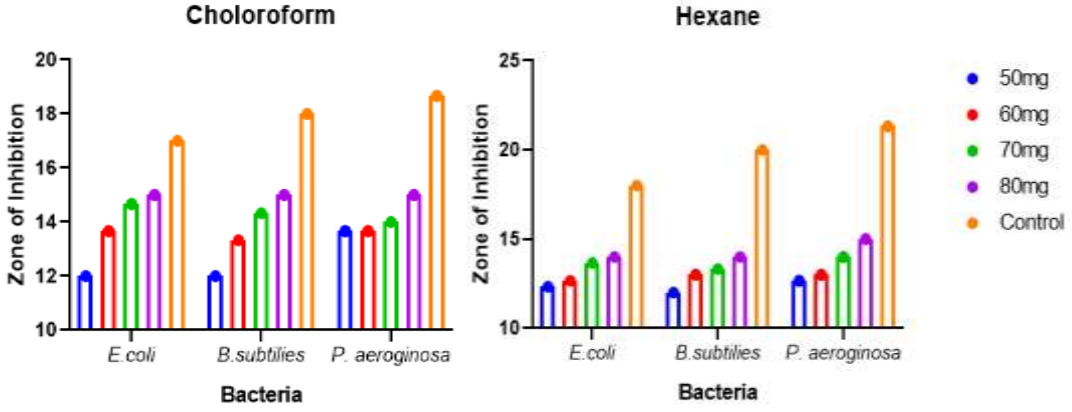

Figure. 2:- Antibacterial activity of pomegranate peel extracts of chloroform and hexane

3.2. Antifungal activity of different fruit peels extract

After evaluation of antifungal activity of extract at different concentrations (50mg/ml, 60mg/ml, 70mg/ml and 80mg/ml) was done against different fruits peel and crown leaves extract. The comparative study of methanol, ethanol, chloroform and n-hexane extract of pomegranate peel, sweet lime peel, banana peel, pineapple crown leaves, corn husk and custard apple peel were tested against *Aspergillus niger*, *Trichoderma viridens* and *Candida albicans* and the antibiotic fluconazole as a positive control. The pomegranate peel, the 80mg/ml concentration was found to be the most effective against *Aspergillus niger, Trichoderma viridens* and *Candida albicans.* The pomegranate peel extract exhibited the maximum inhibition activity of methanol ±22mm against *Candida albicans,* while other extract like ethanol ±22mm, chloroform ±23mm and hexane ±24mm, in case of *Tricoderma viridens* and *Aspergillus niger*.

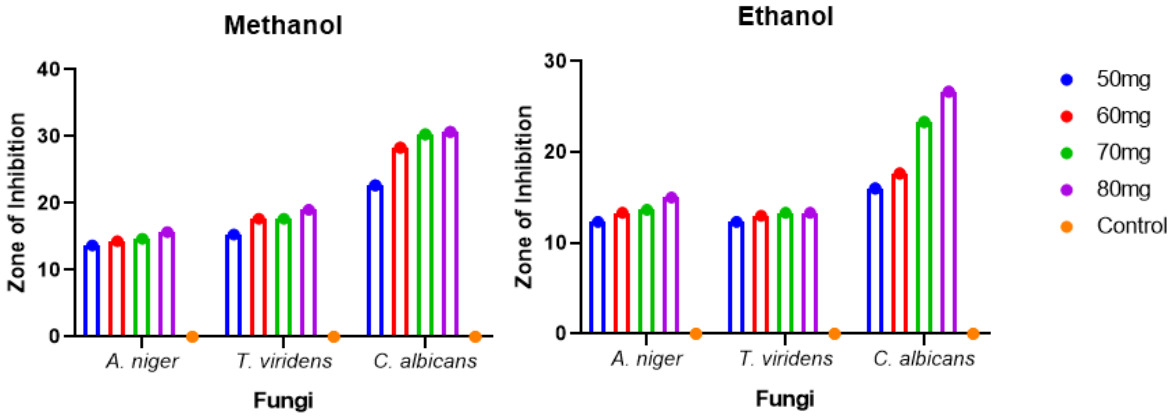

Figure 3:- Antifungal activity of pomegranate peel extracts of methanol and ethanol

The antifungal potency of pomegranate extract was determined by the agar well diffusion method and maximum antifungal activity was found in methanolic and ethanolic extract. The comparative study for methanol, ethanol, chloroform and n- hexane extracts of pomegranate peels extract against *Aspergillus niger, Trichoderma viridens and Candida albicans*. The antifungal potency of four different concentrations (50mg/ml, 60mg/ml, 70mg/ml and 80mg/ml), and fluconazole used as control. The methanolic and ethanol extract 80mg/ml concentration most potent against *Candida albicans,* in compared to other two fungal species like *Aspergillus niger* and *Trichoderma viridens.* In chloroform *and* n-hexane extract, 80mg/ml effective against *Candida albicans* to other two fungal *Aspergillus niger* and *Trichoderma viridens*.

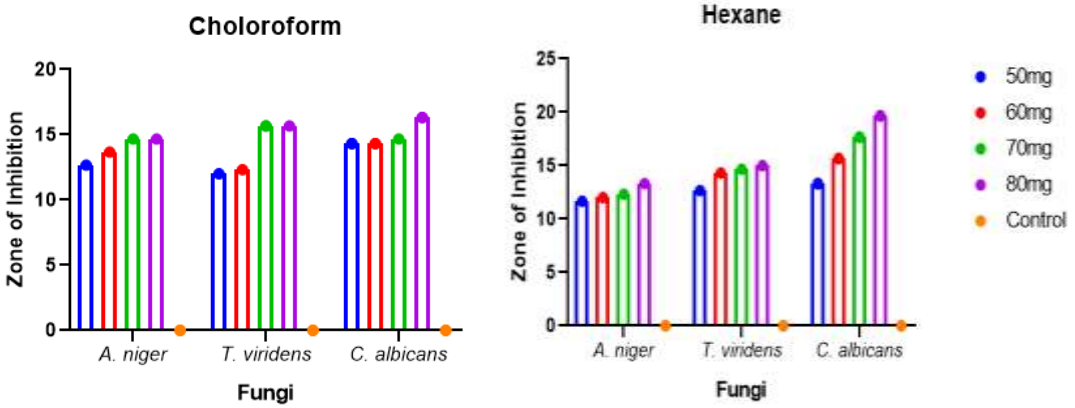

Figure. 4:- Antifungal activity of pomegranate peel extracts of chloroform and hexane

3.3. Minimum inhibitory activity

The methanol extract showed significant antimicrobial activity against various microorganisms. *E. coli* inhibition varied significantly (P=0.0378, F=3.861), while *B. subtilis* exhibited highly

significant inhibition (P=0.0001, F=20.56). *P. aeruginosa* and *A. niger* showed significant inhibition with P values of 0.0402 (F=3.775) and 0.0031 (F=8.353), respectively. *Trichoderma viridens* and *Candida albicans* also had highly significant inhibition with P values of 0.0001 (F=59.72 and F=34.23).

Table 1:- Minimum inhibitory activity of methanolic extract against Bacteria and fungi

	Methanol extract					
	50 µg/ml	**100 µg/ml**	**150 µg/ml**	**200 µg/ml**	**P Value**	**F value**
E.coli	0.42±0.05	**0.24±0.07**	0.30±0.06	0.28±0.07	0.0378	3.861
B. subtilis	0.54±0.03	0.47±0.03	0.33±0.09	**0.30±0.10**	0.0001	20.56
P. aeruginosa	0.35±0.06	0.41±0.22	0.42±0.04	**0.33±0.27**	0.0402	3.775
A. Niger	**0.45±0.23**	0.67±0.03	0.64±0.04	0.87±0.01	0.0031	8.353
Trichoderma viridens	0.81±0.06	0.76±0.01	0.66±0.01	**0.37±0.06**	0.0001	59.72
Candida albicans	0.57±0.15	0.80±0.06	**0.20±0.05**	0.36±0.04	0.0001	34.23

The ethanol extract demonstrated significant antimicrobial activity. *E. coli* showed highly significant inhibition (P=0.0001, F=71.48), as did *B. subtilis* (P=0.0003, F=14.76). *P. aeruginosa*, *A. niger*, *Trichoderma viridens*, and *Candida albicans* also exhibited significant inhibition with P values of 0.0001 (F=26.02, F=19.21, F=22.44) and 0.0002 (F=16.60), respectively.

Table 2:- Minimum inhibitory activity of ethanol extract against Bacteria and fungi

	Ethanol extract					
	50 µg/ml	**100 µg/ml**	**150 µg/ml**	**200 µg/ml**	**P Value**	**F Value**
E.coli	0.53±0.05	0.36±0.02	**0.29±0.03**	0.32±0.08	0.0001	71.48
B. subtilis	0.82±0.14	0.51±0.07	0.41±0.09	**0.39±0.04**	**0.0003**	**14.76**
P. aeruginosa	0.50±0.08	**0.32±0.09**	0.33±0.02	0.39±0.05	0.0001	26.02
A. Niger	**0.55±0.03**	0.71±0.11	0.73±0.01	0.75±0.03	0.0001	19.21
Trichoderma viridens	0.73±0.04	0.68±0.03	**0.47±0.05**	0.63±0.07	0.0001	22.44
Candida albicans	**0.53±0.05**	0.65±0.04	0.71±0.04	0.66±0.06	0.0002	16.60

The chloroform extract exhibited significant antimicrobial activity. *E. coli* showed significant inhibition (P=0.0005, F=13.45), and *B. subtilis* had a moderate response (P=0.0456, F=3.602). *P. aeruginosa*, *A. niger*, and *Trichoderma viridens* displayed significant inhibition with P values of 0.0027 (F=8.673), 0.0001 (F=95.34), and 0.0085 (F=6.301), respectively. *Candida albicans* showed no significant inhibition (P=0.2708, F=1.513).

Table 3:- Minimum inhibitory activity of Chloroform extract against Bacteria and fungi

	Chloroform extract					
	50 µg/ml	**100 µg/ml**	**150 µg/ml**	**200 µg/ml**	**P Value**	**F Value**
E.coli	0.72±0.21	0.52±0.11	0.71±0.06	**0.31±0.02**	0.0005	13.45
B. subtilis	**0.23±0.09**	0.59±0.33	0.56±0.08	0.49±0.09	0.0456	3.602
P. aeruginosa	**0.30±0.09**	0.60±0.26	0.75±0.05	0.37±0.03	0.0027	8.673
A. Niger	0.30±0.11	**0.18±0.02**	0.24±0.04	0.43±0.00	0.0001	95.34
Trichoderma viridens	**0.72±0.03**	0.81±0.00	0.77±0.02	0.75±0.04	0.0085	6.301
Candida albicans	0.82±0.08	0.77±0.07	**0.72±0.11**	**0.72±0.11**	0.2708	1.513

The n-hexane extract demonstrated significant antimicrobial activity. *E. coli* showed highly significant inhibition (P=0.0001, F=57.23), while *B. subtilis* had a moderate response (P=0.0120, F=5.662). *P. aeruginosa, A. niger,* and *Candida albicans* also exhibited significant inhibition with P values of 0.0072 (F=6.599), 0.0001 (F=69.44), and 0.0001 (F=35.09), respectively. *Trichoderma viridens* showed no significant inhibition (P=0.3760, F=1.182).

Table 4:- Minimum inhibitory activity of n-Hexane extract against Bacteria and fungi

n-Hexane extract						
	50 µg/ml	**100 µg/ml**	**150 µg/ml**	**200 µg/ml**	**P Value**	**F Value**
E.coli	0.84±0.10	0.45±0.03	0.28±0.09	**0.27±0.01**	0.0001	57.23
B. subtilis	0.62±0.12	0.49±0.16	0.45±0.02	**0.40±0.10**	0.0120	5.662
P. aeroginosa	0.43±0.15	**0.31±0.16**	0.44±0.09	0.48±0.05	0.0072	6.599
A. Niger	**0.46±0.02**	0.52±0.02	0.61±0.06	0.62±0.04	0.0001	69.44
Trichoderma viridens	0.76±0.03	0.76±0.06	**0.74±0.07**	0.75±0.05	0.3760	1.182
Candida albicans	**0.56±0.02**	0.58±0.04	0.63±0.02	0.63±0.04	0.0001	35.09

4. DISCUSSION

The present study evaluated the antimicrobial efficacy of various solvent extracts from pomegranate peel against a range of pathogenic microorganisms, including *Escherichia coli*, *Bacillus subtilis*, *Pseudomonas aeruginosa, Aspergillus niger, Trichoderma viridens,* and *Candida albicans*. The findings demonstrate that pomegranate peel extracts possess significant antimicrobial properties, with variations in efficacy depending on the solvent used for extraction. The methanol extract exhibited considerable antimicrobial activity across all tested microorganisms. This high efficacy can be attributed to the polarity of methanol, which facilitates the extraction of a wide range of bioactive compounds, including phenolics, flavonoids, and tannins. These compounds are known for their antimicrobial properties, potentially disrupting microbial cell membranes and interfering with enzyme activities essential for microbial survival (Duman, Ozgen, Dayisoylu, Erbil, & Durgac, 2009). Similarly, the ethanol extract showed substantial antimicrobial effects, particularly against *E. coli* and *P. aeruginosa*. Ethanol, like methanol, is a polar solvent capable of extracting significant amounts of bioactive constituents from plant materials. Previous studies have reported that ethanol extracts of pomegranate peels are rich in ellagitannins and punicalagins, which exhibit strong antimicrobial activities (Mehdinia & Rostami, 2020). The results of this study align with these findings, highlighting the potential of ethanol extracts in combating bacterial infections. The chloroform extract demonstrated moderate antimicrobial activity, especially against fungal strains like *Aspergillus niger* and *Candida albicans*. Chloroform, being a non-polar solvent, extracts lipophilic compounds, including essential oils and terpenoids, which have been associated with antifungal properties. The lower efficacy against bacterial strains compared to methanol and ethanol extracts suggests that the antimicrobial compounds extracted by chloroform are more effective against fungi. The n-hexane extract showed notable activity against *E. coli* and *Candida albicans*. Hexane, another non-polar solvent, is effective in extracting lipophilic components such as fatty acids and essential oils. These components can integrate into microbial lipid bilayers, causing disruption and leading to cell death (Machado et al., 2002). However, the overall antimicrobial activity of the n-hexane extract was less pronounced compared to the methanol and ethanol extracts, likely due to the limited range of bioactive compounds extracted by this solvent. The antimicrobial properties of pomegranate peel extracts observed in this study are consistent with previous research. For instance, (Al-Zoreky, 2009) demonstrated the effectiveness of pomegranate peel extracts against a broad spectrum of microorganisms, including multidrug-resistant bacterial strains and pathogenic fungi. Another study by (Duman et al., 2009) reported significant antimicrobial activity of pomegranate peel extracts against several bacterial and fungal species. The current study further supports these findings and provides additional insights into the comparative efficacy of different

solvent extracts. The significant antimicrobial activity of pomegranate peel extracts, particularly those obtained using methanol and ethanol, underscores their potential as natural antimicrobial agents. These extracts could be incorporated into pharmaceuticals, food preservatives, and cosmetics to enhance microbial safety and shelf life. Future research should focus on identifying and isolating specific bioactive compounds responsible for the antimicrobial effects, as well as evaluating the extracts' efficacy in vivo.

5. CONCULSION

The study highlights the potent antimicrobial efficacy of pomegranate peel extracts, notably those extracted using methanol and ethanol, against a diverse range of pathogens. These include bacterial strains such as *Escherichia coli*, *Bacillus subtilis*, and *Pseudomonas aeruginosa*, as well as fungal species like *Aspergillus niger*, *Trichoderma viridens*, and *Candida albicans*. Additionally, chloroform and n-hexane extracts demonstrated considerable effectiveness, particularly against fungal strains. These findings underscore the promising potential of pomegranate peel extracts as natural antimicrobial agents, applicable in pharmaceuticals and food preservation. Further research should aim to isolate specific bioactive compounds and evaluate their efficacy in vivo.

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