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Original research paper

IMPACT OF DRYING METHODS AND SOLVENT EXTRACTION ON THE ANTIBACTERIAL PROPERTIES OF POMEGRANATE (*PUNICA GRANATUM* L.) PEEL

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Abstract: The current study aims to study the effects of three drying methods (freeze, tray, sun) and five solvents (methanol, ethanol, water, acetone, hexane) on the antibacterial properties of the by-product (peel) of pomegranate fruits against four bacterial strains (*Pseudomonas aeruginosa*, *Escherichia coli*, *Bifidobacterium bifidum* and *Enterococcus faecalis*). The results demonstrated that these extracts had a substantial antibacterial impact against the tested bacterial species. All pomegranate peel extraction fractions, containing methanol, ethanol, water and acetone, inhibited the growth of several Gram-positive and Gram-negative bacteria. The combination of freeze-drying process and methanol as extractive solvent demonstrated significantly higher antimicrobial activity ($p<0.05$) in pomegranate peel powder against *P. aeruginosa* (24.16 mm), *E. coli* (24 mm) and *E. faecalis* (24 mm) at a concentration of 35 µL. Hexane was found unsuitable as an extraction solvent because it lacked antibacterial activity, while acetone, although it exhibited limited extraction efficiency for pomegranate peel, produced an extract with relatively better antibacterial activity. This study will help in achieving sustainability and a circular economy by utilizing pomegranate peel waste as a natural active agent for further applications in the food processing and packaging industry to develop value-added products and edible packaging for food preservation and efficient use of pomegranate peel as a natural antibacterial agent in pharmaceuticals, food preservation and biomedical applications.

Keywords: a*nti-microbial activity, drying, pomegranate peel, solvent extraction, ultrasonication*

INTRODUCTION

Food wastage is a significant concern worldwide due to its contribution to the growth of landfills and the emission of greenhouse gases (GHGs) into the environment. Approximately one-third of the total food production is discarded as food waste throughout the supply chain, resulting in a decrease in both the quality and quantity of food (Ishangulyyev, Kim & Lee, 2019; Marra et al., 2022). In the horticulture commodity, pomegranate fruits are one of the most important fruits, also known as the 'super fruit', due to their nutritional and me-

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dicinal health benefits. It is consumed as a whole, jam and juice around the world. The pomegranate fruit contains around 40-50 percent as waste (peel) of the total fruit weight (Marra et al., 2022; Kumar et al., 2022a). The pomegranate peel (PGP) exhibited good amounts of nutrients such as carbohydrates, pectin, protein content and bioactive or polyphenolic compounds such as ellagic acid, gallic acid, punicalagin A, punicalagin B, punicalin, catechin, quercetin, corliagin, gallocatechin, etc. (Cano-Lamadrid et al., 2022; Man, Xu, Wang, Liao & Xu, 2022; Kumar, Pratibha et al., 2022). The PGP exhibits good antimicrobial, anti-inflammatory, antioxidant, anticancer, antiviral and antifungal activities, which may be attributed to the bioactive compounds present in it (Valero-Mendoza et al., 2023). Antimicrobial agents derived from pomegranate peel waste can be utilized as stabilizers, preservatives, and inhibitors of microbial growth in the food and medical sectors (Viuda-Martos, Ruiz-Navajas, Fernández-López, & Pérez-Álvarez, 2008).

Recently, the use of pomegranate peel extract and powder has been widely increased for the development of active edible packaging for shelf-life extension of fruits and vegetables by retarded the oxidation and free radical scavenging activity (Kumar et al., 2023; Bodana et al., 2024). Previously, numerous researchers studied and reported the antimicrobial efficiency of PGP against several microorganisms, such as *Klebsiella pneumoniae*, *Candida albicans*, *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli*, *Enterococcus faecalis*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Streptococcus thermophilus*, *Bacillus subtilis*, *Bifidobacterium bifidum*, *Shigella flexneri*, *Trichophyton rubrum*, *Salmonella typhimurium*, and *Salmonella anatum*, etc. (Kumar, Daniloski, D'Cunha, Naumovski & Petkoska, 2022; Alharbi et al., 2024; Mirhaj et al., 2024). The recovery of bioactive constituents from PGP also depends on the type of pomegranate fruit peel, drying conditions, extraction solvents and methods (Mphahlele, Fawole, Makunga & Opara, 2016; Kumar, Pratibha et al., 2022b). Furthermore, there are very limited studies available on the influence of drying conditions and solvents on the antimicrobial efficiency of PGP. The evaluation and identification of the antimicrobial activity of pomegranate peel are important to evaluate their efficiency against retarded growth of food-borne pathogens. Therefore, in the present study, the effects of different drying methods (lyophilization, tray and sun drying) and various extraction solvents (methanol, ethanol, water, hexane and acetone) were evaluated to assess the antimicrobial properties of pomegranate peel against four microbial strains (*P. aeruginosa, E. coli, B. bifidum* and *E. faecalis*). This study aims to contribute to achieving sustainability and a circular economy by utilizing pomegranate peel waste as a natural active agent for further applications in the food processing and packaging industries, thereby developing value-added products and edible packaging for food preservation. Pomegranate peel can be considered a natural source of antimicrobial agents and a potential alternative to chemical fungicides. Further, the present study will also provide critical insights into the influence of drying techniques and solvent extraction on the antimicrobial efficacy of pomegranate peel. Optimizing these parameters enhances the potential use of pomegranate peel as a natural antimicrobial agent in pharmaceuticals, food preservation and biomedical applications. The findings will contribute to the sustainable utilization of waste and the development of eco-friendly antimicrobial alternatives.

MATERIALS AND METHODS

Preparation of pomegranate peel powder

The garden-fresh pomegranate (cv. Bhagwa) fruits were collected from Azadpur Mandi, Delhi. The fruits were carefully peeled and then dried. The pomegranate peel was dried using three methods such as lyophilization (Benchtop, Vir Tis, USA) at –45 ºC for 94 h, tray-drying at 60 ºC for 29 h and sun-drying for 72 h (Kumar, Pratibha et al., 2022; Aditya, Neeraj & Bhatia, 2024; Aditya, Neeraj, Bhatia, Jarial & Jarial, 2025). The dried fine pomegranate peel powder was kept refrigerated for future analysis.

Ultrasonic-assisted extraction

Pomegranate peel powder was extracted using an ultrasonic bath to get a clear and transparent extract for the purpose of evaluating its antibacterial activity. The extract was prepared in different solvents such as Me, Et, Wa, Ac and He, respectively (Kumar & Neeraj, 2018). A total of 0.2 g of finely ground pomegranate

peel powder was immersed in 10 mL of solvent and treated with ultrasonic waves in an ultrasonic bath (CUB-5, Citizen, 40 kHz, 220-240 V) at a temperature of 45 ºC for a duration of 30 min. The mixture was then subjected to centrifugation (Sigma, 3-18, KS, Germany) at 5 ºC and 8654 rpm for 10 min and then filtered using Whatman filter paper No. 1. The pomegranate peel extract samples were stored in amber-colored glass bottles (PW1150, Himedia, Maharashtra, India) under refrigeration (4±1 ºC) to preserve their stability and determination of their antibacterial efficacy.

Bacterial strains evaluated

The microbial strains, *viz*., *P. aeruginosa* (NCDC-105), *B. bifidum* (NCDC-229), *E. faecalis* (NCDC-223) and *E. coli* (NCDC-134) were obtained from NIFTEM-K, India, in pure lyophilized form. The microbial culture was inoculated in liquid culture broth (nutrient broth) media and incubated at 37 ºC for 2-6 h to reach the logarithmic stage. The turbidity of the cultures was adjusted to an absorbance of 625 nm, resulting in a final concentration of approximately 2×10^8 CFU/ml of bacteria.

Antimicrobial activity

The agar well diffusion method was employed to investigate the antimicrobial activity of pomegranate peel extract (PGPE), as described by Kumar, Neeraj and Kaur (2019). The bacterial culture suspension (100 µL) was spread onto the surface of Muller-Hinton agar medium. The sterilized stainless-steel borer was used to make a 6 mm diameter well in the medium. The different volumes (20, 25, 30 and 35 µL) of PGP extract (PGPE) were added to the wells. The plates were incubated at 35 ºC for 12–48 h, and antimicrobial activity was assessed by measuring the diameter of the inhibition zone. The ampicillin (10 µg/disk, CLSI standard) was used as a positive control to determine antimicrobial activity in PGPE and was purchased from Himedia, Maharashtra, India (SKU: SD002).

Statistical analysis

The average value (n = 5) of the data, along with its standard deviation, was reported as the result of the experiments. The data were statistically analyzed using ANOVA with the Duncan multiple range test ($P<0.05$).

RESULTS AND DISCUSSION

Standard zone of inhibition for ampicillin (positive control)

The standard zone of inhibition in terms of sensitivity, intermediate and resistance against tested bacterial strains of ampicillin antibiotic (10 µg) has been presented in Table 1. It was used as a positive control to examine the sensitivity, resistance and intermediate in terms of zone inhibition (mm) in PGPE against the tested bacterial species.

Antibacterial activity against *P. aeruginosa* (NCDC-105)

The results of evaluating the effectiveness of ampicillin (10 µg) against selected bacterial strains are presented in Table 1.

For ampicillin, the size of the zone of inhibition, in terms of sensitivity, resistance, and intermediate response, varies among the selected bacterial strains due to differences in their cell wall composition, antibiotic uptake mechanisms, and intrinsic or acquired resistance mechanisms. Gram-positive and Gram-negative bacteria exhibit varying susceptibility based on factors such as outer membrane permeability, efflux pumps and the presence of β-lactamase enzymes that degrade ampicillin (Kumar et al., 2019).

The antibacterial activity of PGPE obtained through freeze, tray and sun drying methods, and extracted using selected solvents, was evaluated against *P. aeruginosa*.

Table 1.
Activity of antibiotic (ampicillin 10 µg) as a standard positive control for different strains as per CLSI

S. no.	Bacterial species	Ampicillin (10 µg) (mm)		
		Sensitive	Intermediate	Resistant
1.	*Pseudomonas aeruginosa (NCDC-105)*	22	16-21	15
2.	*Escherichia coli (NCDC-134)*	17	14-16	13
3.	*Bifidobacterium bifidum (NCDC-229)*	30	25	20
4.	*Enterococcus faecalis (NCDC-223)*	17	-	16

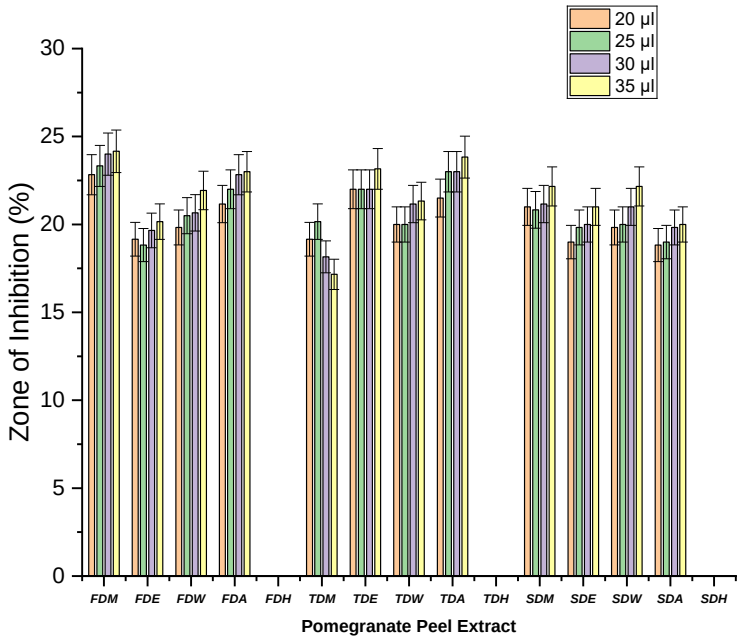

Figure 1. Antimicrobial activity of pomegranate peel extract against *P. aeruginosa.* An average value (n = 5) with a significant difference has been reported. FD = Freeze dried PGP; TD = Tray dried PGP; SD = Sun dried PGP; M = Methanol; E = Ethanol; W = Water; A = Acetone; H = Hexane

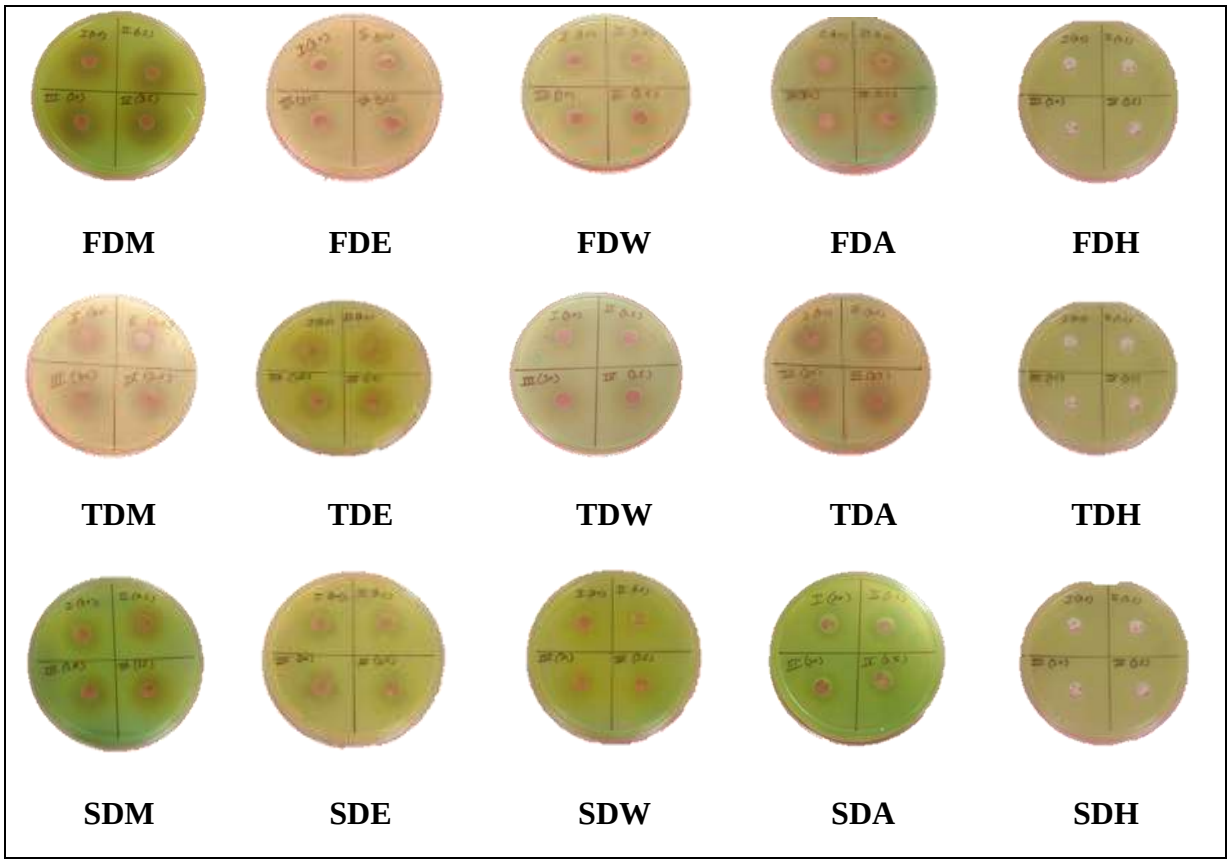

Figure 2. Inhibitory activity of pomegranate peel extract against *P. aeruginosa.*
F= Freeze dried, T= Tray dried, S= Sun dried, M= Methanol, E= Ethanol, W= Water, A= Acetone, H=Hexane

The extracts were tested at concentrations of (20, 25, 30 and 35 µL) with ampicillin (10 µg) serving as a standard positive control. The freeze-dried (FD) methanol extract (35 µL) ex-

hibited potential antibacterial activity against *P. aeruginosa*.

Antibacterial activity was also observed at concentrations of 20 µL, 25 µL and 30 µL (Fig. 1 & 2). Additionally, the FD acetone extract (20 µL) and the FD water extract (35 µL) demonstrated intermediate antibacterial activity. Notably, as the concentration of the extract increased, the antibacterial activity also increased, indicating a dose-dependent response.

Similarly, tray-dried (TD) acetone extracts at concentrations of 25 µL, 30 µL and 35 µL exhibited antibacterial activity, with zone sizes of 23 mm and 23.83 mm. In the case of sun-dried (SD) extracts, SD methanol and SD water at a concentration of 35 µL exhibited antibacterial activity, with a zone size of 22.16 mm (Table S1).

However, the hexane extracts (FDH, TDH and SDH) were found to be inactive, as no significant antibacterial activity was observed based on the zone of inhibition, compared to the standard (ampicillin 10 µg). The lack of antibacterial activity in the hexane extracts may be attributed to the non-polar nature of hexane, which limits its ability to extract polar and semi-polar bioactive compounds such as phenolics, flavonoids and tannins (Abraham, Abdulazeez, Seun & Ogonna, 2019).

Since most antimicrobial compounds in pomegranate peel are more soluble in polar solvents such as methanol, ethanol and water, hexane extraction likely yielded fewer bioactive constituents, resulting in minimal or no inhibition against the tested bacterial species.

The present findings demonstrated that the antibacterial efficacy of pomegranate peel extracts was contingent upon the drying technique and solvent employed.

The freeze-dried methanol extract (35 µL) demonstrated the most significant action against *P. aeruginosa*, but the acetone and water extracts displayed moderate effects. Hexane extracts exhibited inactivity, highlighting the significance of solvent polarity in the extraction of bioactive compounds. These findings underscore the potential of pomegranate peel as a natural antibacterial agent.

Antibacterial activity against *E. coli* (NCDC-134)

The results of the antibacterial effects of PGPE against *E. coli* are presented in Figs. 3 and 4. The results indicated that PGPE extracted using various solvents exhibited sensitivity at all tested concentrations (20, 25, 30, and 35 µL) against *E. coli* strains. The highest zone of inhibition (22.66 mm) was observed at a 20 µL concentration of pomegranate peel extract in ethanolic extraction, followed by TD water (22 mm), FD acetone (21.66 mm), FD methanol (21.33 mm) and TD acetone (21 mm). The TD ethanol and TD water extracts did not show a significant variance between them; however, the remaining values showed significant differences in antibacterial activity.

The freeze-dried acetone extract exhibited the highest zone of inhibition (23 mm), followed by TD acetone (20 mm), TD water (20 mm) and methanolic extraction of freeze-dried pomegranate peel (21.66 mm) at a 25 µL concentration. While FD methanol (23.33 mm) shows the highest inhibition, followed by FD acetone (23 mm), TD ethanol (23 mm), and TD acetone (23 mm), with no significant variance among them. Among aqueous extracts at 30 µL, FD water (22.16 mm), TD water (22.83 mm), and SD water (22 mm) exhibited notable inhibition against *E. coli*. The methanolic fraction of freeze-dried pomegranate peel at 35 µL showed the highest inhibition zone (24 mm) compared to other concentrations (Table S2).

The results indicate that increasing the concentration of PGPE enhances its antibacterial efficacy against *E. coli*, highlighting its potential as a natural antibacterial agent.

Antibacterial activity against *B. bifidum* (NCDC-229)

The results for the antibacterial efficacy of pomegranate peel extract against *B. bifidum* are presented in Fig. 5 and 6. The tray-dried PGP extract with water exhibited higher inhibition at various concentrations. The FD methanol and TD acetone at 20 µL demonstrated antimicrobial resistance against *B. bifidum*, with inhibition zones of 21.90 mm and 21.80 mm, respectively.

The positive control exhibited a 20 mm zone of inhibition against *B. bifidum*. The TD water

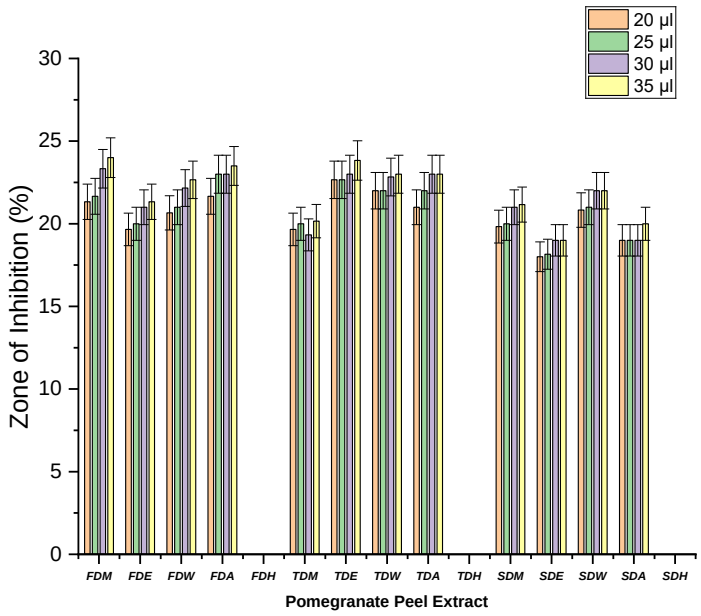

Figure 3. Antimicrobial activity of pomegranate peel extract against *E. coli.* An average value (n = 5) with a significant difference has been reported. FD = Freeze dried PGP; TD = Tray dried PGP; SD = Sun dried PGP; M = Methanol; E = Ethanol; W = Water; A = Acetone; H = Hexane

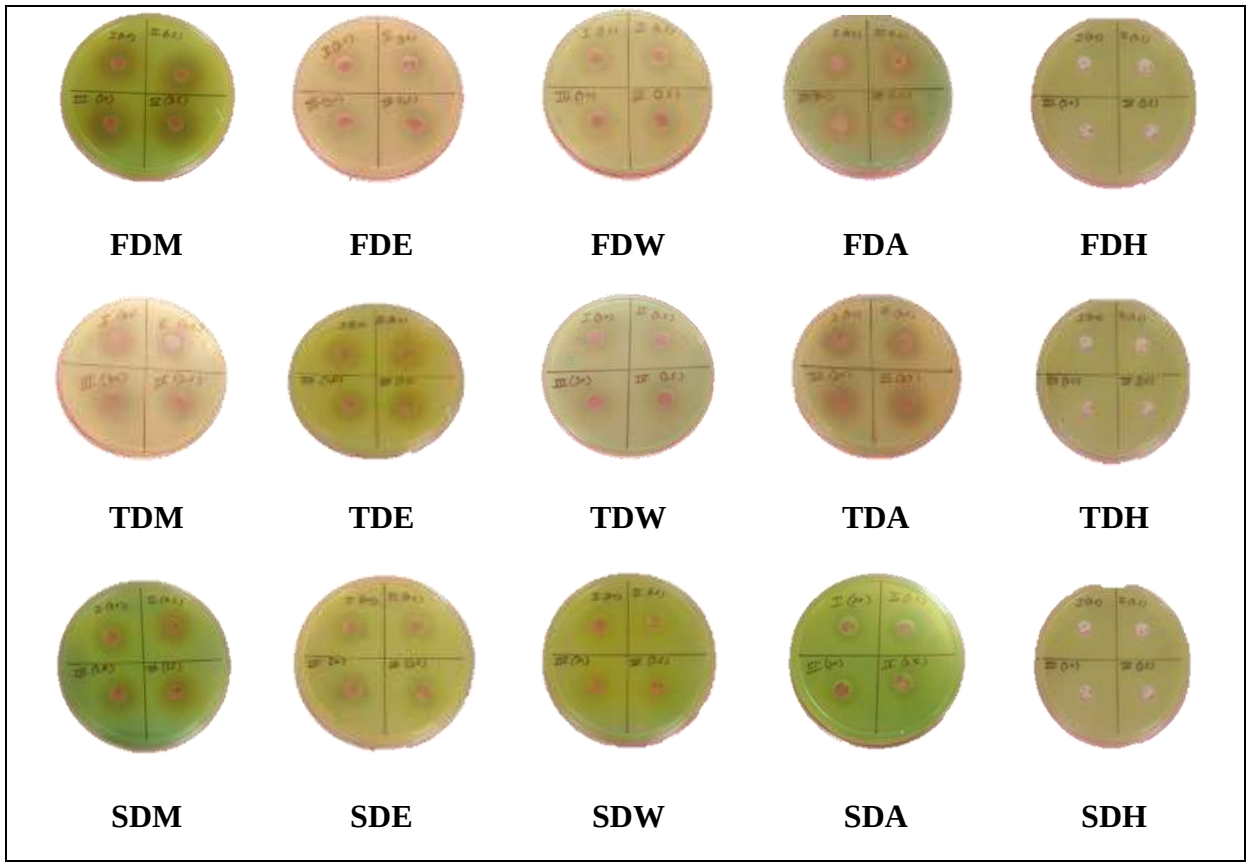

Figure 4. Inhibitory activity of pomegranate peel extract against *E. coli.*
(F= Freeze dried, T= Tray dried, S= Sun dried, M= Methanol, E= Ethanol, W= Water, A= Acetone, H=Hexane)

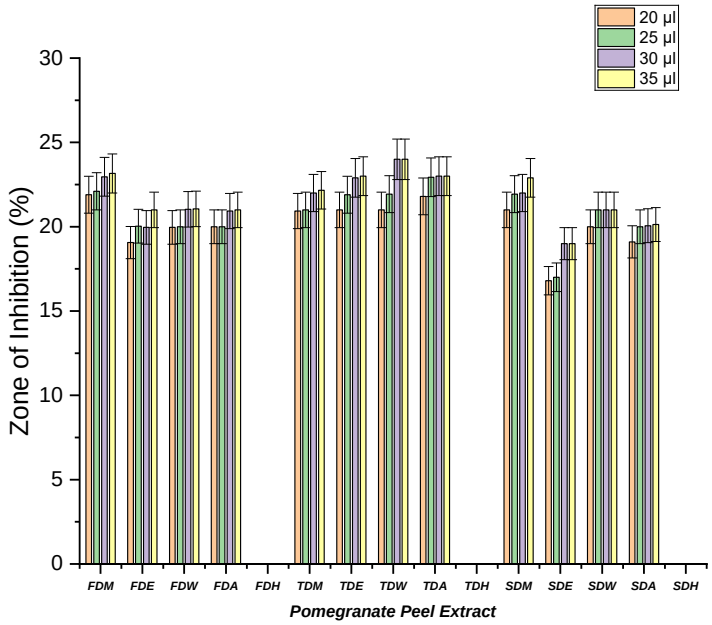

Figure 5. Antimicrobial activity of pomegranate peel extract against *B. bifidum.* An average value (n = 5) with a significant difference has been reported. FD = Freeze dried PGP; TD = Tray dried PGP; SD = Sun dried PGP; M = Methanol; E = Ethanol; W = Water; A = Acetone; H = Hexane

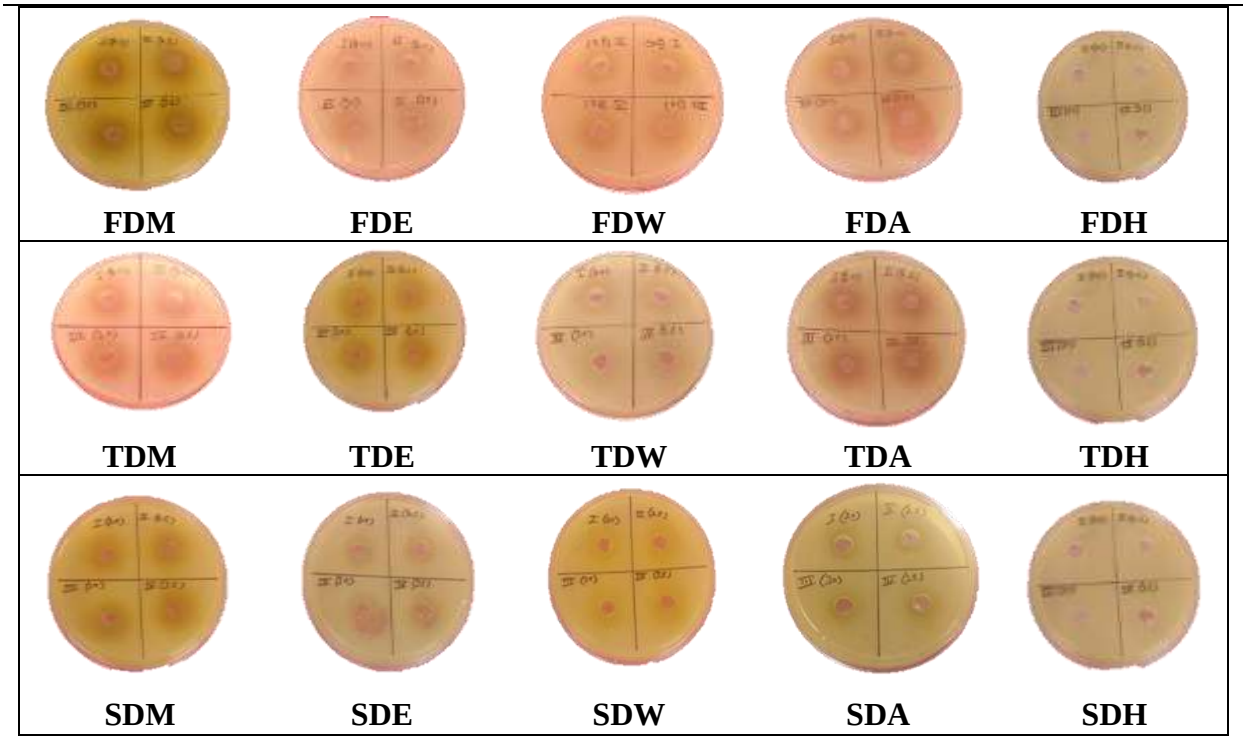

Figure 6. Inhibitory activity of pomegranate peel extract against *B. bifidum.*
F= Freeze dried, T= Tray dried, S= Sun dried, M= Methanol, E= Ethanol, W= Water, A= Acetone, H=Hexane)

and TD ethanol showed similar inhibition zones (21 mm), followed by the methanolic extract of tray-dried PPE (20.93 mm). The TD ethanol and TD water extracts did not show a significant variance from each other. The aqueous extract (FD water), acetone extract of freeze-dried PPE, and methanolic extract of sun-dried PPE (SD methanol) also exhibited resistance against *B. bifidum*, with inhibition zones of 19.96 mm, 20 mm, and 20 mm, respectively. The hexane extract of pomegranate peel did not exhibit any antibacterial activity

against *B. bifidum* at a concentration of 20 µL. The TD acetone extract showed the highest zone of inhibition, followed by the methanolic extract of freeze-dried PPE, which exhibited a zone of inhibition of 22.10 mm. The aqueous extracts from different drying methods showed inhibition zones ranging from 20 mm to 21.93 mm. Acetone extracts of both freeze-dried (FD acetone) and sun-dried (SD acetone) peels demonstrated a similar inhibition zone of 20 mm. The freeze-dried extracts in ethanol, water, and acetone exhibited a significant correlation in their inhibition zones. The hexane extract remained inactive at a 25 µL as well as 30 µL concentration against *B. bifidum*. At 30 µL, the aqueous extract (TD water) of tray-dried PPE exhibited the highest inhibition zone (24 mm), followed by FD methanol (22.96 mm), TD acetone (23 mm) and TD ethanol (22.9 mm). The methanolic extracts of sun-dried and tray-dried pomegranate peel exhibited similar inhibition zones, each with a diameter of 22 mm (Table S3).

Compared to the positive control (ampicillin), the ethanolic extract (FD) and sun-dried peel powder showed a lower inhibition zone, indicating their ineffectiveness in inhibiting the growth of *B. bifidum*. Tray-dried PGP extract with water exhibited the highest inhibition against *B. bifidum*, while FD methanol and TD acetone also showed significant antimicrobial activity. Hexane extracts remained inactive, and ethanolic extracts demonstrated lower inhibition compared to the positive control (ampicillin), indicating limited effectiveness against *B. bifidum*.

Antibacterial activity against *E. faecalis* (NCDC-223)

The antibacterial activity of pomegranate peel extract against *E. faecalis* is shown in Figures 7 and 8. The methanolic extract of freeze-dried and the ethanolic extract of tray-dried PPE exhibited the highest sensitivity, with inhibition zones of 21.9 mm and 22 mm, respecttively, at a concentration of 20 µL. This was followed by FD acetone (21 mm), SD methanol (21 mm), TD water (20 mm), TD acetone (20 mm), SD water (20 mm) and FD water (19.93 mm), as measured by the inhibition zone diameter. The hexane fraction did not exhibit any antibacterial effect against *E. faecalis*. The FD ethanol (19.03 mm), TD methanol (19 mm) and sun-dried extracts in both ethanol and acetone showed similar inhibition zones of 18 mm. At a 25 µL concentration, the freeze-dried peel extract in methanol exhibited the highest sensitivity, with an inhibition zone of 23.06 mm, followed by TD ethanol (22 mm), SD methanol (22 mm), FD acetone and TD water (21 mm) and FD water (20.96 mm). The extracts in methanol, ethanol, acetone and water exhibited sensitivity against *E. faecalis*. However, the hexane extracts of pomegranate peel showed no effect on *E. faecalis* growth at a 25 µL concentration. The highest sensitivity was observed at concentrations of 30 µL and 35 µL in the freeze-dried methanolic extract, with inhibition zones of 23.06 mm and 24 mm, respectively. At 30 µL, the TD ethanol extract exhibited the second-highest inhibition activity (22.93 mm) after the FD methanol. The acetone extracts of FD, TD and SD also showed sensitivity against *E. faecalis*, with inhibition zones of 22.03 mm, 22 mm, and 18 mm, respectively. The TD ethanol extract exhibited higher inhibition activity (22.93 mm) compared to FD ethanol (20 mm) and SD ethanol (19 mm) at a 30 µL concentration. At a 35 µL concentration, the methanol, acetone, ethanol and water extracts demonstrated higher inhibition activity compared to lower concentrations of pomegranate peel extract. The freeze-dried methanolic extract of pomegranate peel exhibited the highest inhibition activity, with a zone of 24 mm, followed by TD ethanol (23 mm) and TD acetone (23 mm). Significant variations were observed among FD water (22 mm), FD acetone (22 mm), TD water (22 mm), and SD methanol (22 mm) in their inhibition zones against *E. faecalis*. The ethanolic extract of freeze-dried pomegranate peel powder and the aqueous extract of sun-dried pomegranate peel exhibited inhibition zones of 21.23 mm and 21 mm, respectively, at a 35 µL concentration (Table S4). The hexane fraction at the same concentration showed no inhibitory activity against *E. faecalis*.

The antibacterial activity of pomegranate peel extract against *E. faecalis* was highest in the freeze-dried methanolic extract, followed by the TD ethanol and TD acetone extracts, particularly at higher concentrations. While methanol, ethanol, acetone and water extracts exhibited antibacterial effects, the hexane fraction showed no inhibition against *E. faecalis* at any of the tested concentrations.

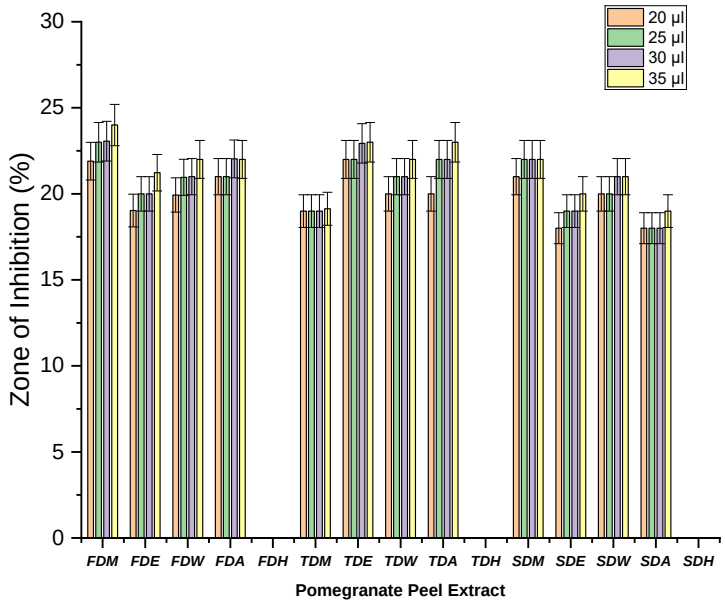

Figure 7. Antimicrobial activity of pomegranate peel extract against *E. faecalis.* An average value (n = 5) with a significant difference has been reported. FD = Freeze dried PGP; TD = Tray dried PGP; SD = Sun dried PGP; M = Methanol; E = Ethanol; W = Water; A = Acetone; H = Hexane

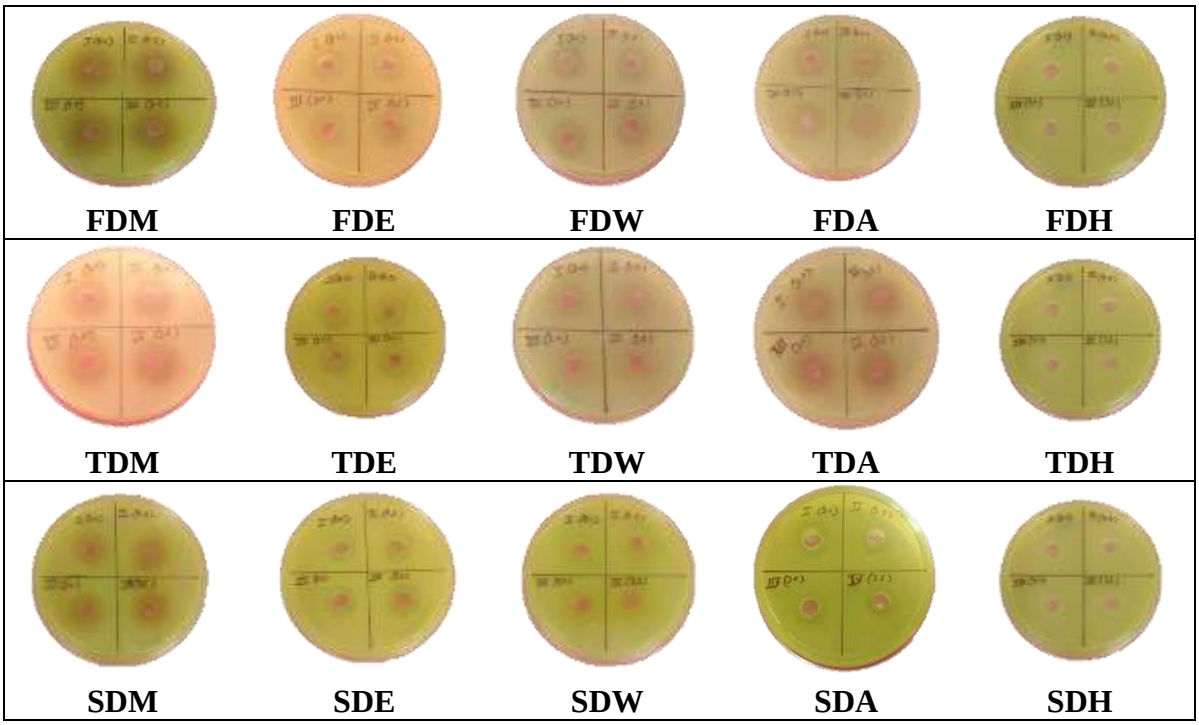

Figure 8. Inhibitory activity of pomegranate peel extract against *E. faecalis.*
F= Freeze dried, T= Tray dried, S= Sun dried, M= Methanol, E= Ethanol, W= Water, A= Acetone, H=Hexane

The antibacterial efficacy of pomegranate peel powder against various bacterial species has garnered significant attention in recent years, underscored by its rich composition of phenolic compounds, tannins and flavonoids. Pomegranate peel powder and its extracts are rich in bioactive phytochemicals, predomi-nantly hydrolysable tannins (punicalagins and punicalins), ellagic acid, gallic acid and flavonoids, which collectively define their chemical

profile. These compounds are primarily responsible for the strong antimicrobial activity through mechanisms such as membrane disruption, protein precipitation and inhibition of microbial enzymes. The synergistic action of phenolic acids and tannins enhances broad-spectrum antimicrobial efficacy against foodborne and clinical pathogens. Consequently, pomegranate peel exhibits significant potential as a natural antimicrobial agent for food preservation and biomedical applications. Studies have shown that pomegranate peel possesses substantial antimicrobial properties, inhibiting the growth of both Gram-positive and Gram-negative bacteria. Extracts derived from pomegranate peel have demonstrated notable antibacterial activity against *S. aureus* and *P. aeruginosa*, with MIC values affirming their potency (Nozohour, Golmohammadi, Mirnejad & Fartashvand, 2018). The activity of pomegranate peel against these bacteria correlates with the presence of phytochemicals such as gallic acid and ellagic acid, which possess significant antibacterial properties (Kumar, Daniloski et al., 2022). The high tannin content present in pomegranate peels has been reported to synergistically enhance the antimicrobial effects, further contributing to the reduction of bacterial loads in food products (Shalaby, Dawood, Hefni & Murad, 2019). Moreover, studies have also shown that incorporating pomegranate peel powder into food products not only extends shelf life but also significantly reduces microbial contamination. In sausages supplemented with varying concentrations of pomegranate peel powder, there was a progressive reduction in aerobic plate counts during storage, indicating its effective role as a natural preservative (Abuzaid, Shaltout, Salem & El-Diasty, 2020). Similar trends have been observed in other food applications, where pomegranate peel has been utilized to mitigate spoilage bacteria, including *L. monocytogenes* and *E. coli*, providing a dual benefit of enhancing food safety while prolonging shelf life (Hayrapetyan, Hazeleger & Beumer, 2012). Mechanistically, the antibacterial action of pomegranate peel may involve the chelation of iron and the disruption of bacterial cell walls, depriving pathogens of critical nutrients necessary for their growth (Rummun, Somanah, Ramsaha, Bahorun, Neergheen, 2013). As a result, the pomegranate peel not only inhibits microbial proliferation but also may exert protective effects in various food matrices, thereby enhancing both sensory and nutritional qualities (Lacivita, Incoronato, Conte & Nobile, 2021). Physiological and cultural conditions, such as nutrient media composition, pH and temperature, significantly influence microbial colonization, growth rate, and metabolic activity. These parameters are crucial for optimizing accurate and reproducible outcomes in *in vitro* antimicrobial studies (Aditya, Jarial & Jarial, 2022; Aditya, Jarial & Jarial, 2023).

The results of this study indicate that the drying method of pomegranate peel and the choice of extraction solvent, *viz*., ethanol, water, methanol, acetone and hexane, significantly influence its antibacterial activity against selected pathogens, including *E. coli, E. faecalis, B. bifidum* and *P. aeruginosa.* Among the tested methods, freeze-drying combined with methanolic extraction demonstrated the highest inhibitory effect on microbial growth and sensitivity. In contrast, hexane proved ineffective as an extractive solvent for antimicrobial activity. Among the five freeze-dried PGP extracts, the methanolic extract at a concentration of 35 µL exhibited the highest antimicrobial efficacy, producing a zone of inhibition of 24.16 mm against *P. aeruginosa*, surpassing the standard sensitivity threshold of 22 mm. These findings align with previous research by Kaur, Kaushal & Sharma, (2018), Nozohour et al. (2018), Alexandre et al. (2019), Kumar et al. (2019) and Singh, Singh, Kaur & Singh, (2019), who have reported the antimicrobial properties of pomegranate peel extract against various bacterial species.

Pomegranate peel extracts exhibited antimicrobial activity against *E. coli* and *B. bifidum*, with efficacy varying depending on the solvent and drying method used. Higher concentrations of PGPE led to greater inhibition. Freeze-dried pomegranate peel extracts in ethanol, aqueous and acetone solvents showed inhibition zones of 21.33 mm, 22.66 mm and 23.50 mm, respectively, against *E. coli*. The lowest inhibition (19 mm) was observed in the ethanolic extract of sun-dried peel, followed by the SD acetone extract (20 mm) and the TD methanol extract (20.16 mm). Hexane fractions were found inactive against *E. coli*. Against *B. bifidum*, the aqueous extract of tray-dried peel exhibited the highest inhibition (24 mm), followed by freeze-dried methanol extract (23.16 mm) at 35 µl concentration. The hexane frac-

tion and sun-dried ethanol extract (19 mm) showed no inhibition. The SD ethanol sample failed to resist *B. bifidum* due to low inhibition concentration. Freeze-dried and sun-dried extracts (FDA, FDE, FDW and SDW) exhibited resistance inhibition, with no significant variation in inhibition zones. All PGPE concentrations were effective against *B. bifidum*, except for specific extracts at lower concentrations. Hexane extracts remained inactive across all concentrations. These results align with previous findings by Alexandre et al. (2019), Kumar et al. (2019) and Singh et al. (2019), confirming PGPE's antimicrobial activity against various bacterial strains.

The antibacterial activity of pomegranate peel extract against *E. faecalis* indicated that the methanolic extract of freeze-dried and ethanolic extract of tray-dried PPE have exhibited the highest sensitivity against *E. faecalis* strain at 20 µl concentrations, followed by FD acetone, SD methanol, TD water, TD acetone, SD water and FD water, respectively. Hexane solvent did not exhibit antibacterial activity against *E. faecalis*. The results indicated that all extractions of different pomegranate peels, performed using methanol, ethanol, water and acetone, were found to be sensitive against *E. faecalis*. The results of the present study align well with those of previous studies by Prasetyo et al. (2021) and Balaban, Koc, Sar and Akbas (2021), which reported the antimicrobial activity of white pomegranate peel extract and *Hicaznar* pomegranate peel extract against *E. faecalis.* The results of the present study showed that the various drying methods and extraction solvents such as methanol, ethanol, water and acetone had the potential to inhibit the growth of several Gram-positive and Gram-negative bacteria, including *P. aeruginosa*, *E. coli* and *E. faecalis*. The combination of freeze-drying process and methanol as extractive solvent, demonstrated significantly higher antimicrobial activity ($p<0.05$) in pomegranate peel powder against tested bacterial strains at a concentration of 35 µL. The hexane solvent proved ineffective against the microbial strain, while acetone was a poor solvent for extracting pomegranate peel extract with better antibacterial activity. The findings of this study highlight the potential of pomegranate peel waste as a valuable natural antimicrobial agent, promoting its utilization in food processing and packaging to enhance product safety and shelf life. This valorization supports sustainable waste management while opening avenues for eco-friendly, bioactive packaging solutions, contributing to a greener and safer food industry.

CONCLUSIONS

Pomegranate peel is rich in a wide range of bioactive compounds known for their antioxidant and antimicrobial properties. Key components include phenolic, ellagitoanines, flavonoids, tannins, alkaloids, organic acids, vitamins, minerals, polysaccharides and dietary fibers, making it a promising candidate for natural preservation and therapeutic applications. The present highlights its potential by evaluating different drying methods combined with various solvent extractions for their effectiveness against various pathogens. The results indicate that freeze, tray and sun drying, combined with solvent extracts using ethanol, water, methanol, and acetone, showed significant inhibition against targeted bacterial species. Among these, freeze-drying and methanolic extraction proved to be the most effective in inhibiting *E. coli*, *E. faecalis*, *B. bifidum* and *P. aeruginosa*. Conversely, hexane proved ineffective as an extraction solvent due to its lack of antimicrobial activity.

These results indicate that pomegranate peel extract holds strong potential as a natural antimicrobial agent, supporting its application in food preservation, nutraceutical formulations and functional product development.

By adopting appropriate industrial processing and standardization, pomegranate peel extract could be developed into a viable alternative to conventional antimicrobial drugs. Its natural bioactive properties make it a promising candidate for food preservation, helping to extend shelf life and reduce reliance on synthetic preservatives. These antimicrobial properties could be harnessed for active packaging, ensuring food safety and quality. Additionally, its potential applications in healthcare align with the growing demand for eco-friendly anti-microbial solutions, contributing to sustainable and safer therapeutic practices.

SUPPORTING INFORMATION

This article contains additional supporting material available online.

Table S1. Antibacterial activity of pomegranate peel extract against *P. aeruginosa*
Table S2. Antibacterial activity of pomegranate peel extract against *E. coli*
Table S3. Antibacterial activity of pomegranate peel extract against *B. bifidum*
Table S4. Antibacterial activity of pomegranate peel extract against *E. faecalis*

AUTHOR CONTRIBUTIONS

Conceptualization, N. and N. K.; Methodology, N. and N. K.; Investigation, formal analysis, validation, writing-original draft preparation, N., N. K., A. and P.; Writing-review and editing, N. K., A. and P.; Supervision, N.

DATA AVAILABILITY STATEMENT

Data is contained within the article.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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UTICAJ METODA SUŠENJA I EKSTRAKCIJE RASTVARAČIMA NA ANTIBAKTERIJSKA SVOJSTVA KORE NARA *(PUNICA GRANATUM* L.*)*

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Sažetak: Ova studija ima za cilj da ispita efekte tri metode sušenja (liofilizacija, sušenje na tacni, sušenje na suncu) i pet rastvarača (metanol, etanol, voda, aceton, heksan) na antibakterijska svojstva nusproizvoda (kore) ploda nara protiv četiri bakterijska soja (*Pseudomonas aeruginosa, Escherichia coli, Bifidobacterium bifidum* i *Enterococcus faecalis)*. Rezultati su pokazali da ovi ekstrakti imaju značajan antibakterijski efekat protiv testiranih bakterijskih vrsta. Sve frakcije ekstrakcije kore nara, koje su sadržale metanol, etanol, vodu i aceton, inhibirale su rast više Gram-pozitivnih i Gram-negativnih bakterija. Kombinacija procesa liofilizacije i metanola kao ekstrakcionog rastvarača pokazala je statistički značajno višu antimikrobnu aktivnost ($p<0,05$) praha kore nara protiv *P. aeruginosa* (24,16 mm), *E. coli* (24 mm) i *E. faecalis* (24 mm) pri koncentraciji od 35 µL. Heksan se pokazao nepogodnim kao ekstrakcioni rastvarač jer nije ispoljio antibakterijsku aktivnost, dok je aceton, iako je imao ograničenu efikasnost ekstrakcije kore nara, proizveo ekstrakt sa relativno boljom antibakterijskom aktivnošću. Ova studija doprinosi postizanju održivosti i cirkularne ekonomije kroz korišćenje otpada od kore nara kao prirodnog aktivnog agensa za dalju primenu u prehrambenoj industriji i industriji ambalaže, radi razvoja proizvoda sa dodatom vrednošću i jestive ambalaže za očuvanje hrane, kao i efikasne upotrebe kore nara kao prirodnog antibakterijskog agensa u farmaceutskoj industriji, konzervisanju hrane i biomedicinskim aplikacijama.

Ključne reči: *antimikrobna aktivnost, sušenje, kora nara, ekstrakcija rastvaračem, ultrasonikacija*

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