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ANTIBACTERIAL EFFECTS OF POMEGRANATE PEEL EXTRACT ON *Staphylococcus*, *Bacillus*, AND *Salmonella* SPP.

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Abstract: This study examined the physicochemical properties and antibacterial activity of fresh fruit peels of the local Zivzik pomegranate (*Punica granatum* L.) grown in the Şirvan district of Siirt. Total phenolic content, antioxidant capacity (CUPRAC and DPPH), pH, oxidation-reduction (O/R) potential, water activity (a_w), and soluble dry matter content were analyzed in extracts prepared from the fresh peels. Antibacterial activity was evaluated against *Staphylococcus aureus*, *Bacillus subtilis*, and *Salmonella Typhimurium* and compared with reference antibiotics. The total phenolic content of the peel extract was 6.827±0.495 mg GAE/mL, while antioxidant capacity measured 17.872±0.541 mg TE/mL (CUPRAC), and DPPH radical scavenging activity reached 1.332±0.003 mg AAE/mL. The extracts exhibited a pH of 3.657±0.031, an O/R potential of 201.200±0.721 mV, and an a_w of 0.952±0.001. Soluble dry matter contents were 17.833±0.208 °Brix for the ethanol extract and 11.400±0.541 °Brix for the methanol extract. Microbiological assessments showed that the ethanol extract produced the strongest inhibition against *Bacillus subtilis* (14.33 mm; ++), while *Salmonella Typhimurium* exhibited the lowest sensitivity (10.33 mm; +). A statistically significant interaction between solvent type and extract concentration was observed only for *B. subtilis* ($P<0.05$), indicating species-specific variability in response to extract composition. The extracts also generated inhibition levels greater than several standard antibiotics evaluated under identical conditions. Overall, the findings indicate that Zivzik pomegranate peel constitutes an effective natural antimicrobial source with practical applicability in food processing systems seeking to enhance microbial safety through non-synthetic preservation strategies.

Keywords: Antimicrobial activity, Food safety, Foodborne pathogens, *Punica granatum* L. cv. Zivzik, Fresh pomegranate peel

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1. Introduction

Pomegranate (*Punica granatum* L.) is a major fruit species belonging to the Lythraceae family, cultivated extensively across temperate climate zones. It is characterized by a flavor profile that varies from sweet to sour depending on the cultivar, and it holds significant importance as a long-established horticultural crop. Statistical records for 2024 show a 0.6% decline in the number of fruit-bearing pomegranate trees in Türkiye, accompanied by a 10.1% reduction in total production, which reached 703,425 tons. Within this national context, Siirt province, producing 9,559 tons annually, ranks 11th across the country and 4th within the Southeastern Anatolia Region. The Zivzik pomegranate, a cultivar native to Siirt, is recognized as one of the region's most commercially valuable local genotypes owing to its high fruit quality, consumer-preferred sensory attributes, and extended postharvest durability. These characteristics collectively contribute to its intensive cultivation and growing market prominence (Pakyürek et al., 2020; Dilmen et al., 2022; TSI, 2025).

Pomegranate (*Punica granatum* L.) is widely recognized as a fruit of considerable nutritional and pharmacological significance, often classified as both a "functional food" and a "medicinal plant." The fruit contains approximately 15% carbohydrates, 0.8% protein, and about 12% tannins, together with essential micronutrients including vitamins B1, B2, and C, as well as minerals such as calcium, phosphorus, potassium, and iron (Saxena et al., 1987). The pharmacological activity of pomegranate is primarily attributed to its repertoire of bioactive polyphenolic compounds, particularly ellagitannins such as punicalagin, which are known for their pronounced antioxidant capacity and broad-spectrum antimicrobial effects (Poyrazoğlu et al., 2002; Newman and Lansky, 2007; Bele et al., 2009). The antibacterial, antifungal, antiviral, and anthelmintic activities reported for pomegranate peel extracts have positioned this material as a promising candidate for alternative therapeutic applications (Bele et al., 2009; Foss et al., 2014). The antimicrobial mechanism is primarily associated with the high concentrations of phenolic compounds localized in the peel tissue, which act by disrupting the structural integrity of microbial cell walls and inhibiting key

enzymatic systems essential for microbial survival (Tağı et al., 2010).

Antimicrobial resistance is increasingly recognized as a major global public health challenge (WHO, 2024), intensifying the need for new antimicrobial agents of natural origin that could serve as alternatives to conventional synthetic compounds (Saxena et al., 1987). In this context, pomegranate peel, an abundant by-product of the fruit juice industry, has attracted considerable scientific interest, both because it is produced in large quantities as waste and because it contains a substantial pool of bioactive phenolic constituents (Fischer et al., 2011; Topkaya and Isik, 2019). A review of the existing literature shows that most studies have concentrated on the dried peels of different pomegranate cultivars or on fruit juice concentrates. However, research that comparatively examines the bioactive potential and antimicrobial profile of fresh peels from high-value local genotypes, such as the Zivzik pomegranate, is quite limited.

Although numerous studies have examined the antimicrobial potential of pomegranate peel, research specifically addressing the antibacterial activity of extracts derived from the fresh peels of locally adapted cultivars, particularly the Zivzik pomegranate, remains limited. The present study therefore aims to provide a quantitative evaluation, under controlled in vitro conditions, of the antibacterial activity of fresh Zivzik pomegranate peel extracts obtained using solvents of differing polarities, employing the disk diffusion assay against selected bacterial strains.

2. Materials and Methods

The plant material used in this study was the Zivzik pomegranate (*Punica granatum* L. cv. Zivzik), a cultivar extensively cultivated in orchard plantations located in the villages of Zivzik, Pirinçli, Sarıdana, and Kapılı within the Şirvan district of Siirt. Fruit samples were collected from a commercial orchard in Pirinçli village at full harvest maturity, using a random sampling strategy to obtain representative material.

Analytical procedures were conducted using reagents of certified analytical grade, including gallic acid and trolox standards and the Folin-Ciocalteu reagent. Given the stringent purity requirements of both the extraction protocol and subsequent quantification steps, HPLC-grade methanol and ethanol were selected as extraction solvents.

For the antibacterial activity assays, three bacterial strains representing major foodborne pathogens, the central focus of this study, were employed. The Gram-positive strains consisted of *Bacillus subtilis* ATCC 6633 and *Staphylococcus aureus* ATCC 29213, while the Gram-negative strain was *Salmonella Typhimurium* ATCC 14028. For calibration of antimicrobial response and to ensure assay validity, reference antibiotic discs (Oxoid) were incorporated as positive controls under identical test conditions. The antimicrobial panel comprised erythromycin (15 µg), amoxicillin/clavulanic acid (30 µg), penicillin (10 µg), cephalexin (30 µg), and streptomycin (10 µg), each applied at CLSI-recommended potencies to permit standardized comparison of inhibition responses.

2.1. Phytochemical Analysis

2.1.1. Extraction procedure

For the extraction procedure conducted prior to the phytochemical analyses, 10 g of mechanically fragmented fresh pomegranate peel was accurately weighed using an analytical balance, and the volume was brought to 100 mL by adding 70% ethanol (Sigma-Aldrich, USA). The mixture was shaken until homogenized and then left to macerate at room temperature for 24-48 hours. Upon completion of the extraction period, the mixture was filtered through Whatman No. 1 filter paper (China) to remove coarse particulate matter. The resulting filtrate was subsequently centrifuged at 12,000 rpm for 35 minutes in a refrigerated centrifuge set to +4°C (Thermo Scientific/Megafuge 16R, USA). The clarified supernatant obtained after centrifugation was collected and reserved for use in the phytochemical analyses.

2.1.2. Determination of total phenolic content and antioxidant capacity

The total phenolic content (TPC) was determined using the Folin-Ciocalteu colorimetric method described by Singleton and Rossi (1965), and the results were expressed as gallic acid equivalents (mg GAE/100 g). For the determination of total antioxidant capacity (TAC), the FRAP (Ferric Reducing Antioxidant Power) assay developed by Benzie and Strain (1996) was employed. The measurements obtained were calculated as trolox equivalents (µmol TE/100 g) using a standard calibration curve. Absorbance readings for both analyses were performed using a UV–Vis spectrophotometer (Shimadzu, Kyoto, Japan).

2.2. Physicochemical Analyses

Fresh pomegranate peels were separated from the fruit tissue using a sterile knife, mechanically fragmented, and homogenized. The pH and oxidation–reduction potential (ORP) of the prepared homogenates were measured using a calibrated pH meter/ionometer (Mettler Toledo SevenCompact™ S220, China). The soluble solids content of the samples was determined with a digital refractometer (Hanna HI96801, Romania) and expressed in °Brix (Cemeroğlu, 2013). Water activity (a_w) was measured using a water activity meter (Novasina LabTouch, Lachen, Switzerland) based on the procedure recommended by Welti-Chanes et al. (2007).

2.3. Preparation of Antibacterial Extracts

Pomegranate peels were mechanically cut into small pieces and subjected to maceration for 24 hours in 70% ethanol and methanol at a solid-to-solvent ratio of 10% (w/v). At the end of the maceration period, the mixtures were filtered through Whatman No. 1 filter paper to remove particulate matter. The organic solvents present in the filtrates were then evaporated under controlled conditions in an oven set to 50 °C. The resulting solvent-

free crude extracts were diluted with sterile distilled water to obtain four concentrations, consisting of an undiluted stock and serial dilutions at 1/2, 1/4, and 1/8. All extracts were stored at +4°C until the antibacterial assays were performed.

2.4. Antibacterial Susceptibility Tests

For the evaluation of the antibacterial effects of the pomegranate peel extracts, the well diffusion method was employed, whereas susceptibility testing of the reference antibiotics was conducted using the disk diffusion method as described by Temiz (2010). The bacterial strains used in the study (*Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 29213, and *Salmonella Typhimurium* ATCC 14028) were first activated by incubation on Tryptic Soy Agar (TSA, Merck, Germany) at 37 °C for 18–24 hours. The density of the resulting bacterial suspensions was then adjusted to the 0.5 McFarland turbidity standard (1.5×10^8CFU mL^{-1}) using a densitometer (Biosan DEN-1, Latvia) in sterile physiological saline.

Sterile Petri plates containing Mueller Hinton Agar (MHA) were inoculated with the standardized bacterial suspensions, which were evenly spread across the agar surface using a Drigalski spatula. The plates were then left at room temperature for 10-15 minutes to allow absorption of the inoculum into the medium. After absorption, the reference antibiotic discs, erythromycin (15 µg), streptomycin (10 µg), penicillin (10 µg), amoxicillin/clavulanic acid (30 µg), and cephalexin (30 µg), were placed onto the agar surface under aseptic conditions with a minimum distance of 20 mm between them. For the analysis of the extracts, wells with a diameter of 5 mm were created in the agar using a sterile cork borer, and 30 µL of each extract concentration was dispensed into the corresponding wells. The plates were held at room temperature for 15-20 minutes to facilitate the diffusion of the extracts into the agar and were subsequently incubated under aerobic conditions at 37 °C for 18-24 hours. At the end of the incubation period, the inhibition zone diameters surrounding both the antibiotic discs and the extract-filled wells were measured in millimeters using a digital caliper. The results were evaluated according to the classification criteria proposed by Ponce et al. (2003). Based on the inhibition zone diameters obtained in the assays, the criteria used to classify the antimicrobial activity levels of the extracts are presented in Table 1.

2.5. Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) values of the extracts were determined using the liquid dilution (tube dilution) technique based on the method described by Temiz (2010). The analysis was conducted in two stages. In the first phase of the procedure, two-fold serial dilutions of the pomegranate peel extracts were prepared in sterile liquid growth medium. Each dilution tube was inoculated with approximately 10 µL of an active bacterial suspension adjusted to the 0.5 McFarland turbidity standard, after which the contents were mixed thoroughly using a vortex mixer. All tubes were then incubated at 37 °C for 18-24 hours.

Following incubation, the second phase involved verifying bacterial viability in order to determine the MIC. Subculturing was performed from the tubes that exhibited no visible turbidity, an indication of potential inhibition, onto selective solid media appropriate for each test organism. For this purpose, Bacillus cereus Selective (BCS) Agar (Oxoid, UK) was used for Bacillus subtilis, Baird-Parker Agar (Merck, Germany) for Staphylococcus aureus, and Salmonella-Shigella (SS) Agar (Lab M, UK) for Salmonella Typhimurium. The inoculated plates were incubated at 37 °C for 18-24 hours and examined for the presence or absence of viable colonies. The MIC was defined as the lowest extract concentration for which no macroscopic colony formation was observed on the selective medium during this subculture step.

2.6. Statistical Analysis

All experiments were conducted in triplicate. In the antibacterial activity assays, inhibition zone diameters were measured along a single axis for symmetrical zones, whereas for irregularly shaped zones, measurements were taken along three different axes, and the arithmetic means were recorded. Statistical evaluation of the data was performed using the SPSS 22.0 software package (IBM Corp., Armonk, NY, USA). Differences among groups were analyzed using One-Way Analysis of Variance, and the significance of differences between means was determined using Duncan's Multiple Range Test. A significance level of $P<0.05$ was adopted, and all results were reported as Mean ± Standard Deviation.

3. Results and Discussion

3.1. Phytochemical Profile of Pomegranate Peel: Phenolic Compound Content and Antioxidant Activity

Analyses performed to determine the bioactive potential of the fresh peels of the Zivzik pomegranate (*Punica granatum* L. cv. Zivzik) revealed quantitative values for total phenolic content and antioxidant capacity (FRAP). These results are presented in Table 2.

Table 1. Antimicrobial activity classification according to inhibition zone diameter (Ponce et al., 2003)

Inhibition Zone Diameter (mm)	Antimicrobial Impact	Symbol
Diameter < 8.00	Ineffective	-
9.00 < Diameter < 14.00	Slightly Effective	+
15.00 < Diameter < 19.00	Effective	++
Diameter >20.00	Highly Effective	+++

Table 2. Total phenolic content and antioxidant capacity of fresh Zivzik pomegranate peel extracts

Parameter	Mean ± SD
Total phenolic content (mg GAE mL^{-1} ± SD)	6.827±0.495
CUPRAC antioxidant capacity (mg TE mL^{-1} ± SD)	17.872±0.541
DPPH radical scavenging activity (mg AAE mL^{-1} ± SD)	1.332±0.003

The fresh peels of the Zivzik pomegranate exhibited a rich phytochemical profile characterized by high phenolic compound content and substantial antioxidant capacity, both of which are strongly associated with enhanced biological activity parameters (Table 2). This pronounced bioactive potential supports the utilization of pomegranate peel, an agro-industrial by-product, as a natural and functional additive with applicability in the food, feed, cosmetic, and pharmaceutical industries, offering an alternative to synthetic agents.

A comparative assessment of the present results within the context of previous research shows a clear alignment with established findings. Kanatt et al. (2010) reported that pomegranate peel extracts display markedly stronger antioxidant and antimicrobial capacities than seed-derived extracts and even outperform the synthetic antioxidant BHT (butylated hydroxytoluene). Consistent with this observation, Aliyari et al. (2020) and Habib et al. (2018) documented a concentration-dependent enhancement in antioxidant activity across various food systems. Nevertheless, the relationship between total phenolic content and biological activity is not universally linear. For example, Orak et al. (2011) found that the Hicaznar cultivar, despite exhibiting the highest phenolic concentration, demonstrated the lowest antibacterial performance. Such findings collectively indicate that antimicrobial performance is governed not only by the overall quantity of phenolic compounds but also by their qualitative composition and the interactions they form with other constituents of the plant matrix, particularly organic acids. In line with this interpretation, De Oliveira et al. (2010) demonstrated that phenolic compounds alone exhibit limited inhibitory activity against Staphylococcus aureus, whereas their combination with organic acids such as lactic or acetic acid produces a substantially stronger antimicrobial effect.

The lower values obtained in the present study compared with those reported by Nuamsetti et al. (2012) using hot-water extraction may be attributed to several methodological and material-dependent factors. The variation observed is likely influenced by the extraction protocol employed (70% ethanol at room temperature), genotypic differences (cv. Zivzik), and the physical form of the plant material (fresh peel in coarse fragments), as well as the particle size used during extraction. It is also well established that the agro-ecological conditions under which the plant is grown affect its secondary metabolite profile. Accordingly, extraction efficiency and the composition of bioactive constituents can vary substantially depending on the combined effects of these parameters.

The preservative potential of pomegranate peel extracts has been demonstrated not only through in vitro assays but also in various food systems. Naveena et al. (2008) reported that the incorporation of pomegranate peel extract into cooked chicken patties was more effective in inhibiting lipid oxidation, based on TBARS values, than both pomegranate juice and the commercial synthetic antioxidant BHT (butylated hydroxytoluene). In addition to this antioxidant effect, Aliyari et al. (2020) found that the inclusion of the extract in sausage formulations resulted in a statistically significant ($P<0.05$) reduction in total viable bacterial load. The collective evidence from these studies demonstrates that pomegranate peel has the capacity to function as a natural preservative with "dual-acting" properties, effectively inhibiting both oxidative deterioration and microbial growth in food matrices, particularly in meat products.

3.2. Physicochemical Characterization

Physicochemical analyses were conducted to determine the characteristic properties of the fresh peels of the Zivzik pomegranate as well as the extracts obtained from this material. The results of these analyses are summarized in Table 3.

Table 3. Selected physicochemical properties of fresh Zivzik pomegranate peel and its extracts

Parameter	Mean ± SD
Properties of fresh pomegranate peel	
pH	3.657±0.031
Oxidation/Reduction potential (mV)	201.200±0.721
Water activity (a_w)	0.952±0.001
Soluble solids content of extracts (°Brix)	
Ethanol extract	17.833±0.208
Methanol extract	11.400±0.436

3.2.1. pH value and oxidation-reduction (O/R) potential

The pH value, a limiting factor in microbial growth kinetics, was measured as 3.657±0.031 in the fresh pomegranate peel samples. This result indicates that the peel exhibits a distinctly acidic character. Considering that most pathogenic microorganisms require a pH of 4.5 or higher for growth (Temiz, 2015), and that species such as *S. aureus*, *E. coli*, and *B. cereus* have minimum pH thresholds of approximately 4.0, 4.3, and 4.9, respectively (Karapınar and Aktuğ, 2015), the low pH value identified in the present study can be regarded as an intrinsic barrier that suppresses bacterial proliferation. This intrinsic acidity is therefore considered to contribute directly to the antimicrobial potential of the material.

The pH value identified in this study is consistent with the ranges reported in the literature. Kennas et al. (2020) documented a pH of 3.82 for pomegranate peel powder,

while Jalal et al. (2018) reported a value of 4.83, both confirming the acidic nature of the material. Similarly, Hallaç and Kılınççeker (2024) and Hallaç et al. (2022) reported pH values between 3.68 and 3.95 and noted that the decrease in pH observed with increasing extract concentration enhanced the antimicrobial potential through synergistic effects. From an industrial perspective, the findings of Naveena et al. (2008) demonstrated that incorporating pomegranate peel extract into food matrices does not induce a substantial shift in product pH, which represents a technological advantage by helping maintain the sensory and physicochemical stability of the final product.

The oxidation-reduction (O/R) potential of the samples was measured as 201.200±0.721 mV. This positive and relatively high value indicates that the medium exhibits an oxidative character. Such elevated redox potential is considered to exert an inhibitory influence by imposing oxidative stress on the metabolic processes and homeostasis of microorganisms, thereby contributing to enhanced microbial stability.

3.2.2. Water activity (a_w)

Water activity (a_w), a fundamental parameter reflecting the availability of free water in a food matrix for microbial metabolism and chemical reactions, is thermodynamically defined as the ratio of the vapor pressure of a food sample (P) to that of pure water (P_0) at the same temperature (P/P_0). Fresh plant materials generally exhibit a_v values above 0.99, a range in which microbial proliferation and enzymatic or chemical degradation accelerate, whereas decreasing a_v enhances shelf-life stability. With respect to critical thresholds, bacterial growth is completely arrested below a_w<0.60, yeast proliferation is substantially limited, and only certain xerophilic molds retain the capacity for restricted growth under such conditions. Consequently, food matrices dehydrated to a_v levels below 0.60 maintain long-term microbiological stability when stored under suitable environmental conditions (Temiz, 2015).

The mean a_w of the fresh Zivzik pomegranate peel samples analyzed in this study was determined as 0.952±0.001 (Table 2). This high value indicates that biological material possesses a matrix highly susceptible to microbial and enzymatic degradation. Accordingly, storing fresh peels without a stabilization process such as drying is expected to result in rapid deterioration, underscoring the necessity of performing extraction promptly after harvest. In addition, this finding is important in demonstrating that the antimicrobial activity assessed in the present study is based on metabolites extracted from fresh plant tissue, which retains its natural form and high water activity, rather than from dried powder.

3.2.3. Soluble solids content (°Brix) and solvent efficiency

The soluble solids content (°Brix) of the extracts was evaluated as an indicator of the efficiency of each solvent in isolating soluble bioactive constituents from the pomegranate peel matrix. Examination of the analytical results revealed that the °Brix value of the ethanol extract (17.833±0.208 °Briks) was significantly higher than that of the methanol extract (11.400±0.436 °Briks) (P<0.05). This finding demonstrates that 70% ethanol is a more effective solvent than methanol for isolating soluble solids, including phenolic compounds, from the fresh peel of the Zivzik pomegranate. The relatively high concentration of soluble material detected in the ethanol extract, reflecting greater mass transfer, is considered to increase the density of bioactive constituents per unit volume. This, in turn, is regarded as one of the primary factors contributing to the stronger antibacterial activity exhibited by the ethanol-based extract.

These results are consistent with the findings reported by Naveena et al. (2008), who observed that the increase in dry matter content associated with higher extract concentrations led to statistically significant (P<0.05) changes in the proximate composition of the product, including crude protein, ether extract, ash, and moisture. In this context, both studies support the view that dry matter content serves as a critical indicator for characterizing the density of functional constituents in a product and, consequently, its potential biological activity.

3.3. Antibacterial Susceptibility of Pomegranate Peel and Standard Antibiotics

3.3.1. Antimicrobial activity of reference antibiotics

The inhibition zone diameters generated by the standard antibiotics employed as positive controls are summarized in Table 4, and their comparative activity patterns are shown in Figure 1. Statistical analysis using one-way ANOVA demonstrated a pronounced variation in the inhibitory performance of the antibiotics across the tested bacterial strains, with the differences found to be highly significant P<0.05.

Table 4. Susceptibility levels of reference antibiotics against the test bacteria (Inhibition zone diameter, mm)

Antibiotics	*S. aureus*	*B. subtilis*	*S. Typhimurium*
E15	31.333±0.657^{a}	29.333±0.540^{a}	31.667±0.434^{a}
AMC30	0^{b}	8.333±0.425^{a}	8.333±0.384^{a}
P10	0^{b}	0^{b}	7.333±0.364^{a}
CL30	12.333±0.480^{b}	13.667±0.651^{a}	11.333±0.450^{c}
S10	20.667±0.360^{b}	21.667±0.847^{a}	15.667±0.297^{c}

$^{a-c:}$ Means followed by different letters within the same row differ significantly according to the multiple comparison test (P<0.05).

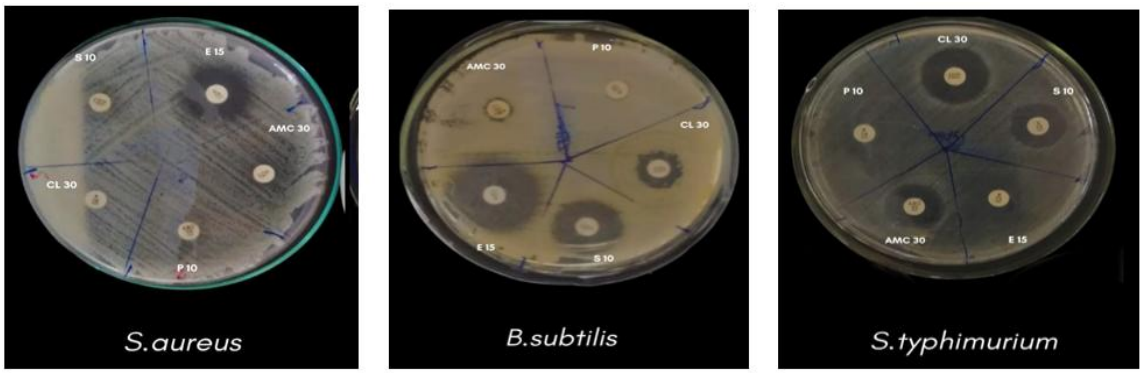

Figure 1. Antibacterial inhibition zones formed by reference antibiotics on *Staphylococcus aureus*, *Bacillus subtilis*, and *Salmonella Typhimurium* under standardized disk diffusion conditions.

Table 5. Antimicrobial activity of different solvent types and extract concentrations against the test bacteria (Inhibition zone diameter, mm)

Solvents	Dilution Ratio	Bacteria		
		S. aureus	*B. subtilis*	*S. Typhimurium*
Ethanol	1	11.667	15.333 [a]	10.333
	0.5	10.667	13.000 [b]	9.333
	0.25	9.333	8.667 [d]	8.667
	0.125	8.000	8.333 [d]	7.667
Methanol	1	12.333	13.333 [b]	10.333
	0.5	9.667	11.333 [c]	9.667
	0.25	9.000	10.333 [c]	8.667
	0.125	7.333	8.333 [d]	8.333

Mean values denoted by different letters within the same column differ significantly (P<0.05).

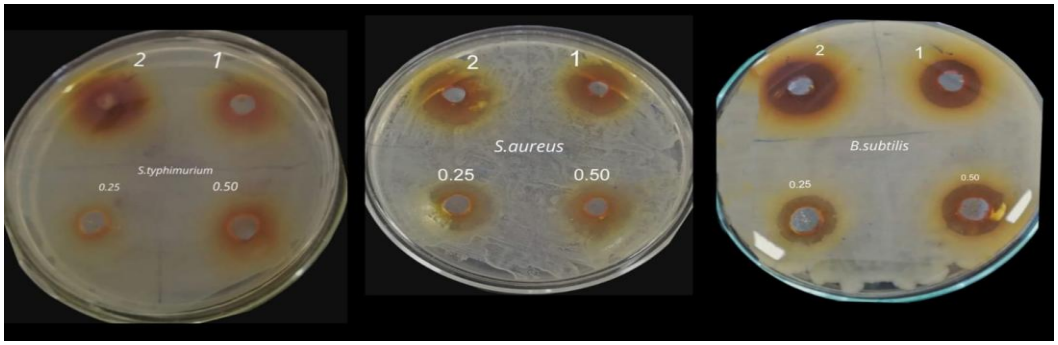

Figure 2. Inhibition zones produced by fresh Zivzik pomegranate peel extracts of varying concentrations and solvents on *Salmonella Typhimurium*, *Staphylococcus aureus*, and *Bacillus subtilis*.

When the inhibition zone diameters were interpreted according to the reference criteria listed in Table 1, erythromycin (E15) demonstrated a high level of antibacterial effectiveness (+++) against all tested bacterial strains. Streptomycin (S10) and cephalexin (CL30) generally fell within the effective (++) category, whereas the activity of amoxicillin/clavulanic acid (AMC30) and penicillin (P10) remained either limited (+) or ineffective (–), depending on the strain, indicating reduced susceptibility or resistance.

3.3.2. Antimicrobial activity of pomegranate peel extracts

The antibacterial activity potential of the extracts prepared from fresh Zivzik pomegranate peel is presented graphically in Figure 2. Detailed quantitative data describing the effects of solvent type and extract concentration (dilution ratio) on the inhibition of the target bacteria are provided in Table 5.

Statistical analyses demonstrated that, particularly for the *Bacillus subtilis* strain, both the solvent type and the extract concentration exerted a highly significant influence on antimicrobial activity (P<0.05). Examination of the overall activity pattern showed that ethanol-based extracts exhibited a statistically superior antimicrobial performance compared with methanol extracts. In addition, inhibition zone diameters increased in parallel with extract concentration, indicating a positive dose-dependent relationship consistent with previous reports in the literature. When bacterial susceptibility patterns were evaluated individually, distinct variations were

observed among the strains. *Staphylococcus aureus* showed the highest level of resistance (smallest inhibition zones) to the ethanol extract, whereas *Salmonella Typhimurium* displayed the greatest resistance to the methanol extract.

The findings obtained in this study are largely consistent with the existing body of literature documenting the antimicrobial potential of pomegranate peel. Akarca and Başpınar (2019) reported that pomegranate peel extracts produced a substantial inhibition zone of 20.22 mm against *Staphylococcus aureus*, indicating a strong antibacterial effect. Similarly, Al-Zoreky (2009) demonstrated that extracts prepared using a methanol-water mixture were capable of inhibiting even methicillin-resistant *S. aureus* (MRSA) isolates, which are of considerable clinical relevance. Furthermore, the observation that the highest antibacterial activity in the present study was achieved with the ethanol extract, particularly against *Bacillus subtilis*, agrees with the findings of Nuamsetti et al. (2012), who identified ethanol as the most efficient solvent for extracting bioactive constituents and maximizing antibacterial activity.

The antimicrobial spectrum of pomegranate peel extracts varies depending on the target microorganism. Previous studies have shown that the extracts exhibit strong inhibitory activity against Gram-positive bacteria such as *Staphylococcus aureus* and *Bacillus cereus*, whereas their effectiveness against Gram-negative bacteria, including *Escherichia coli* and *Salmonella Typhimurium*, tends to be comparatively lower. Despite this variation, the potential applicability of pomegranate peel as a natural preservative in food systems remains substantial. Kanatt et al. (2010) reported that incorporating pomegranate peel at levels of 0.1–0.5% into chicken meat extended the product's shelf life by 2-3 weeks. Likewise, Hayrapetyan et al. (2012) found that the application of the extract significantly suppressed the growth of *Listeria monocytogenes* within a food matrix.

The antimicrobial activity exhibited by pomegranate peel is largely attributed to the presence of bioactive phenolic constituents within the plant matrix, particularly high–molecular weight tannins such as punicalagin (Topkaya and Isik, 2019). The variation observed in the effectiveness of these compounds against Gram-positive and Gram-negative bacteria is thought to arise from structural differences between these groups, including cell wall morphology and membrane permeability, especially the lipopolysaccharide outer membrane that provides an additional barrier in Gram-negative bacteria (Orak et al., 2011). In addition, the literature reports antifungal properties (Foss et al., 2014) and wound-healing activity (Topkaya and Isik, 2019) associated with pomegranate peel extracts, further supporting the therapeutic potential of this material. Findings reported by Ahmad and Beg (2001), Machado et al. (2003), Braga et al. (2005), Shan et al. (2007), and more recent studies by Hallaç et al. (2022) and Hallaç and Kılınççeker (2024) collectively confirm the inhibitory capacity of pomegranate peel against a broad spectrum of pathogenic microorganisms. An assessment of the effects of different solvent types and extract concentrations on bacterial inhibition revealed that the analysis of variance demonstrated a strongly significant difference, particularly for the *Bacillus subtilis* strain ($P<0.05$) (Table 5). For this bacterium, ethanol-based extracts exhibited markedly higher antimicrobial activity compared with methanol extracts. In addition, a progressive increase in inhibition zone diameters was observed as extract concentration increased within both solvent groups, confirming that the antibacterial effect followed a clear dose-dependent pattern. The findings obtained in this study are consistent with the results reported by Nuamsetti et al. (2012). The authors demonstrated that both the target bacterial species and the type of solvent used play a statistically significant role ($P<0.05$) in determining the antibacterial performance of pomegranate extracts, and that the highest level of bioactivity was observed in the ethanol fractions. Evaluation of the extract activity levels showed that bacterial susceptibility exhibited species-specific variation. *Staphylococcus aureus* displayed the highest resistance to the ethanol extract, followed by *Bacillus subtilis* and *Salmonella Typhimurium*. Under methanol extract treatments, the lowest inhibition (highest resistance) was recorded for *S. Typhimurium* (Table 5).

3.4. Minimum Inhibitory Concentration (MIC) and Physicochemical Parameters

3.4.1. Evaluation of MIC values

The minimum inhibitory concentration (MIC) results were consistent with the findings obtained from the disk diffusion assay. The ethanol extract exhibited lower MIC values, reflecting higher biological activity, against all tested bacterial strains compared with the methanol extract (Table 6). When evaluated on a strain-specific basis, the strongest inhibitory effect of the ethanol extract (lowest MIC) was recorded for *Staphylococcus aureus* at a concentration of 11.144 mg/mL (1/16 dilution). This was followed by *Bacillus subtilis* at 22.288 mg/mL (1/8) and *Salmonella Typhimurium* at 44.575 mg/mL (1/4). In contrast, MIC values for the methanol extract were markedly higher. For *S. aureus*, MIC values ranged between 14.25 and 28.50 mg/mL (1/4–1/8); for *B. subtilis*, the value was 28.50 mg/mL (1/4); and for *S. Typhimurium*, the MIC reached 57.00 mg/mL (1/2) (Table 5). Comparative analysis of the MIC values obtained in the present study with those reported in the literature reveals the presence of considerable variation. For *Staphylococcus aureus*, the MIC value determined in this study (11.144 mg/mL) was lower, indicating greater inhibitory effectiveness, than the values reported by Prashanth et al. (2001), Novitri and Kurniati (2021), and Nuamsetti et al. (2012), but higher than those documented by Demir et al. (2019) and Peršurić et al. (2020). A similar pattern was observed for *Bacillus subtilis*.

Table 6. Minimum inhibitory concentration (MIC) values (mg/mL) of pomegranate peel extracts against the tested bacterial strains

Solvents	Bacteria	Dilution Ratio							
		1	1/2	1/4	1/8	1/16	1/32	1/64	1/128
Ethanol	*S. aureus*	-	-	-	-	+	+	+	+
	B. subtilis	-	-	-	+	+	+	+	+
	S. Typhimurium	-	-	+	+	+	+	+	+
Methanol	*S. aureus*	-	-	+	+	+	+	+	+
	B. subtilis	-	-	+	+	+	+	+	+
	S. Typhimurium	-	+	+	+	+	+	+	+

(-)= no bacterial growth observed (Inhibition), (+)= bacterial growth observed (growth present). The MIC (Minimum Inhibitory Concentration) value corresponds to the dilution step immediately preceding the lowest concentration at which macroscopic growth is detected (i.e., the lowest concentration at which inhibition is achieved). In the table, MIC values are indicated in bold type.

Table 7. Dry matter content, pH, and oxidation-reduction potential (O/R) (mV) values of the solvents and extract dilutions

Solvents	Dilution Ratio	Properties		
		Dry Matter (°Brix)	pH	O/R
Ethanol	1	18.633 ^{a}	4.247 ^{a}	168.900 ^{h}
	0.5	11.867 ^{b}	3.830 ^{d}	191.933 ^{e}
	0.25	5.567 ^{d}	3.683 ^{h}	199.000 ^{a}
	0.125	2.667 ^{e}	3.693 ^{g}	198.200 ^{b}
Methanol	1	6.867 ^{c}	4.230 ^{b}	170.933 ^{g}
	0.5	6.700 ^{c}	3.870 ^{c}	189.333 ^{f}
	0.25	3.200 ^{e}	3.740 ^{f}	196.900 ^{c}
	0.125	1.600 ^{f}	3.760 ^{e}	195.400 ^{d}

Mean values denoted by different letters within the same column differ significantly ($P<0.05$).

The MIC value identified in this study (22.288 mg/mL) was higher than those reported by Prashanth et al. (2001), Demir et al. (2019), and Peršurić et al. (2020), yet lower than the values documented by Nuamsetti et al. (2012) and Dey et al. (2012). In the case of *Salmonella Typhimurium*, the MIC value recorded in this study (44.575 mg/mL) was substantially lower than the 62.5 mg/mL value reported by Akarca and Başpınar (2019) for water-based extracts. The variability observed across studies is likely attributable to the combined influence of methodological factors, including differences in extraction protocols, solvent polarity, plant genotype, intrinsic susceptibility of the bacterial strain, and inoculum density. The observation reported by Emam-Djomeh et al. (2015), that Gram-positive bacteria exhibit higher susceptibility to pomegranate peel extracts compared with Gram-negative bacteria, is fully aligned with the low MIC values determined in the present study for *Staphylococcus aureus* and *Bacillus subtilis*, both of which belong to the Gram-positive group.

3.4.2. Effect of solvent type and dilution ratio on physicochemical parameters

The effects of solvent type and extract concentration on dry matter content, pH, and oxidation-reduction (O/R) potential were found to be highly significant $P<0.05$ (Table 7).

A clear and systematic shift in the physicochemical properties of the extracts was observed as concentration increased. Higher extract concentrations were consistently associated with proportional increases in soluble dry matter (°Brix) and pH, whereas the oxidation-reduction (O/R) potential declined in an inversely related manner. Comparisons made at equivalent dilution levels showed that the ethanol extracts exhibited significantly higher soluble dry matter and pH values than the methanol extracts. The elevated dry matter content in the ethanol fractions reflects a greater capacity of this solvent to recover soluble constituents from the plant matrix, including bioactive phenolic compounds, indicating superior extraction efficiency. This enhanced extraction performance is aligned with the stronger antibacterial activity observed for the ethanol extracts, suggesting a positive relationship between extraction yield and biological efficacy.

4. Conclusion

This study provides a comprehensive assessment of the phytochemical composition and antibacterial activity of fresh peel extracts from the Zivzik pomegranate (*Punica granatum* L.). The peel exhibited a rich bioactive profile, with high total phenolic content (6.827±0.495 mg GAE mL^{-1}) and substantial antioxidant capacity (17.872±0.541 mg TE mL^{-1}). Ethanolic extracts showed superior extraction efficiency, lower MIC values, and wider inhibition zones than methanolic extracts, demonstrating ethanol's suitability for isolating functional constituents. The acidic nature of the peel

contributed to antimicrobial activity, while the high water activity (0.952±0.001) indicates the necessity of immediate processing of the fresh material.

Antibacterial tests confirmed a concentration-dependent response, with *S. aureus* and *B. subtilis* displaying greater sensitivity than *S. Typhimurium*. These results demonstrate that Zivzik pomegranate peel, currently treated as an agro-industrial residue, represents a promising natural antimicrobial resource with potential use in food preservation and waste-valorization strategies.

Further research is needed to clarify the extract's mode of action through molecular and structural analyses, to expand microbial spectrum testing to additional pathogens and spoilage organisms, and to evaluate performance within real food matrices through shelf-life studies. Safety assessments using *in vitro* and, when required, in vivo models are essential prior to industrial application. Investigation of green extraction technologies and conversion of extracts into stable powder formulations may enhance process efficiency and facilitate commercial use. Collectively, these efforts will support the safe and functional integration of Zivzik pomegranate peel into food systems as a value-added ingredient.

Author Contributions

The percentages of the authors' contributions are presented below. All authors reviewed and approved the final version of the manuscript.

	M.P.	B.H.
C	50	50
D	50	50
S	50	50
DCP	50	50
DAI	50	50
L	50	50
W	60	40
CR	50	50
SR	60	40
PM	60	40
FA	60	40

C= concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management, FA= funding acquisition.

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

Ethics committee approval was not required for this study because of there was no study on animals or humans.

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