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DETERMINATION OF ANTIBACTERIAL EFFECTS OF PEEL POWDERS OBTAINED FROM ZIVZIK POMEGRANATE GROWN IN SOUTHEAST TÜRKİYE AGAINST SOME PATHOGENIC BACTERIA

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ABSTRACT

In this study, the antibacterial effects of peels obtained from Zivzik pomegranate against Staphylococcus aureus, Escherichia coli, Bacillus cereus, Enterococcus faecalis, and Pseudomonas aeruginosa were investigated. The pomegranate peel was ground into powder form and their control (0%), 2%, 4% and 8% solutions were prepared, and firstly pH, Oxidation/Reduction (O/R) and electrical conductivity (EC) values were measured. Then, these bacteria were inoculated with selected standard antibiotics according to the disc diffusion method and with pomegranate peel powder according to the well/hole method and the zone diameters formed because of incubation were determined. In addition, the counts of bacteria formed after incubation was determined by inoculating these bacteria and each peel solution on special media. In analyses, the pH value of the 0% concentration solution was determined as 5.96, O/R value 83.17 mV and EC value 0.13 µS cm-1. However, the pH decreased to 3.7 in the 8% concentration solution, O/R and EC also increased to195.67 mV and 0.42 µS cm-1, respectively. These changes increased the antibacterial effect of solutions prepared from pomegranate peel powder. Pomegranate peel solutions showed similar effects to most of the standard antibiotics used. Also, by influencing the numbers of bacteria used, especially the 8% solution provided about 1.5-2 log reduction compared to the control. Consequently, while it was determined that 4% and 8% solutions of the powders obtained from this pomegranate peel could be used against these bacteria, it was understood that it would be beneficial for the food industry to conduct research against different microorganisms.

1.Introduction

Pomegranate is one of the fruits whose planting has become widespread in Türkiye recently due to agricultural support. Botanically, it is in the *Punicaceae* family, and both the plant and its fruit exhibit different characteristics from many fruits physically and chemically (Akarca and Başpınar, 2019; Akhtar *et al*., 2019).

This fruit, which is native to a wide region stretching from North India to Iran, is being cultivated in Türkiye, especially in the Aegean, Mediterranean and South-eastern Anatolia regions. The fact that it is not very selective in terms of soil properties, and that it is rich in tannins and many phenolic compounds with functional properties supports this increase in cultivation (Kurt and Şahin, 2013; Akhtar *et al*., 2019).

The parts of the pomegranate fruit such as the seed and peel can be shown as a source of different anthocyanins, hydroxycinnamic acids, hydroxy benzo acids, minerals, essential lipids,

complex carbohydrates, and tannins with hydrolysis ability. Most of these substances are compounds with antimicrobial or antioxidant properties (Tunç et al., 2013; Demir et al., 2019).

However, while the use of antioxidant and antimicrobial substances in the food sector is common, the cost of the use of synthetic materials, their allergic effects and carcinogenicity have led researchers to natural resources. In addition, the increase in the resistance of pathogenic microorganisms to drugs due to randomly used antibiotics can be counted among the reasons for this trend (Golge *et al*., 2018; Kilinççeker and Kurt, 2018; Akarca and Başpınar, 2019; Saeed *et al*., 2019).

As mentioned before, the functional compounds in the pomegranate have caused this fruit to be the subject of different scientific studies. In many studies, it has been revealed that pomegranate fruit or pomegranate peel can be used as antioxidant and antimicrobial material. Especially, while it is emphasized that the anthocyanin, ellagic acid, gallic acid, punicalagin and many other polyphenols in the pomegranate peel may have an antimicrobial effect, it has been stated that studies on this subject are insufficient and different studies are needed (Tunç *et al*., 2013; Morsy *et al*., 2018; Akarca and Başpınar, 2019; Karagecili *et al*., 2023).

Depending on what has been mentioned, the peel of the Zivzik pomegranate, which is native to Türkiye's Southeast Anatolian region (Siirt province), was studied in this study. Fruit inner grains of Zivzik pomegranate are larger and redder than other pomegranate varieties. Its acid rate is low and it can last for a long time without spoiling (Cetinkaya *et al*., 2013; Hallaç *et al*., 2022; Karagecili *et al*., 2023).

Although this pomegranate variety is a registered fruit, there is not much scientific study about the fruit parts. Therefore, after the peels obtained from this pomegranate were dried and pulverized, some biochemical properties of their solutions are prepared with water at different concentrations and their antibacterial effects against five different pathogens that are important in the food industry were investigated.

2.Materials and methods

The pomegranate peels used in the study were obtained from Zivzik pomegranates grown in Siirt province (Türkiye). The pomegranate peels were first washed with clean water and then dried with a clean cotton cloth. Then, the peels were dried in an oven at 70 °C for 1-2 days and ground. After this process, pomegranate powder solutions were prepared at the rates of 0% (control), 2%, 4%, and 8% with distilled water. While some physicochemical measurements mentioned below were made in these solutions, the antibacterial effects of some standard antibiotics and powder solutions were also determined.

The pH and O/R values of the solutions were measured using a pH-meter (Cemeroglu, 2013). Determination of electrical conductivity (EC) value Hanna HI2002 edge®, Romania brand device was used. The measurements were carried out by modifying the method used by Acir et al. (2019). The reading was made by dipping the probe into the samples diluted with distilled water, and the value found was determined as µS cm^{-1}.

2.1. Susceptibility of standard bacterial strains to standard antibiotics

The bacterial strains tested (*S. aureus* ATCC 29213, *E. coli* ATCC 25922, *B. cereus* ATCC 10876, *E. faecalis* ATCC 29242 and *P. aeruginosa* ATCC 8027) were obtained from Giresun University. The disk diffusion method was used to determine the resistance of bacteria to antibiotics (Temiz, 2010). Firstly, bacterial strains were reactivated in Tryptic Soy Agar (TSA, Merck) medium for18-24 hours at 37 °C. Pure cultures were adjusted according to the McFarland standard at a concentration of 0.5 ($1.5x10^8$ CFU) in test tubes containing physiological solution. Immediately after this process, 100 µL of bacterial solution were taken under aseptic conditions and spread on petri dishes with Mueller-Hinton (Merck) medium, and the solution was absorbed into the medium. Then, standard antibiotics (Erythromycin 15µg (Oxoid, E15), Streptomycin 10 µg (Oxoid, S10), Penicillin 10 µg (Oxoid, P10),

Amoxycillin/Clavulanic acid 30 µg (2:1; Oxoid, AMC 30) and Cephalexin 30 µg (Oxoid, CL 30)) were placed on the medium with a minimum distance of 2 cm according to the disc diffusion method. After this process, the petri dishes were incubated at 37 °C for 18-24 hours under aerobic conditions, and transparent zone diameters formed at the end of the incubation were evaluated by measuring with a digital calliper (Temiz, 2010).

2.2. Determination of the antibacterial effect of pomegranate peel

Well agar diffusion method was used for this analysis. In this method; each bacterial strain was inoculated into a Mueller-Hinton (Merck) medium, and after the bacterial solution was absorbed into the medium, wells were opened with a diameter of 0.5 cm and at least 2 cm between each well on the medium. 30 µL of each of the prepared pomegranate peel powder solutions were transferred to the wells and absorbed into the medium for approximately 20 minutes. Petri dishes were then incubated at 37 °C for 18-24 hours under aerobic conditions. The transparent zone diameters formed at the end of the incubation were measured with a digital calliper and evaluated (Ponce *et al*., 2003). Table 1 shows the values used for the interpretation of the antimicrobial effect depending on the zone diameter.

Table 1. Antimicrobial effect depending on zone diameter (Ponce et al., 2003)

Zone diameter (mm)	Antimicrobial effect	Determination
Diameter<8.00	Ineffective	-
9.00<Diameter<14.00	Low effect	+
15.00<Diameter<19.00	Effective	++
Diameter>20.00	Overly effective	+++

2.3.Counting of bacteria inoculated into pomegranate peel powder solutions

100µL of bacterial strains were transferred to each prepared pomegranate peel powder solution (0%, 2%, 4% and 8%) and homogenized. These solutions obtained as the main dilution were diluted up to 10^8 and other dilutions were prepared. Then, taking 100 µL from each dilution, they were inoculated on Baird Parker (Merck) for *S. aureus* (Tallent *et al*., 1998), Eosine-Methylene Blue (EMB, Oxoid) for *E. coli* (Feng *et al*., 1998), *Bacillus cereus* selective (BCS, Oxoid) for *Bacillus cereus* (Harrigan, 1998), Slanetz-Barley (Oxoid) for *E. faecalis* (Halkman, 2019), and Cetrimide (Merck) agar for *P. aeruginosa* (Harrigan, 1998). They were then incubated under aerobic conditions at 37°C for 18-24 hours. At the end of the incubation, the typical colonies that developed on the mediums were counted and evaluated.

The study was carried out in three replications and three parallels. Measurements were made from a single point in zones with proper shapes around the discs, and from 3 different points in zones that were not formed properly. The results of microbiological analyses were evaluated by taking their logarithms. Analysis of variance (ANOVA) was performed by taking the average of the measurements, and Duncan's multiple comparison test was applied when significance was found ($P<0.05$; SPSS 16.0, CHICAGO, IL, USA). The results were expressed as mean±standart deviation.

3.Results and discussions

The results of mentioned physicochemical attributes were presented in Table 2, and it is understood that as the ratio of pomegranate peel powder in the solution increased, the pH value decreases, while the O/R and EC values increased ($P<0.01$). While pH value was the lowest value as 3.75 in the solution containing 8% pomegranate peel powder, the value for O/R in this sample was higher as 195.67 mV than in the other. Additionally, the EC property is measured as 0.39 µS $cm^{-1}$and 0.42 µS cm^{-1}

higher in samples containing 4% and 8% powder than in other solutions (Table 2).

Table 2. Some physicochemical properties of pomegranate peel powder solutions at different concentrations

Concentration	pH	O/R (mV)	EC (µS cm^{-1})
Control	5.96±0.04^{a}	83.17±2.07^{d}	0.13±0.02^{c}
2%	3.95±0.01^{b}	185.93±0.35^{c}	0.33±0.03^{b}
4%	3.80±0.001^{c}	193.23±0.23^{b}	0.39±0.01^{a}
8%	3.75±0.001^{d}	195.67±0.21^{a}	0.42±0.01^{a}

$^{a-c}$ Different letters in the same column indicate significant differences among the concentration (P<0.05).

Table 3. Antibacterial effects of standard antibiotics on some food pathogenic microorganisms (mm)

Microorganism	Erythromycin	Streptomycin	Penicillin	Amoxycillin/ Clavulanic acid	Cephalexin
S. aureus ATCC 29213	30.33±0.58^{a}	18.00±0.0^{c}	30.33±0.58^{a}	27.33±0.58^{b}	29.67±0.58^{a}
E. coli ATCC 25922	11.33±0.58^{d}	21.33±0.58^{b}	0±0.00^{e}	20.33±0.58^{c}	22.33±0.58^{a}
B. cereus ATCC 10876	29.33±0.58^{a}	20.33±0.58^{b}	0±0.00^{d}	0±0.00^{d}	8.67±1.15^{c}
E. faecalis ATCC 29242	25.33±0.58^{b}	28.33±0.58^{a}	22.67±0.58^{c}	26.33±0.58^{b}	20.67±0.58^{d}
P. aeruginosa ATCC 8027	13.67±0.58^{c}	19.67±0.58^{b}	0±0.00^{d}	19.67±0.58^{b}	24.33±0.58^{a}

$^{a-c}$Different letters in the same row indicate significant differences among the standard antibiotics on bacteria(P<0.05).

In addition, selected bacteria are some important pathogens that can often be found in foods. The results showing the antimicrobial effects of the antibiotics used against pathogenic bacteria were presented in Table 3. According to the results, it was understood that these antibiotics have important effects on all pathogens used (P<0.01). While the large diameter of the disc formed indicates that the antimicrobial effect is high, Erythromycin, Penicillin, Amoxycillin/Clavulanic acid, and Cephalexin were found to be overly effective (+++) against *S. aureus*. Streptomycin, on the other hand, had an effective (++) force against this pathogen. Streptomycin, Amoxycillin/Clavulanic acid, and Cephalexin were overly effective (+++) against *E. coli*, while Erythromycin was low effective (+). Erythromycin and Streptomycin were overly effective (+++) against *B. cereus* whereas Cephalexin had a low effect (+). While all antibiotics were overly effective (+++) against *E. faecalis*, lastly antibiotics other than erythromycin and penicillin were found to be overly effective (+++) against *P. aeruginosa* (Table 3).

The results of well agar method measurement showing the antibacterial effect of solutions prepared from pomegranate peel powder, which is the subject of this study, against pathogenic bacteria were shown in Table 4. As can be seen from the table, the antimicrobial effects of all solutions against all pathogens were found to be significant at the level of P<0.01. While 2% and 4% solutions were effective (++) against *S. aureus* and *E. coli*, 8% solution was overly effective (+++). While all solution levels that contained powder were effective (++) against *B. cereus*, they showed an overly effective (+++) antimicrobial potential against *E. faecalis*. However, 4% and 8% of solutions were low effective (+) against *P. aeruginosa* whereas

other treatments were observed to be ineffective (-).

Table 4. Antibacterial effects of different levels of pomegranate peel powder solutions on selected food pathogen microorganisms (mm)

Microorganism	Control	2%	4%	8%
S. aureus ATCC 29213	0±0.0^{d}	15.33±0.58^{c}	19.33±0.58^{b}	20.67±0.58^{a}
E. coli ATCC 25922	0±0.0^{d}	14.67±0.58^{c}	16±1.00^{b}	19.67±0.58^{a}
B. cereus ATCC 10876	0±0.0^{c}	15±0.0^{b}	15.33±1.15^{b}	18.33±0.58^{a}
E. faecalis ATCC 29242	0±0.0^{c}	22.33±0.58^{b}	23.33±1.15^{b}	27.33±0.58^{a}
P. aeruginosa ATCC 8027	0±0.0^{c}	0±0.0^{c}	10.33±0.58^{b}	13.33±0.58^{a}

$^{a-c}$Different letters in the same row indicate significant differences among the antibacterial effects with pomegranate peel powder solutions ($P<0.05$).

Table 5. The effect of pomegranate peel powder solutions on the amounts formed as result of incubation of some food pathogenic bacteria (log CFU mL^{-1})

Microorganism	Control	2%	4%	8%
S. aureus ATCC 29213	7.12±0.60^{a}	6.18±1.03^{a}	5.45±0.36^{a}	5.11±0.18^{a}
E. coli ATCC 25922	8.40±0.03^{a}	8.12±0.07^{b}	7.35±0.01^{c}	6.33±0.06^{d}
B. cereus ATCC 10876	7.84±0.05^{a}	7.05±0.22^{b}	6.79±0.28^{b}	6.14±0.02^{c}
E. faecalis ATCC 29242	7.48±0.01^{a}	6.30±0.00^{b}	6.18±0.02^{b}	5.80±0.09^{c}
P. aeruginosa ATCC 8027	8.75±0.28^{a}	8.77±0.25^{a}	8.14±0.18^{ab}	7.69±0.25^{b}

$^{a-c}$Different letters in the same row indicate significant differences among the pomegranate peel powder solutions antibacterial effects ($P<0.05$).

The results of the analysis performed to determine the number of microorganisms formed in suitable media for each bacterium is presented in Table 5. Statistical analysis showed that pomegranate peel solutions were not significantly effective on *S. aureus* count ($P>0.05$), they were effective on *E. coli*, *B. cereus* and *E. faecalis* at a level of $P<0.01$whereas on *P. aeruginosa* count at the level of $P<0.05$. Generally, it was observed that the number of microorganisms decreased as the ratio of pomegranate peel powder in the solution increased in pathogenic bacteria except for *S. aureus*. While the counts of *S. aureus* were founded in the range of 5.11-7.12 log CFU/mL, the lowest microorganism numbers were determined as 6.33 log CFU/mL for *E. coli*, 6.14 log CFU/mL for *B. cereus*, 5.80 log CFU/mL for *E. faecalis* and 7.69 log CFU/mL for *P. aeruginosa* in the samples inoculated with 8% solution (Table 5).

3.1.Discussions

The pH value is one of the important parameters that affect the growth of microorganisms in a medium. Determination of acidic or basic properties is important in terms of properties such as quality, safety, and processing in foods (Kılınççeker *et al*., 2015; Kurt and Kılınççeker, 2011). In terms of microorganisms, moulds can grow in the pH range of 1.2-4.5, yeasts in the pH range of 1.5-

4.0 and bacteria in the pH range of 4.5-6.5, while it is known that pathogenic bacteria generally cannot grow at low pH (Temiz, 2015; Kim *et al*., 2018). In the study, as the concentration increased, the amount of organic acid passing from the pomegranate peel to the solution increased and decreased the pH values of the solutions (Table 2). Therefore, these low pH values in the solutions adversely affected the growth of microorganisms, as can be understood from Table 4 and Table 5. Like our results, Kennas *et al*., (2020) found the pH value of pomegranate peel powder to be 3.82, and Jalal *et al*., (2018), on the other hand, measured it as 3.83 and they emphasized that it is an acidic material.

The O/R potential value is one of the important internal factors that affect the development of microorganisms, such as pH. It has a positive value under aerobic conditions and a negative value under anaerobic conditions (Temiz, 2015). In this study, the O/R values of the solutions in the range of 83.17-195.67 mV are perceived as an indicator of the aerobic environment. For this reason, it was thought that especially the growth of anaerobic microorganisms might be adversely affected by the increase in the ratio of pomegranate peel powder in the solutions. Accordingly, the numbers of *S. aureus* and *E. coli* showing facultative anaerobic properties could be reduced, especially (Table 2).

The EC value is defined as the ability of a food or solution to conduct an electrical current. This value is a function of the type and amount of ingredients in foods, and electrolyte-containing materials such as salts, acids, gums, and thickeners have a significant effect on the electrical conductivity of foods (Singh and Heldman, 2015). It also shows a linear relationship between temperature and water/ion content (Jha *et al*., 2011). It is widely used to determine contaminants and microbial activity in water in the food industry (Kaptan and Kayisoglu, 2016). It was observed that the EC range in the investigated solutions increased depending on the increase in concentration, and this was attributed to the increase in the amount of substance or ionization. As can be seen in Table 2, an increase in the EC value was observed with the increase of the pomegranate peel concentration, suggesting that the transfer of antimicrobial substances into the solution increased, especially. Looking at the results, it is understood that the amount of pomegranate peel powder used is effective on the differences in pH, O/R and EC values in solutions and this also affects the antimicrobial strength.

According to results in Table 3 and Table 4, When the antibacterial effect of pomegranate peel powder is compared with standard antibiotics in terms of *S. aureus*; The solutions with 2% and 4% concentrations showed similarity with Erythromycin in that they were effective (++), while the 8% solution was overly effective (+++) as other antibiotics.

The antibacterial effect of 8% pomegranate peel powder solution in terms of *E. coli* was similar to that of Streptomycin, Amoycillin/Clavulanic acid and Cephalexin being overly effective (+++). Solutions containing 2% and 4% powder were determined as effective (++) compared to ineffective (-) Penicillin and low-effective (+) Erythromycin.

It was determined that all pomegranate peel powder solutions were effective (++) against *B. cereus*. However, they created a difference from standard antibiotics in that Penicillin and Amoxycillin clavulanic acid was ineffective (-), cephalexin was the low effect (+), and erythromycin and streptomycin were over effective (+++).

All solutions were overly effective (++) against *E. faecalis*, and these results were like that of standard antibiotics.

The ineffectiveness (-) of 2% pomegranate peel powder solution and Penicillin against *P. aeruginosa*, and the low effective (+) of 4% and 8% peel powder solutions were similar only to Erythromycin. However, they were also understood to be less effective (+) than other standard antibiotics.

Like our results in Table 4, Akarca and Başpınar (2019) stated that the zone diameters formed by the water extracts of the pomegranate peel in the disc diffusion method they applied

against seven pathogenic bacteria, were between 11.15-25.68 mm and the strongest effect was obtained especially against *S. aureus* and *B. cereus*. Balaban *et al*., (2021), when they apply pomegranate peel extracts as a biofilm at different densities, they found that the antimicrobial effect increased as the density increased, and the zone diameters formed were 9-17 mm for *B. cereus* and 0-13 mm for *E. faecalis*. In conclusion, they emphasized that pomegranate peel extracts can be used as an antimicrobial material for the food industry. Al-Zoreky (2009) observed that aqueous and ether extracts of pomegranate peel did not form an inhibition zone in a trial against eleven pathogens, but the water-methanol extract formed zones in the range of 12-20 mm and he said that these values were the result of a significant antimicrobial effect. Also, Dahham *et al*., (2010) measured the resulting disc diameters in the range of 18-25 mm in their study to determine the antimicrobial effect of pomegranate peel extract for seven bacteria. According to this result, while they said that pomegranate peel extract could be an important antimicrobial source, stated constituents such as phenols, tannins and flavonoids found in the extracts as the cause of this antimicrobial activity. In another study, it was said as a material with antimicrobial activity against many microorganisms due to components such as punicalagin in the pomegranate fruit peel structure (Rongai *et al*., 2019).

Lastly, examples of similar studies in which the pomegranate peel powders used in this study were effective at different concentrations and in support of the microorganism count results given in Table 5 can be given as follows. Rasuli *et al*., (2021) stored buffalo meats marinated with 0%, 0.5%, 1% and 1.5% pomegranate peel extract in cold storage. In the analyses made during the storage, they determined that the total viable counts in the samples decreased as the extract concentration in the sample increased. Morsy *et al*., (2018) added 0%, 1% and 1.5% pomegranate peel nanoparticles to meat patties and they applied cold storage for 15 days. In the microbiological analysis of meatballs, they said that the total bacteria, psychrophilic bacteria and lipolytic bacteria counts of the samples containing pomegranate peel nanoparticles were lower than control during storage and the lowest counts were at high concentrations (1.5%). Mahajan *et al*., (2015) determined that in cheeses prepared with pomegranate peel extract at 0%, 1% and 2% concentrations, the numbers of total viable, psychrophilic organisms and yeast-moulds in the samples containing the extracts decreased. They also stated that the increase in the ratio of pomegranate peel extract supports this decrease. As a result, it has been emphasized that the pomegranate peels used in these studies can be used as a natural antimicrobial agent for the food industry due to the anthocyanin, punicalagin, ellagic acid, gallic acid and some other phenolic compounds it contains. It can be said that the results of our study are in accordance with these studies. Solutions showed a reducing effect on the counts of pathogenic bacteria in our study depending on the functional components in the structure of the pomegranate peel used and the physicochemical properties given in Table 2.

4.Coclusion

As a result of the study, it was understood that the solutions prepared from the peel powders obtained from the Zivzik pomegranate can be used as an antibacterial material against *S. aureus, E. coli, B. cereus, E. faecalis* and *P. aeruginosa*. As the pomegranate peel concentration in the solutions increased, the amount of antibacterial substance transferred to the medium increased, decreased the pH values and increased the O/R and EC values. These changes increased the antibacterial effects of pomegranate peel powder solutions. It was thought that the antibacterial effect increased with the increase in the passage of bioactive substances and organic acids that decrease the pH value in the solution. As a matter of fact, when the 0% concentration is increased to 8%, it has been determined that there is a decrease in the number of microorganisms up to approximately 1.5-2 log levels. While it has been observed that solutions of different

concentrations have similar effects to most standard antibiotics used, it has been understood that the use of solutions with 4% and 8% concentrations can be recommended especially against these pathogens.

5.References

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