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Antibacterial Effect of Pomegranate Peel Extract Against Some Bacterial Isolates

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ABSTRACT

One of the most common opportunistic nosocomial pathogens is Pseudomonas aeruginosa, which is well-known for its strong ability to build biofilms and inherent resistance to antibiotics. In recent years, there has been a greater focus on finding plant-based substitutes. Peel from pomegranates (Punica granatum L.) is a significant source of polyphenolic chemicals that have been shown to have biological action. Soxhlet extraction and rotary evaporation were used to create a pomegranate peel methanol extract. Using the agar well diffusion method, antibacterial activity was assessed at doses of 200, 300, 400, 500, and 1000 µg/mL against five clinical isolates of P. aeruginosa (P1–P5). The crystal violet microtiter plate test was used to measure the biofilm-forming capacity. This study observed that the extract exhibited significant concentration-dependent antibacterial activity. At a concentration of 1000 µg/mL, the inhibition zones ranged from 18 mm for P5 to 25 mm for P3. At 300 µg/mL, all tested strains showed resistance to the extract. Among the 5 tested strains, 4 were high biofilm-producing strains, and 1 was a moderate biofilm-producing strain. Methanol extract from pomegranate peels has strong, concentration-dependent antibacterial activity against clinical isolates of P. aeruginosa. The isolates' great propensity to form biofilms highlights the pathogen's clinical importance and encourages more research on plant extracts as antibiofilm agents.

Keywords: *Pseudomonas aeruginosa; Punica granatum, pomegranate peel; Soxhlet extraction; antibacterial activity; well diffusion, biofilm; polyphenols.*

1. INTRODUCTION

One of the most important nosocomial diseases in the world, Pseudomonas aeruginosa is a Gram-negative, non-fermenting opportunistic bacterium. It is a major cause of hospital-acquired infections, especially in patients with cystic fibrosis, burn victims, immunocompromised patients, and those in need of mechanical ventilation [1]. P. aeruginosa's exceptional ability to develop multidrug resistance (MDR) through a variety of mechanisms, such as overexpression of efflux pumps, decreased outer membrane permeability, production of beta-lactamases, and horizontal gene transfer, increases the clinical threat it poses [2].

The capacity of P. aeruginosa to create organized biofilms on both biotic and abiotic surfaces is a crucial component of its pathogenicity. Compared to their planktonic counterparts, biofilm-embedded bacteria are up to 1000 times more resistant to drugs and are shielded from host immune responses [3]. Strong biofilm development in clinical isolates is linked to treatment failure, extended hospital stays, chronic infections, and worse patient mortality [4]. We really need to find ways to fight germs that are better than the medicines we have now. This is because a type of germ called MDR P. Aeruginosa is very common in hospitals and it forms groups that're hard to kill. MDR P. Aeruginosa is a problem because it forms these groups, which are called biofilms in hospitals. We need to find ways to fight MDR P. Aeruginosa because it is very good at forming biofilms, in hospital environments.

Plants are a source of things that can help us. Pomegranate is a plant that people have studied a lot. It is very good for us because it has a lot of things that can fight off bacteria. The Pomegranate plant has good things in it that can help our bodies. It has a lot of polyphenols which're good for us. Pomegranate is a useful plant and that is why scientists are very interested, in it [5]. Pomegranate fruit peels have a lot of stuff in them like hydrolysable tannins and flavonoids

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and phenolic acids. The peels are a part of the pomegranate fruit they make up around 40 to 50 percent of the whole fruit. Pomegranate fruit peels and the compounds in them like tannins and flavonoids and phenolic acids are really good, at fighting off bad things that can hurt us. They have antioxidant properties and strong antimicrobial properties [6].

Pomegranate peel extracts are really good at fighting off bacteria. They work against a lot of kinds of bacteria like P. Aeruginosa. The pomegranate peel extracts do this by messing up the bacteria cells stopping them from working and getting in the way of how they talk to each other to form groups. This helps stop the bacteria from growing and forming groups that're hard to kill. Pomegranate peel extracts are very good at this because they can disrupt the bacteria cells and disable the things that help the bacteria survive. Pomegranate peel extracts are also good, at interfering with how the bacteria form groups, which makes them easier to fight off [7]. Methanol-based Soxhlet extraction is really good at getting components out of plants. This method is very helpful for finding out what is in these plants. Methanol-based Soxhlet extraction lets us see what these components can do. We can use methanol-based Soxhlet extraction to learn more about the activity of these phenolic components, from plants.

Although there is an increasing amount of evidence on the antibacterial efficiency of pomegranate peel, there are few comparative studies which evaluate efficacy of peel against numerous clinical isolates of P. aeruginosa and measurement of its ability to build biofilms. The present research thus intended to:

(1) Preparation of methanol extract of pomegranate peel by Soxhlet extraction.

(2) evaluate its antibacterial efficacy by agar well diffusion technique against five clinical isolates of P. aeruginosa.

(3) To assess the capacity of the isolates to generate biofilms using the crystal violet microtiter plate test.

2. Materials and Methods

2.1 Plant Material and Methanol Extract Preparation

Fresh pomegranate fruits or Punica granatum L. were bought from a market. The peels were carefully taken out from the pomegranate fruits cleaned with water to remove any dirt on the surface and then dried in a hot air furnace at 40°C for 72 hours until they had the same weight. After they were ground into a powder using an electric grinder the dried pomegranate peels were kept in closed containers at room temperature until they were needed.

Methanol was taken out using a machine. The liquid used to extract be 350 milliliters of pure methanol and the machine was filled with fifty grams of dried and ground pomegranate skin. The extraction was done for six hours at 65°C to complete the cycle. A special machine was used to remove the liquid from the extract under pressure after it was filtered through a special paper and it worked at 40°C. The pomegranate extract made with methanol was mixed with a dimethyl sulfoxide to make solutions with the right amount of pomegranate extract for tests, on bacteria and their growth after the extract was weighed to see how much was obtained from the pomegranate fruits.

2.2 Isolates of Bacteria

We looked at five Pseudomonas aeruginosa isolates, which we called P1 to P5 during this study. To figure out what type of Pseudomonas aeruginosa we were dealing with we used some microbiology techniques. We did things like Gram staining. We looked really closely at the shape of the colonies. We also did some tests to see what was going on. We took Pseudomonas aeruginosa from things, like patient samples and other clinical materials.

Prior to testing made Pseudomonas aeruginosa suspensions in sterile normal saline. made sure the Pseudomonas aeruginosa suspensions had a turbidity, to the 0.5 McFarland standard, was 1.5 × 108 CFU/mL of Pseudomonas aeruginosa.

2.3 Antibacterial Activity

A study sees how good the pomegranate peel solution was at killing bacteria. The Clinical and Laboratory Standards Institute told us how to do it so we followed their instructions from 2023. First, we used a cotton swab to pick up some Pseudomonas aeruginosa bacteria. We got 100 µL of it. Then we spread it out evenly on some plates called Mueller-Hinton Agar plates, used a tool to make holes in the plates that had the bacteria on them. These holes were 6 mm across.

After that we put 100 µL of the pomegranate peel solution into each hole. We made the solution, in strengths and mixed it with a little DMSO, which was 10% of the mix. After 24 hours of incubation at 37°C, the diameter of each inhibition zone was measured. All results are reported as the mean of three independent replicate experiments. A tested sample was determined to be resistant if no inhibition zone formed outside the well.

2.4 Biofilm Formation assay

Using the crystal violet (CV) microtiter plate method as outlined by O'Toole (2011), the biofilm-forming ability of the five P. aeruginosa clinical isolates was quantitatively evaluated. This study used the crystal violet staining method to detect bacterial biofilms, with the specific procedure as follows: add 200 µL of bacterial suspension in TSB medium containing 1% glucose to each well of a 96-well flat-bottom polystyrene microplate, then conduct static incubation at 37°C for 24 hours; aspirate the culture medium to remove planktonic cells, wash the plate 3 times with sterile PBS at pH 7.4, fix the samples with 99% methanol for 15 minutes, stain with 0.1% crystal violet at room temperature for 20 minutes, rinse with distilled water and let the plate air-dry, then add 95% ethanol to dissolve the bound stain. All parameters are clearly defined to support direct replication. A Bio-Tek microplate reader was used to detect optical density at 630 nm (OD_{630}). Biofilm generation was categorized as weak ($OD_{630} \leq 0.100$), moderate ($0.100 < OD_{630} \leq 0.200$), or strong ($OD_{630} > 0.200$). Every test was run in triplicate [8].

3. Results

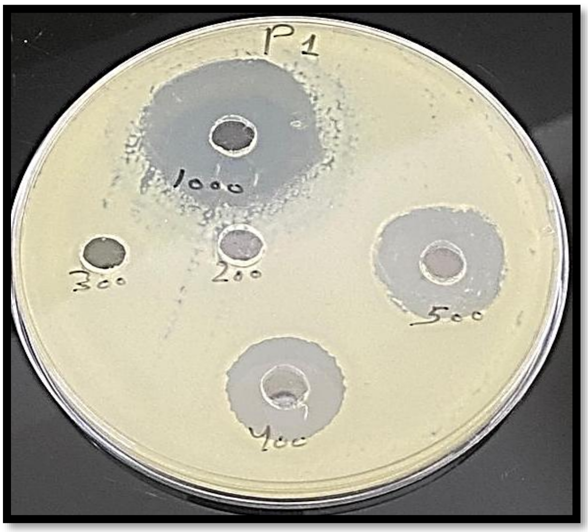
3.1 Pomegranate Peel Methanol Extract's Antibacterial Activities

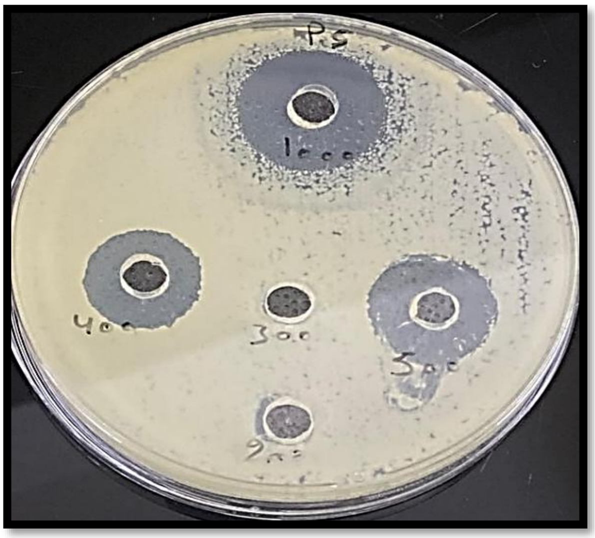
All antibacterial activity data measured in this study for the methanol extract of pomegranate peel against 5 clinical isolates of Pseudomonas aeruginosa are fully summarized in Table 1 and Figure 1. All isolates showed definite concentration-dependent antibacterial activity from the extract. Inhibition zones varied from 18 mm (P5) to 25 mm (P3) at the highest tested concentration (1000 µg/mL), with an average inhibition zone of 22.0 ± 2.6 mm. Inhibition zones varied from 14 mm (P3) to 18 mm (P1 and P4) at 500 µg/mL, and between 14 and 16 mm at 400 µg/mL. Additionally, all five isolates showed complete resistance (R) at 300 µg/mL and below, suggesting that the lowest inhibitory threshold concentration that the well diffusion method can detect in this system is 400 µg/mL. In comparison to most other isolates, isolate P3 consistently produced the highest inhibition zones at 1000 µg/mL (25 mm) and 500 µg/mL (14 mm), while isolate P5 showed the lowest susceptibility overall.

Table 1. Pomegranate peel methanol extract inhibition zone diameters (mm) against five clinical isolates of P. aeruginosa using the agar well diffusion assay

Concentration µg/mL	P 1 (mm)	P 2 (mm)	P 3 (mm)	P 4 (mm)	P 5(mm)
1000	24	23	25	20	18
500	18	15	14	18	16
400	16	14	15	16	14
300	R	R	R	R	R
200	R	R	---	R	R

R: Resistant (no inhibition zone seen beyond the well edge)


P1
1000
300
200
500
400


P5
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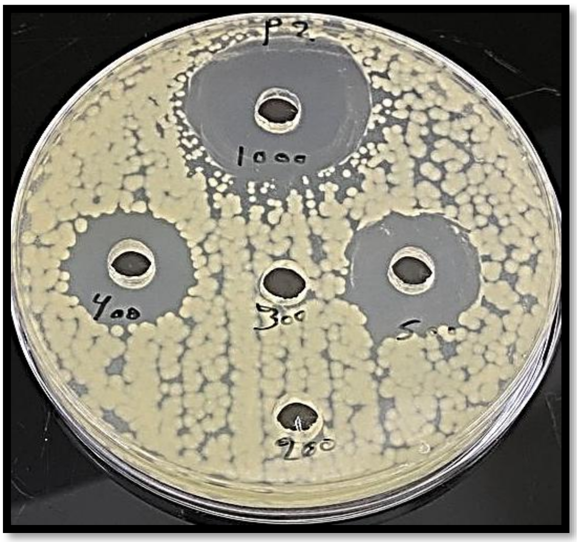
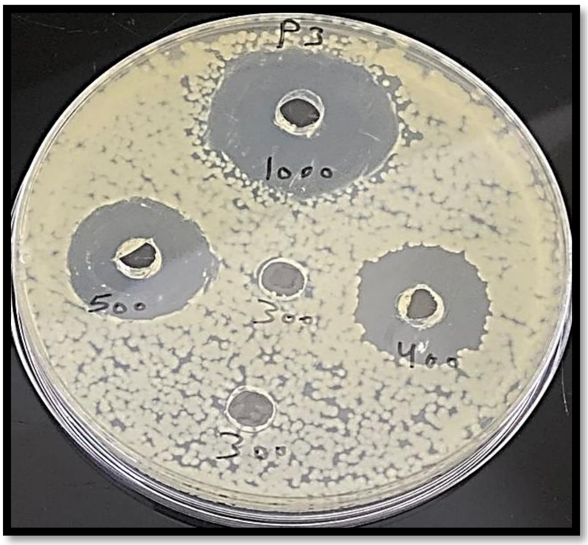
400
300
500


P2
1000
400
300


P3
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500
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400
300

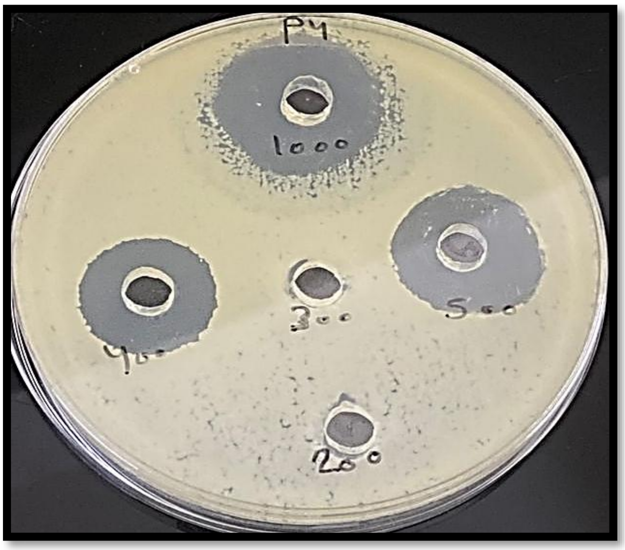

Figure 1. shows the inhibition zones created by pomegranate peel methanol extract against clinical isolates of Pseudomonas aeruginosa (P1–P5) at doses between 200 and 1000 µg/mL using the agar well diffusion method

3.2 Biofilm Formation assay

The biofilm formation capacity data for the 5 clinical Pseudomonas aeruginosa isolates numbered P1-P5 tested in this study are recorded in Table 2 and Figure 2. This indicator was obtained by measuring the OD_{630} value through the crystal violet microtiter plate assay. Among the isolates, P1, P2, P4, and P5 are strong biofilm producers, with OD values of 0.205, 0.286, 0.421, and 0.425, respectively. Only P3 is a moderate biofilm producer, with an OD value of 0.172. The OD values of P4 and P5 are twice the threshold for super-strong biofilm production. Biofilm strength showed a significant negative correlation with antimicrobial susceptibility. P3 consistently produced the largest inhibition zones at the two highest concentrations of the tested extract.

Table 2. ability of P. aeruginosa clinical isolates to create biofilms using the crystal violet microtiter plate assay (OD_{630})

Isolate	Biofilm OD_{630}	Biofilm Category
P1	0.205	Strong
P2	0.286	Strong
P3	0.172	Moderate
P4	0.421	Strong
P5	0.425	Strong

OD_{630}: Optical density at 630 nm. Classification: Strong (OD > 0.200); Moderate (0.100 < OD ≤ 0.200); Weak (OD ≤ 0.100).

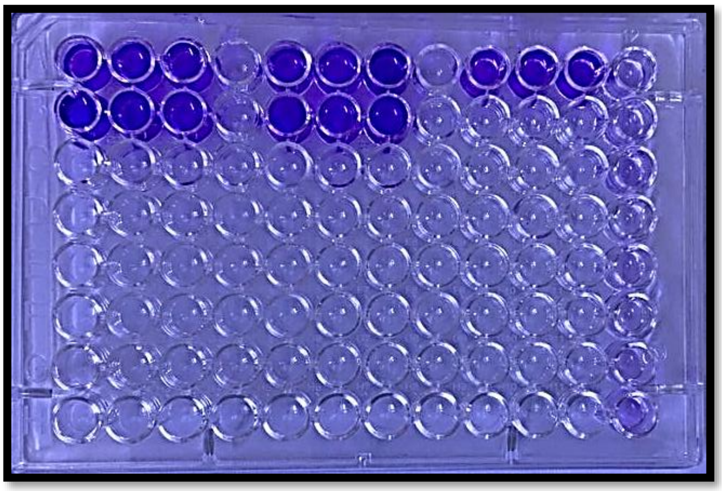

Figure 2. Pseudomonas aeruginosa clinical isolates (P1–P5) produce biofilms in a 96-well microplate stained with crystal violet. Strong biofilm formation is indicated by intense blue coloring, and moderate to mild biofilm growth is indicated by lesser staining.

4. Discussion

This study assessed the ability of five clinical isolates of Pseudomonas aeruginosa to produce biofilms and the antibacterial activity of a methanol extract of pomegranate (Punica granatum L.) peel prepared by Soxhlet. At doses of 400 µg/mL and above, the extract showed strong concentration-dependent antibacterial activity. P3 (25 mm) and P1 (24 mm) showed the largest inhibition zones at 1000 µg/mL. These results agree with earlier studies by Scaglione et al. (2024), which showed significant antibacterial activity of pomegranate peel extracts against P. aeruginosa with inhibition zones in the range of 18–26 mm at comparable concentrations [9].

Pomegranate peel methanol extract's high polyphenolic composition, which comprises hydrolyzed tannins (Punicalagin, Ellagic acid, Gallic acid), flavonoids (Quercetin, Catechin), and hydroxycinnamic acids (Ferulic acid, Caffeic acid), is responsible for its antibacterial effectiveness. These compounds work against bacteria by breaking down the cell wall of the bacteria. They stop the bacteria from making things it needs to survive. The compounds also get in the way of the bacteria making copies of itself. They even take away metals that the bacteria needs to grow. The compounds do all of this and more to stop the bacteria from growing. They have ways of working together to stop the bacteria. These compounds are really good, at stopping bacteria because they use methods to do so [10]. The activity we saw was really strong. This was probably because the Soxhlet system used methanol to get the stuff out. The methanol helps get all the components that are polar and moderately polar. The Soxhlet system is good at this because it uses methanol. The methanol is what makes sure we get all the components, from the Soxhlet system [11].

The phenotypic and genotypic variety present in clinical P. aeruginosa strains is reflected in the variation in inhibitory zones between the five isolates at equivalent extract doses. The sensitivity of isolates to plant-derived antimicrobial agents is known to be influenced by differences in lipopolysaccharide composition, efflux pump expression, and outer membrane permeability [12]. All isolates showed complete resistance at doses $\leq$ 300 µg/mL, which is in line with P. aeruginosa known inherent resistance to a variety of chemical agents.

This study used a biofilm assay to test 5 clinical isolates of Pseudomonas aeruginosa. Among the tested strains, 4 isolates (P1, P2, P4, and P5) were identified as strong biofilm producers, with OD_{630} values ranging from 0.205 to 0.425. Studies by Kabir et al. (2024) show that the prevalence of this bacterium's strong biofilm phenotype in hospital settings reaches 60%-80%, which is consistent with the pattern observed in this study [13].

The discovery of a link between how well bacteria are affected by antibacterial agents and how strongly they form biofilms was really interesting. The bacteria isolate P3, which's the only one that forms biofilms at a moderate level always had the biggest areas where bacteria were killed at 1000 and 500 µg/mL. When bacteria form strong biofilms it stops the antibacterial compounds from getting to and spreading into the bacterial cells. This means that the amount of the compound that actually gets into the cells is lower which makes it less effective at killing the bacteria. The biofilm formation is, like a shield that protects the bacteria from the agents [14]. The evidence that is there shows this pattern. The connection between these things is also backed up by the fact that a lot of extract was needed to stop anything from happening in P4 and P5. These are the ones that're really good at making biofilms. It makes sense that P4 and P5 would need a lot of extract to stop biofilms from forming because P4 and P5 are very good, at making biofilms.

The results show that the part of the pomegranate peel that is extracted using methanol is a thing that can fight bacteria. It works against Pseudomonas aeruginosa that is found in clinics. To make this extract more useful in clinics we need to do studies. We should find out the amount of the extract that can stop the bacteria from growing. We should also see if it can stop bacteria from forming groups at concentrations. The methanolic extract of pomegranate peel should be tested with antibiotics to see if it works better together. We need to check if the methanolic extract of pomegranate peel is bad, for living things and if it really works in living bodies. The methanolic extract of pomegranate peel is what we are talking about here.

REFERENCES

Elfadadny, A., Ragab, R. F., AlHarbi, M., Badshah, F., Ibáñez-Arancibia, E., Farag, A., ... & Nageeb, W. M. (2024). Antimicrobial resistance of Pseudomonas aeruginosa: navigating clinical impacts, current resistance trends, and innovations in breaking therapies. *Frontiers in microbiology*, *15*, 1374466.

Michaelis, C., & Grohmann, E. (2023). Horizontal gene transfer of antibiotic resistance genes in biofilms. *Antibiotics*, *12*(2), 328.

Hawas, S., Verderosa, A. D., & Totsika, M. (2022). Combination therapies for biofilm inhibition and eradication: a comparative review of laboratory and preclinical studies. *Frontiers in cellular and infection microbiology*, *12*, 850030.

Saha, P., Kabir, R. B., Ahsan, C. R., & Yasmin, M. (2025). Multidrug resistance of Pseudomonas aeruginosa: do virulence properties impact on resistance patterns?. *Frontiers in Microbiology*, *16*, 1508941.

Peppoloni, S., Colombari, B., Tagliazucchi, D., Odorici, A., Ventrucci, C., Meto, A., & Blasi, E. (2022). Attenuation of Pseudomonas aeruginosa virulence by pomegranate peel extract. *Microorganisms*, *10*(12), 2500.

Abutayeh, R. F., Ayyash, M. A., Alwany, R. A., Abuodeh, A., Jaber, K., & Al-Najjar, M. A. (2024). Exploring the antimicrobial potential of pomegranate peel extracts (PPEs): Extraction techniques and bacterial susceptibility. *PloS one*, *19*(12), e0315173.

Khursheed, H., & Qasim, R. (2024). Synergistic Antibiofilm Activity of Probiotic Lactobacillus Acidophilus and Punica Granatum L., Against Pseudomonas Aeruginosa Biofilm. *Journal of Ayub Medical College Abbottabad*, *36*(2), 245-250.

CLSI (2023). Performance Standards for Antimicrobial Susceptibility Testing. 33rd ed. CLSI supplement M100. Wayne, PA: Clinical and Laboratory Standards Institute.

O'Toole, G. A. (2011). Microtiter Bulaşık Biyofilm Oluşumu Testi. *Journal of Visualized Experiments (JoVE)*, (47), e2437.

Scaglione, E., Sateriale, D., Mantova, G., Di Rosario, M., Continisio, L., Vitiello, M., ... & Salvatore, P. (2024). Antimicrobial efficacy of Punica granatum Lythraceae peel extract against pathogens belonging to the ESKAPE group. *Frontiers in Microbiology*, *15*, 1383027.

Salim, A., Deiana, P., Fancello, F., Molinu, M.G., Santona, M., and Zara, S. (2023). Antimicrobial and antibiofilm activities of pomegranate peel phenolic compounds: Varietal screening through a multivariate approach. Journal of Bioresources and Bioproducts, 8(2), 146–161. https://doi.org/10.1016/j.jobab.2022.11.004

Witkowska, A.M., Waśkiewicz, A., and Zujko, M.E. (2023). Phenolic compound profiling of pomegranate peel by HPLC-DAD: influence of solvent and extraction method on recovery. Molecules, 28(6), 2571. https://doi.org/10.3390/molecules28062571

Sendra, E., Fernández-Muñoz, A., Zamorano, L., Oliver, A., Horcajada, J. P., Juan, C., & Gómez-Zorrilla, S. (2024). Impact of multidrug resistance on the virulence and fitness of Pseudomonas aeruginosa: a microbiological and clinical perspective. *Infection*, *52*(4), 1235-1268.

Kabir, R. B., Ahsan, T., Rahman, M. F., Jobayer, M., & Shamsuzzaman, S. M. (2024). Biofilm-producing and specific antibiotic resistance genes in Pseudomonas aeruginosa isolated from patients admitted to a tertiary care hospital, Bangladesh. *IJID regions*, *11*, 100369.