

# Effect of pomegranate peel extract on some virulence factors of *Proteus mirabilis* isolated from pathological cases

Shahd Hussein Rzaig<sup>1</sup>, Mohammad Rafi<sup>2</sup>, Sabaa Muhsen Farhan<sup>3</sup>

<sup>1</sup>P Science college, Bacteriological, Master Medical microbiology.

<sup>2</sup>M.B.ch. B CABSU.

<sup>3</sup>Medical laboratory technic, Almaarif university college

DOI: 10.47750/pnr.2022.13.S01.254

## Abstract

**Background** The most serious infections in bacteria are related to the virulence factors they carry. *Proteus mirabilis* are characterized by various virulence features that enable them to cause many infections, foremost of which is the creation of the biofilm, which plays a vital role in the virulence of these bacteria. This is why our investigation came to uncover an extract that inhibits the synthesis of virulence factors. **Methodology** The bacterial isolates in the current study were identified using the phenotypic and cultural characteristics of *P.mirabilis* bacteria by cultivating them on blood agar to examine the phenomena of swarming. This is the key diagnostic trait of this bacteria, and it has a peculiar odor that recalls the stench of rotting fish and its colonies are solitary and pale in color when grown on the differential media. Optional MaCconky agar and smooth rims medium non-fermenting lactose. Result reveals the influence of pomegranate peel extract in suppressing the formation of protease enzyme in the *proteus mirabilis*. The average productivity of bacteria treated with varied concentrations of pomegranate peels 50,40,30 % is 13,60,11,6.20 % compared to the control 18.80 % where the concentration 50 percent was the best concentration It worked to reduce the production of the protease enzyme by a large percentage compared to other concentration, less than the effect of pomegranate peel extract in the synthesis of protease in % percent concentrations with 40 % concentrations, but significant difference% percent concentration found significant differences with the 50 %t concentrations and the control As for the 50 % concentration, it was determined that there were substantial disparities with all the concentrations and the control ones.

**Keywords:** pomegranate peel extract, virulence factors of *Proteus mirabilis*.

## INTRODUCTION

*Proteus mirabilis* is a Gram-negative bacillus that does not develop spores or capsula, but rather flagella-moving bacilli.(1) They have fimbriae and are found in soil, water, and the intestinal tract of animals and humans (2). These bacteria are among the pathogens of the disease, which are infections of the urinary tract and depend on the urinary tracts (3). Polysaccharides caused by *P. mirabilis* in the expression of different virulence factors, biofilm production and resistance to different classes of antibiotics. Nucleo et al., 2010 (4).Flagella, toxins, fimbriae, adhesions, enzymes, and immune cell resistance are also expressed (5) Urinary tract infections (UTIs) are one of the most common bacterial infections in humans, both in the community and in hospitals. Urinary infections include a group of clinical conditions that range in severity from asymptomatic infections to acute bladder, prostate, pelvic, renal, and urethral infections. It has become one of the most common diseases that require the review of specialized doctors for the purpose of treatment, and it affects people of all ages, from newborns to the elderly, and the most prevalent bacterial species responsible for urinary tract infection and generally arises in the intestine is the Enterobacteriaceae family such as *Proteus* (6). The complications of urinary tract infection include kidney stones (Kidney stone) and recurrent bacteriuria. Urinary tract infection is one of the difficult and widespread problems in the world and affects millions of people annually, and women are more likely than men to contract this disease, as it comes in second place after Respiratory tract infections, as males and females are exposed to this disease, (7). The bacterium *P.mirabilis* causes urinary tract infections, especially for patients hospitalized and using urinary catheters, as well as people who suffer from abnormal functional and anatomical problems of the urinary tracts .

## Methodology

### Collection of specimens

Various samples were collected from urine, otitis media, with sixty samples distributed over 20 samples for each patient case.

### Identification of bacteria

The bacterial isolates in the current study were identified through the phenotypic and cultural characteristics of *P. mirabilis* bacteria by growing them on blood agar to observe the phenomenon of swarming. This is the primary diagnostic characteristic of this bacteria, and it has a distinctive odor that resembles the smell of rotting fish and its colonies are single and pale in color when grown on the differential medium. Optional MacConkey agar and smooth rims medium non-fermenting lactose.

### Biochemical test

Biochemical tests were used as complementary tests for the purpose of initial diagnosis and to confirm the diagnosis of *P. mirabilis*, Colonies of *P. mirabilis* appeared on MacConkey agar in pale yellow color, not fermented with lactose sugar.

#### production Protease

1. (1 g) of casein was added to 90 (mL) of Tris-HCl (PH = 7.5, 0.1 molar).
2. Dissolve casein by heating it at a temperature of (80°C), then add the volume to (100 ml) of the same solution.
3. (5ml) of the bacterial suspension was transferred to the tubes containing the solution of the base material (prepared in the previous paragraph with three replicates for each isolate, and these tubes were tightly closed)
4. The tubes were incubated at 37°C for 30 minutes at a speed of 1500 rpm in a vibrating incubator.
5. After completing the incubation, it was placed in a centrifuge at a speed of (5000 rpm) for 15 minutes.
6. Separate the sediment from the suspended matter and keep the filtrate (enzyme leaching).
7. Casein (0.8 ml) was incubated in a water bath at a temperature of (37°C (0 for 10) minutes), then (0.2 ml) of the filtered enzyme was added and incubated for 20 minutes.
8. Stop the reaction, then add (30 ml) of (5% TCA) previously prepared and centrifuge at a speed of 6000 (rpm) for a period of (15 minutes).
- 9- Control samples were prepared by adding (3 ml) of (5% TCA) to (1% casein) and the same steps were repeated.(8)

### Biofilm formation

Using the heart and brain infusion medium, the *P. mirabilis* bacteria were grown for (24 hours) at 37 C (0), and then (200 l) was introduced to the pits of the calibration dishes, which were done in three repetitions and then repeated. To remove any remaining bacteria that may have stuck to its bottom and sides, this negative control was incubated for (24 hours) at (37 °C), washed with physiological saline solution, and then 200 (microliter) of alcohol was added. Afterwards, it was tested for the presence of bacteria. It is first necessary to dilute the crystal violet dye in (99%) percent methanol for 15 minutes before adding the 95 percent ethanol alcohol to stabilize the cells. The crystal violet dye is then removed with distilled water and dried before the ethanol alcohol at (99%) percent is added to stabilize the cells. Analyzing the optical density of the biofilm using an EIISA reader that has a wavelength of 630 nanometers.(9)

## Result and discussion

Table 1 Effect of pomegranate peel extract on protease production

concentration	Mean	Std. Deviation	Std. Error
30%	13.60	1.140	.510
40%	11.00	3.536	1.581
50%	6.20	1.304	.583
control	18.80	.837	.374

Pomegranate peel extract has been shown to reduce the synthesis of the enzyme protease in the proteus mirabilis.. The average productivity of bacteria treated with different concentrations of pomegranate peels 50,40,30% is 13,60,11,6.20% compared to the control 18.80% where the concentration 50% was the best concentration It worked to reduce the production of the protease enzyme by a large percentage compared to other concentrations that reduced the production of the enzyme by a small percentage The increase or decrease in the activity of the protease enzyme in food media may be due to the components of the medium as well as the quality and concentration of organic and inorganic substances and the role it plays for the production of the protease enzyme in bacteria and as a result of the difference in the productivity and diameter of the degradation in the synthetic medium used (10). The protease enzyme is one of the most important enzymes of virulence factors. P. mirabilis has the ability to break down IgA and IGg, thus losing its immune capacity..

Table 2 LSD value of Effect of pomegranate peel extract on protease production

Dependent Variable: protease production

LSD

(I) concentration of pomegranate peel extract	(J) concentration of pomegranate peel extract	Mean Difference (I-J)	Std. Error	Sig.
30 %	40%	2.600	1.273	.058
	50%	7.400*	1.273	.000
	control	-5.200*	1.273	.001
40%	30 %	-2.600-	1.273	.058
	50%	4.800*	1.273	.002
	control	-7.800*	1.273	.000
50%	30 %	-7.400*	1.273	.000
	40%	-4.800*	1.273	.002
	control	-12.600*	1.273	.000
control	30 %	5.200*	1.273	.001
	40%	7.800*	1.273	.000
	50%	12.600*	1.273	.000

\*. The mean difference is significant at the 0.05 level.

Table 2 shows less than the effect of pomegranate peel extract in the synthesis of protease in the proteases in the proteases, where there is a significant difference for 30% concentrations with 40% concentrations, but significant differences were found in the concentration with 50% and the control, while the 40% concentration found significant differences with the 50% concentrations and the control As for the 50% concentration, it was found that there were significant differences with all the

concentrations and the control ones, as shown in Figure 1. The results showed convergence with (11), in a study conducted by it, which recorded the highest activity (22.667 units / ml), while the results of (12) gave the measured quantity of protease production for the tested isolates, and the highest activity of protease was (8 units / ml), while the lowest activity of protease was 2.5. While the result of the qualitative method for protease production was (40%), and the results of (13) recorded the highest activity of protease (8 units/ml of *P. mirabilis* strains, as well as the percentage of protease activity ranged from (8-0.133 units/ml) and also results were recorded) The specific activity of the protease enzyme ranged between (14.5-0.121 units/ml) in the isolated strains of *P. mirabilis*.

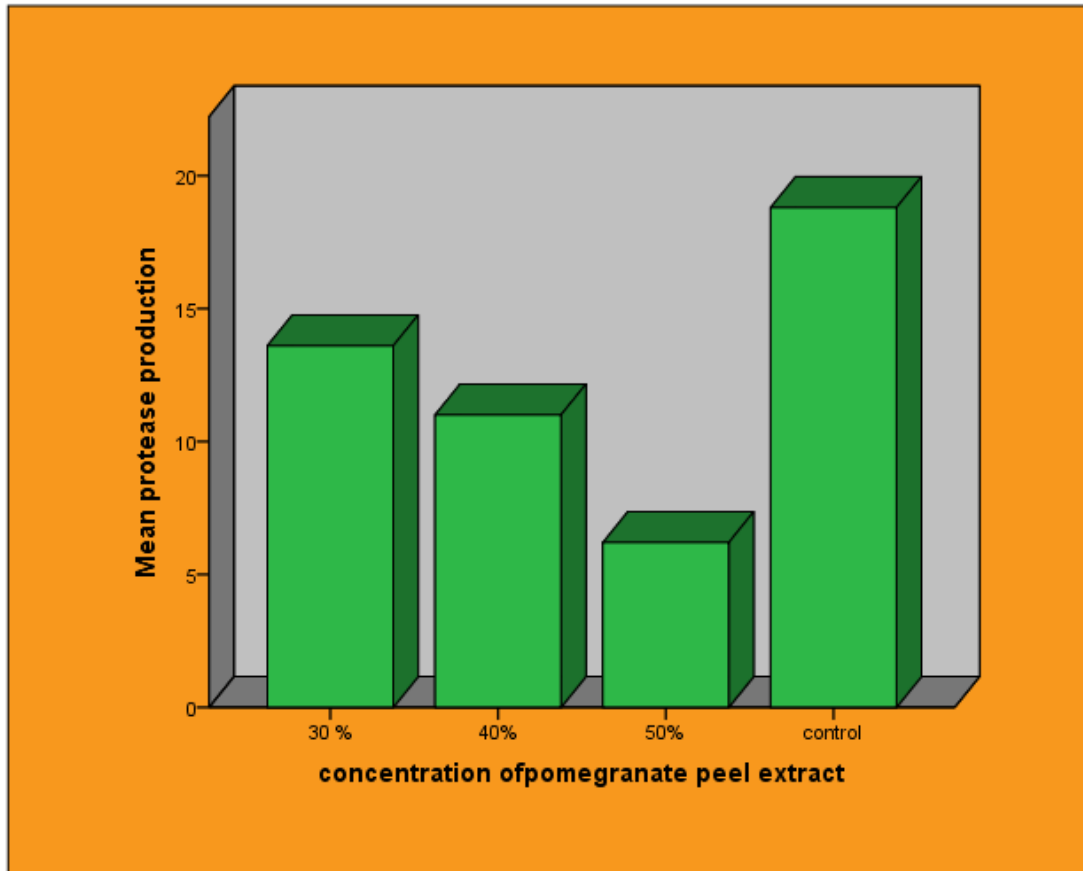


Figure 1 Effect of pomegranate peel extract on protease production

Table 3 Effect of pomegranate peel extract on biofilm production

concentration	Mean	Std. Deviation	Std. Error
30%	11.80	.837	.374
40%	9.40	3.647	1.631
50%	4.20	.837	.374
control	16.60	1.140	.510

In the proteus mirabilis, the extract from pomegranate pips was shown to reduce biofilm formation (see Table 3). Pomegranate peels were used in various concentrations to cure germs, with the average productivity being 11.8, 9.40, 4.20 % higher than the control concentration of 16.60 %. The concentration of 50% was the most effective. Compared to previous concentrations that lowered the development of biofilm, this one reduced it by a substantial percentage. The Tube Method was used to examine the *P. mirabilis* bacteria's susceptibility to biofilm development, and the results showed that a violet layer formed on the bottom and inner walls of the tubes. Pomegranate peel extract inhibited biofilm formation in our study. Bacterial isolates

Table 4 LSD value of Effect of pomegranate peel extract on biofilm production

con.of pomegranate peel extract	con.of pomegranate peel extract	Mean Difference (I-J)	Std. Error	Sig.
30%	40%	2.400	1.265	.076
	50%	7.600*	1.265	.000
	control	-4.800-*	1.265	.002
40%	30%	-2.400-	1.265	.076
	50 %	5.200*	1.265	.001
	control	-7.200-*	1.265	.000
50%	30%	-7.600-*	1.265	.000
	40%	-5.200-*	1.265	.001
	control	-12.400-*	1.265	.000

Table 4 shows less than the effect of pomegranate peel extract on biofilm production

In proteus mirabilis in the biofilm in where there is a significant difference for 30% concentrations with 40% concentrations, but significant differences were found in the concentration with 50% and the control, while the 40% concentration found significant differences with the 50% concentrations and the control As for the 50% concentration, it was found that there were significant differences with all the concentrations and the control ones, as shown in Figure 2. The virulence factors of P. mirabilis act in a synergistic manner to make it more virulent. (15) found that P. mirabilis has the ability to produce the enzyme urease, which makes the medium basic, which in turn leads to an increase in biofilm formation. In two similar studies, it was found that Bio-membrane increases in the basal medium. In addition to the studies of (3) He emphasized the role of mannose-resistant cilia (MR/P) in bio-membrane formation and bacterial cell assembly, thus causing infection and the formation of infections. Which supports the results of this study, which confirms the high ability of Proteus bacteria, which is one of its types, P. mirabilis isolated from different infections, to produce this enzyme, biofilm formation and the agglutination property of red blood cells. life

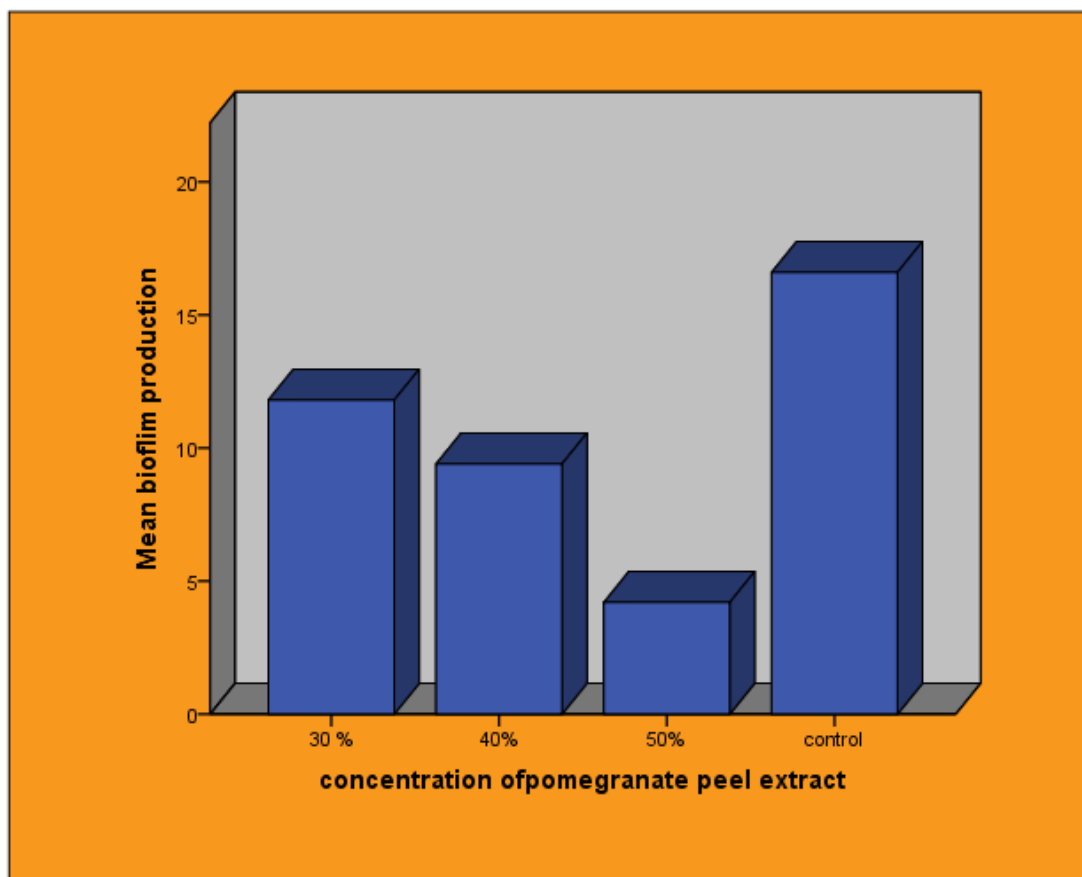


Figure 2 Effect of pomegranate peel extract on biofilm production

## REFERENCES

1. JAISINGHANI, Renu Narendra; MAKHWANA, Shweta; KANOJIA, Ankit. Study on antibacterial and flavonoid content of ethanolic extract of Punica granatum (pomegranate) peel. *Microbiology research*, 2018, 9.1: 7480.
2. MUSHTAQ, Mehvesh, et al. Use of pomegranate peel extract incorporated zein film with improved properties for prolonged shelf life of fresh Himalayan cheese (Kalari/kradi). *Innovative Food Science & Emerging Technologies*, 2018, 48: 25-32.
3. SAAD, Pasent Gharib, et al. Green synthesis of silver nanoparticles using Omani pomegranate peel extract and two polyphenolic natural products: characterization and comparison of their antioxidant, antibacterial, and cytotoxic activities. *Beni-Suef University Journal of Basic and Applied Sciences*, 2021, 10.1: 1-10.
4. DEVANESAN, Sandhanasamy, et al. Antimicrobial and cytotoxicity effects of synthesized silver nanoparticles from Punica granatum peel extract. *Nanoscale research letters*, 2018, 13.1: 1-10.
5. ALABDEEN, Zain, et al. Effect of Pomegranate Peel Extract on the Production of Some Enzymes in Proteus spp Isolated from Different Clinical Samples in Kirkuk city. *Kirkuk University Journal-Scientific Studies*, 2021, 16.3: 1-11.
6. AL-DEFIERY, M. E., et al. Antimicrobial activity of garlic and Pomegranate Peel extracts against some pathogenic bacteria. In: *IOP Conference Series: Earth and Environmental Science*. IOP Publishing, 2021. p. 012017.
7. HASSOON, Shaymaa Jabbar; MOHAMED, Walaah Mahmood; MOHSEN, Alaa. Isolation and Identification of Proteus bacteria from mouth and nose of human and animals with studying the Sensitivity to ethanol: aqueous extracts of pomegranate peel and Lantana cammara leaves. *Journal of Education and Scientific Studies*, 2021, 3.17.
8. HABIBIPOUR, Reza; MORADI-HAGHGOU, Leila; FARMANY, Abbas. Green synthesis of AgNPs@ PPE and its Pseudomonas aeruginosa biofilm formation activity compared to pomegranate peel extract. *International journal of nanomedicine*, 2019, 14: 6891.
9. PALCHYKOV, V. A., et al. Bactericidal, protistocidal, nematocidal properties and chemical composition of ethanol extract of Punica granatum peel. *Biosystems Diversity*, 2019, 27.3: 300-306.
10. SINGH, Balwinder, et al. Antimicrobial potential of pomegranate peel: A review. *International Journal of Food Science & Technology*, 2019, 54.4: 959-965.
11. CHEN, Xinxin, et al. Analysis of chemical compounds of pomegranate peel polyphenols and their antibacterial action against Ralstonia solanacearum. *South African Journal of Botany*, 2021, 140: 4-10.
12. KHALEEL, A. I.; SIJAM, K.; RASHID, T. S. Determination of antibacterial compounds of punica Granatum peel extract by tlc direct bio-autography and GCMS analysis. *Biochem. Cell. Arch*, 2018, 18: 379-384.
13. KHORMALI, Keyvan, et al. Novel Dy2O3/ZnO-Au ternary nanocomposites: Green synthesis using pomegranate fruit extract, characterization and their

- photocatalytic and antibacterial properties. *Bioorganic Chemistry*, 2021, 115: 105204.
14. KUMAR, Nishant, et al. Pomegranate peel extract-a natural bioactive addition to novel active edible packaging. *Food Research International*, 2022, 111378.
  15. ABDEL-AZIZ, S. H., et al. Antibacterial Potential of Pomegranate Peel Extracts on *Escherichia coli* Isolated From Benha Hospital in Egypt. *Benha Journal of Applied Sciences*, 2021, 6.3: 61-64.