

Effect of Alcoholic Extract of Pomegranate Peels on *Klebsiella pneumonia* and *Escherichia coli*

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ABSTRACT

Escherichia coli and *Klebsiella pneumonia* are common human pathogens that are responsible for causing a wide range of human infections, including urinary tract infections and many sexually transmitted diseases, which are raising a severe threat to public health and quality of life. Recently, many naturally occurring substances, including plants, have been interested in the production of therapeutic drugs. Pomegranate plants are proven to have considerable antibiotic and antimicrobial effects. The following study aims to investigate the impact of pomegranate extracts and their antimicrobial effects on *Klebsiella pneumonia* and *Escherichia coli* bacteria. The sensitivity of the tablets was examined on the Mueller Hinton agar medium of the microbe's understudy with a concentration of 10 continuous formation units/ml. The results indicated that the extracts of the pomegranate plants were more effective on *E.coli* as compared to *Klebsiella pneumonia*. However, the effectiveness and the antimicrobial effects of the extracts were evidently observed in both cases. Furthermore, there is a requirement to investigate the adverse effects and associated impacts of pomegranate extracts.



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1. Introduction

In recent times, one of the most common problems associated with medicines and healthcare therapy is antibiotic resistance [1]. The increased resistance to multiple drugs contributes to increased mortality and morbidity of the diseases. It has been observed that naturally occurring substances like plants and other herbs have been an important source of many therapeutic purposes for decades. These remedies and substances are highly effective and induce fewer adverse effects as compared to synthetic drugs. There is incredible evidence of the use of these natural substances as therapeutic products or using them as raw materials in the production of medical drugs. In the production of some pharmaceutical substances, many studies have examined the effect of plant extracts on the growth of microorganisms and thus their potential to be used in the treatment of some diseases caused by various microbial infections. Furthermore, it has been proved safe and effective for clinical use against diseases [2].

It is considered the original place of pomegranate in southwest Asia or Carthage, and its cultivation is widespread in most countries bordering the Mediterranean as well as in northwestern India. Pomegranate contains 78% moisture, 14.5% carbohydrate, 5.1% fiber, 1.6% protein, 0.1% fat and 0.7% mineral elements. Furthermore, pomegranates are majorly comprised of flavonoids, phenolic acids, Procyanidins, Anthocyanins, Gallic acid, Catechin, Ellagic acid, and tannins. These compounds contribute to their antimicrobial properties and other therapeutic effects [3].

The compounds which are extracted from pomegranate peels have a considerable effect against multiple microorganisms, including viruses like herpes virus, HIV, poliovirus, and influenza virus. Additionally, pomegranate peels are widely used in treating multiple clinical conditions like sore throat, cardiovascular diseases, rheumatoid arthritis, osteoarthritis, cancers and symptoms of AIDS [1]. Moreover, its action against the effects of bacteria and the fungi enables it to cure vaginal infections and associated reproductive infections, too [4].

Escherichia coli and *Klebsiella pneumonia* are gram-negative bacteria that belong to the family of Enterobacteriaceae. They are common human pathogens that are responsible for causing a wide range of human infections, including urinary tract infections and many sexually transmitted diseases, which are raising a severe threat to public health and quality of life [5].

The following study aims to investigate the impact of pomegranate extracts and their antimicrobial effects on the human infections causing gram-negative *Klebsiella pneumonia* and *Escherichia coli* bacteria under Mueller Hinton agar.

2. Materials and methods

2.1 Bacterial isolate

To meet the objective of the following research, the isolates of two bacteria were selected, including *Klebsiella pneumonia* and *Escherichia coli* which were obtained from Al-Salam Hospital in Mosul and then re-diagnosed for confirmation through the use of the McConkey medium and the observation of lactose sugar fermentation and the form of colonies and the use of IMVIC examination for diagnosis and examination of the API for an accurate diagnosis.

2.2 Preparing extracts and loosening

Alcoholic extracts of the pomegranate peels were prepared by collecting the pomegranate peels at home and drying them manually in the shade and having a suitable air stream, and then grinding and weighing them. They were used in preparing the alcoholic extract according to the method by Brown et al., 2019 [6]. As for the different concentrations and fears, they were prepared by adding 1 g of an extract of 5 ml of sterile distilled water to obtain 200 mg/ml, from which the different concentrations and fears were prepared. My agencies: (12.5, 25, 50, 100, 200, 300, 400, 500, 600, 750) mg / ml [7].

The sensitivity of the tablets was examined on the Mueller Hinton agar medium of microbe's understudy with a concentration of 10 continuous formation units/ml, and these tablets were made of filter paper with a diameter of 0.5 mm and under sterile conditions, and those tablets were dipped with the concentration mentioned above and then put those tablets on the aforementioned culture media and read Results were recorded 24 hours after a 30-degree bosom. Imipenem (10mg) was also used as a positive comparison and physiological solution NS. As a negative comparison.

3. Results

This study is considered one of the important studies on the effect of pomegranate husk extract on the growth and reproduction of *Klebsiella pneumoniae* and *Escherichia coli* bacteria. The study found that the extracts of the pomegranate fruit pods alcoholic effect noticeable inhibitor on the growth of *Escherichia coli* using tablets sensitivity examination on Mueller Hinton agar medium has been observed Variation in the sensitivity of the aforementioned bacterium to the alcoholic extract, which differed with the concentration of the extract used in the study.

Where Table (1) shows the diameters of inhibition of the growth of this bacterium, as dampening diameters appeared at a concentration (12.5,25,50,100,200,300,400, 500,600,750) mg/ml at a rate of (R, R, 13 13, 15, 15, 20, 18, 22, 22 (mm) for *Escherichia coli*, respectively, where an increase of the inhibition diameter of bacteria is observed, with an increase in the concentration of the extract, and so on for all dilution. 18, 20, 17, 22, and 21) mm, respectively, which indicates the effectiveness of this extract against this type of bacterium, as shown in Table (1) and figure 1.

As for *Klebsiella pneumoniae*, we notice a lesser effect of the extract on the bacteria due to the presence of the capsule, which is one of the virulence factors of the bacteria, and which increases its resistance to life antibodies. No. (2) The results (RR, R, R, 5, 15,15, 15, 17, 18) mm respectively, and when calculating the average of the three repeats, the results were as follows (R, R, R, 3.5, 8.5, 15, 16, 17, 17, and 18) mm, where we note that the higher the concentration of the extract, the greater the inhibition zone of the bacteria, as shown in Table No. 2 (3, 4 and 5). as shown in Table (1) and figure 1.

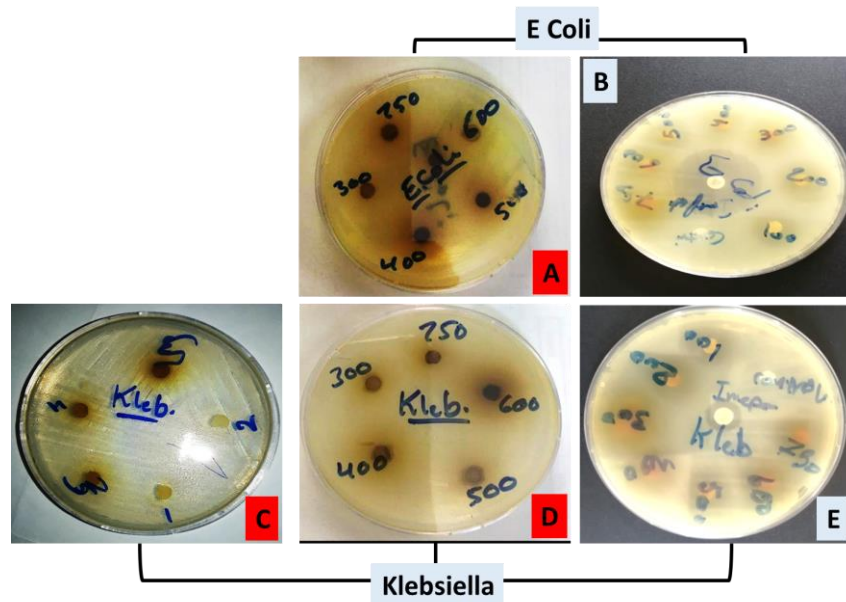


Figure 1. A representative image for the inhibitory zone of pomegranate peel extracts on *Escherichia coli* and *Klebsiella pneumoniae* using a serial dilution of extract as low as 12.5 mg/ml and up to 750mg/ml compared to positive control imipenem 10mg/ml.

Table 1. The Inhibitory Effect of Alcoholic Pomegranate Peel Extracts on *Escherichia coli* compared to Imepenem Antibiotic.

Mean ±SD	E .Coli			Extract Concentration (mg/ml)	K. Pneumonia			Mean ±SD
	Replicates (mm)				Replicates (mm)			
	1	2	3		1	2	3	

R	R	R	R	12.5	R	R	R	R
R	R	R	R	25	R	R	R	R
2.5±15	18	16	13	50	R	R	R	R
3±12	17	11	13	100	R	10	R	R
0.6±15	16	15	15	200	5	11	9	8.5±3
2.5±18	18	20	15	300	15	14	16	15±1
0±20	20	20	20	400	15	16	17	16±1
0.6±17	17	17	18	500	15	17	18	17±1.5
0.6±22	22	21	22	600	17	17	18	17±0.6
0.6±21	21	21	22	750	18	16	19	18±1.53
1±24	24	23	25	Imepenem 10mg	23	22	21	22±1
R=resistant, mm=millimeter inhibition zone								

4. Discussion

The increased incidence of urinary tract infections is highly associated with the growth and exposure of microorganisms. Amongst the most common bacterial infections in humans, urinary tract infections are at the highest rate. It has been found that most UTIs are caused by *Klebsiella* species and *Escherichia coli* [8]. Antibiotic resistance is the leading issue globally which contributes to the increasing cases of bacterial infections and also the increasing severity of the infections. Therefore, multiple approaches are taken to use plants and naturally occurring substances for the purpose of antibacterial and antioxidant effects [9].

In the evaluation of the effects of alcoholic extracts of pomegranate peels. Coil, According to the results of the following study, it has been found that in the case of *Escherichia coli*, the diameter of inhibition has increased in the McConkey medium in all the concentrations. The following result signifies the effectiveness of the extracts against *E.Coli*. These results are supported by multiple other studies, stated in their study that *Punica granatum* has significant antibacterial effects against *Escherichia coli* [10]. The extracts of the following plant are highly impactful in both the aqueous and alcohol forms. Furthermore, pomegranate has been widely used in home remedies to treat multiple diseases, particularly gastrointestinal disorders [11].

Similarly, [12] represented in the results of their study that the alcoholic extracts of the pomegranate plant have strong antimicrobial and anti-oxidative effects. The following results were studied on the isolates of the *E.coli* bacteria, where the least inhibitory concentration of the extracts was 12.5mg/ml and 25 mg/ml. However, the minimum inhibitory concentration and minimum bactericidal concentration of the peel of pomegranate was 25 mg/ml, and the for the seed extracts, it was 50mg/ml. Additionally, studies have shown that both the extracts of the seeds pomegranate and the peel shows significant antibacterial results; however, stronger antibacterial results have been seen from the peel extracts as compared to the seed extracts [5].

In another study, it was said that the diameter of the inhibition zone of the extracts from pomegranate was most commonly against the two bacteria, *Escherichia coli* and *Staphylococcus aureus*. Their effect increased with the increase in the concentration of the extract. On the basis of the solvent, the best and the most effective solvent was alcohol.

Following the other specie which is studied in the text, it has been found that the extracts have been presented to have lesser effects as compared to *e.coli*. The possible reason for the reduced effect is probably the capsule around the bacteria. *Klebsiella pneumonia* is an encapsulated, gram-negative bacteria that primarily causes urinary tract infections, pneumonia and other infections in humans [13].

In the other relevant studies, it has been found that the pomegranate extracts have a significant antibacterial effect on the isolated *Klebsiella pneumoniae*. Furthermore, in the comparative study, it was stated that the peel of pomegranate fruit has an active effect on a broad panel; additionally, the peel of the fruit exhibits a stronger and greater impact as compared to the potable juice [14].

5. Conclusion

Herbal plants have been greatly utilized for treatment of various diseases including infectious diseases. Pomegranate peels shown important antibacterial effects against common infectious diseases induced by *K. Pneumonia* and *E coli* which might be helpful in part in the treatment of infectious diseases overriding antibiotic resistance in general population.

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