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Antimicrobial Activity Evaluation of Punica grantum (Pomegranate) Against Urinary Pathogenic Escherichia Coli (UPEC)

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Abstract: Numerous research have documented the antibacterial, anti-inflammatory, antiparasitic, and anticancer properties of punica grantum in therapeutic settings. Objectives: To ascertain whether punica grantum juice has any potential antibacterial activity in preventing urinary pathogenic Escherchia coli (UPEC) isolates from female outpatients. The antimicrobial effect of punica grantum juice, against urinaty pathogenic Escherichia coli isolates, was studied using well- diffusion method, and the impact of antibiotic-infused Punica grantum juice discs on (UPEC) isolates was also studied using disc- diffusion method, then the statistical study was performed using Statistical Package for the Social Sciences (SPSS) program. Punica grantum juice has antimicrobial activities on UPEC isolates at concentrations of 400mg/ml and 200mg/ml. It showed 1.7 cm of the average inhibition zone at concentration of 400 mg/ml, and 1 cm at concentration of 200 mg/ml against UPEC. There is a statistically significant differences in the diameters of inhibition zones of antibiotic-resistant E. coli after the addition of pomegranate juice at a concentration of 200 mg/ml.

Key Words: punica grantum, juice, escherichia coli; (UPEC), antimicrobial activity

I. INTRODUCTION

Worldwide, the spread of dangerous infectious organisms, such as Escherichia coli, is contributing to a high rate of morbidity and mortality. Furthermore, there has been a noticeable rise in the rate of antibiotic resistance [1], [2].

Escherichia coli, or E. coli, is a typical component of the gut flora in humans. But some E. Coli strains have the ability to produce toxins and become resistant, which could result in dangerous infections [3]. In addition, E.coli is the main cause of urinary tract infections [3], urinary tract infections occur at a higher rate in young women due to the ease of transmission of the pathogen from the intestines [4] and It can also spread to the meninges and the circulation [3]. Numerous investigations have revealed an increase in Escherichia coli multidrug resistance (MDR) globally [5], [6]. Thus, the scientists' decision to try new remedies is prompted by the prior problem. Over time, plants with biological components have demonstrated a significant and minimally hazardous impact on human health. There is a good chance that these natural components will be used to create new medications [7]. Punica grantum juice contains metabolites, primarily phenolic derivatives, which bind to bacterial proteins and reduce pH without causing resistance in recorded cases to impede bacterial growth [8]. Due to the plant's high phenolic chemical concentration, numerous research have documented Punica grantum's therapeutic properties, which include anti-inflammatory, antibacterial, anticancer, and antiparasitic effects [9], [10]. Finding Punica grantum juice's antibacterial efficacy against UPEC was the goal of the current investigation.

II. MATERIALS AND METHODS

This investigation was carried out in a microbiological laboratory, Tishreen University Hospital, Lattakia, Syria, in 2022. The fruits were chilled, not fresh.

A. PREPARATION OF PUNICA GRANTUM JUICE

The fruit washed in a lab under running water, had its exterior surface sanitized with 70% alcohol, washed again with sterile distilled water, was cut with a sterile knife, and had its juice squeezed into a sterile container. To remove the seeds and other tissues, the acquired juice was filtered via filter paper into additional sterile containers. Then the juice was lyophilized, and the rest was weighted.The lyophilized juice

was dissolved in 1 ml of sterile distilled water, to be the first concentration to test. Four extensions were prepared. They were 400 mg/ml, 200mg/ml, 100 mg/ml, 50 mg/ml.

B. MICROBIAL ISOLATES AND ANTIBACTERIAL ACTIVITIES OF PUNICA GRANTUM JUICE

UPEC were detected and isolated by standard biochemical procedures [11] at microbiological laboratory, Tishreen University Hospital, Lattakia, Syria .Ten isolates of urinary pathogenic Escherichia coli (UPEC) were treated with the four concentrations Punica grantum juice, by well diffusion assay on Mollar-Hinton agar dishes. The agar dishes were prepared to perform the juice by opening 6mm wells. The bacterial isolates were transferred into Mollar-Hinton agar petri dishes. 60μl of each concentration were performed into the wells. In addition to sterile distilled water in a different well as a blank and the amikacin disc as a control. the dishes were incubated at 37ºC for 18-24 hours. Using a measuring ruler in centimeters, the inhibition zones were identified following a 24-hour incubation period.

C. THE IMPACT OF ANTIBIOTIC-INFUSED PUNICA GRANTUM JUICE DISCS ON ESCHERICHIA COLI ISOLATES USING DISC DIFFUSION METHOD

Seven antibiotics were selected to study their synergistic effect with pomegranate juice on twenty isolates of (UPEC) of female patients, which are: (Amikacin, Nitrofurantoin, Amoxicillin with Clavulanic Acid, Trimethoprim + Sulfamethoxazole, Cefixime, Levofloxacin, Ciprofloxacin). The antibiotic disks were impregnated with 50 microliters of the concentration (200 mg/ml) of pomegranate juice, and dried in a UV-sterilized chamber then they were performed on 20 E. coli isolates, within Mueller-Hinton agar medium.

D. STATISTICAL STUDY

The data was processed using the statistical program SPSS, and the distribution curve of the variables was evaluated using the Kolmogorove-Sminrove test. The distribution was found to be non-normal, so the Wilcoxon signed-rank test was performed to study the effect of adding the juice within the anti-micro biotic discs on the diameters of inhibition zones induced by the antimicrobials used.

III. RESULTS

A. ANTIBACTERIAL EFFECT OF PUNICA GRANTUM JUICE

The sensitivity of UPEC against Punica grantum juice concentrations was determined. We found two bacterial inhibition zones at concentrations of 400 mg/ml and 200 mg/ml, with average inhibition zone diameters of 1.7 cm and 1 cm respectively. The average diameters of inhibition zones of amikacin disc were 2.3 cm, while for distilled water it was 0 cm. The inhibitory effectiveness of pomegranate juice on Escherichia coli bacteria was obtained in a study (Al Sataf et al. 2021) in Turkey [8], with an inhibition zone diameter of 1.3 cm at a concentration of 125 mg/ml. See Figure 1.

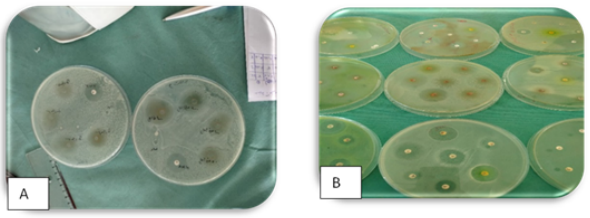

FIGURE 1: Antibacterial effect of Punica grantum juice; A: antibacterial effect of pomegranate juice on UPEC, B: UPEC sensitivity test for antibiotic -infused pomegranate juice discs

B. E. COLI ANTIBIOTIC SENSITIVITY TEST BEFORE AND AFTER APPLICATION OF THE JUICE

The Table 1 shows the mean values of the inhibition diameters induced by antibiotics and demonstrates the change in these values after adding the juice to the antibiotic discs. We observe a shift in the mean diameter inhibition value of amikacin with the juices from resistant (R) to sensitive (S), as well as for ciprofloxacin when adding pomegranate juice. We also find that the mean values of inhibition diameters for amoxicillin with clavulanic acid, and nitrofurantoin, have changed from intermediate (I) to sensitive (S). Also, these values remained (S) but in a higher value for levofloxacin and amikacin discs. The value (R) remained constant before and after the addition of juice to the cefixime disc.

The table 2 shows that the P-value for the inhibition zones induced by the antibiotics used is less than 0.05. Therefore, the variable of inhibition zones before applying the juice follows a non-normal distribution. Thus, we conducted a non-parametric Wilcoxon signed-rank test to study the effect of adding the juice to the discs of antibiotics on the inhibition zones induced by the antibiotics used. The quantitative variables were expressed as mean ± standard deviation.

C. STUDY OF THE IMPACT OF POMEGRANATE ON INHIBITION ZONES INDUCED BY ANTIBIOTICS USING WILCOXON SIGNED RANKS TEST

To investigate the effect of pomegranate juice (200 mg/ml) on the inhibition zones induced by the antibiotics used, we conducted a Wilcoxon signed ranks test, and the results are as shown in the Table **??**.

We notice from the preceding Table **??** that the P-value is <0.05 for pomegranate juice, at a concentration of (200 mg/ml) with all the antibiotics used, which are (trimethoprim+ sulfamethoxazole, amikacin, amoxicillin+ clavulanic acid, cefixime, levofloxacin, ciprofloxacin, nitrofurantoin). This indicates statistically significant differences in the zones of inhibition induced by the antibiotics used before and after adding pomegranate juice. We also note that the Z value in the previous table is negative, hence the addition of pomegranate juice has caused a statistically significant increase in the zones of inhibition.

antibiotics	Mean± Standard deviation	
	Inhibition zones (cm)	Inhibition zones (cm) after adding pomegranate juice
Trimethoprim Sulfamethoxazole	0.55±0.861	1.63±0.886
Amikacin	1.955±0.737	2.49±0.427
Amoxicillin + Clavulanic Acid	1.4±0.988	1.975±0.830
Cefixime	0.51±0.664	1.085±0.695
Levofloxacin	1.825±0.574	2.22±0.718
Ciprofloxacin	1.355±1.11	2.01±0.789
Nitrofurantoin	1.585±0.818	2.16±0.549

TABLE 1: E. coli antibiotic sensitivity test before and after application of the juice

Antibiotics	P-value	
	Inhibition zones (cm)	Inhibition zones (cm) after adding pomegranate juice
Trimethoprim Sulfamethoxazole	<0.0001	0.2
Amikacin	<0.0001	0.2
Amoxicillin + Clavulanic Acid	0.001	0.017
Cefixime	<0.0001	0.033
Levofloxacin	0.007	0.012
Ciprofloxacin	0.004	0.079
Nitrofurantoin	0.023	0.2

TABLE 2: Results of the Kolmogorove-Sminrove test

Antibiotic + Pomegranate Juice	P-VALUE	Z -VALUE
(Trimethoprim + Sulfamethoxazole) + Pomegranate Juice	0.000	-3.62
Amikacin + Pomegranate Juice	0.000	-3.64
Amoxicillin + Clavulanic Acid)+ Pomegranate Juice	0.000	-3.52
Cefixime + Pomegranate Juice	0.001	-3.18
Levofloxacin + Pomegranate Juice	0.003	-.2.9
Ciprofloxacin + Pomegranate Juice	0.000	-3.6
Nitrofurantoin + Pomegranate Juice	0.001	-3.31

TABLE 3: The results of the Wilcoxon signed ranks test study the effect of Pomegranate Juice (200 mg/mL) on the diameters of inhibition zones induced by the used antibiotics

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IV. DISCUSSION

Many research have verified punica grantum's antibacterial, antioxidant, and anticancer properties [9], [10] . Punica grantum possesses flavonoids, anthocyanins, and tannins as its active ingredients against bacteria [8].

Pomegranate juice contains various chemical compounds such as Ellagitannins (soluble tannins), phenols, tannic acid, punicic acid, flavonoids, anthocyanins, phytoestrogenic flavonoid compounds, and flavonols. Tannic acid (abundantly present in pomegranate) is considered toxic to microorganisms, where the hydrophilic portion interacts with the polar region of the bacterial membrane, while the hydrophobic portion is immersed within its non-polar inner region, potentially leading to its instability [12]. In an effort to address the issue of antibiotic resistance, the current study assessed the antibacterial activity of pomegranate juice as a medicinal plant and the extent to which it may be utilized as a therapeutic agent in the treatment of urinary tract infections. However, earlier study reported higher antimicrobial activity of punica grantum juice with different concentration against E. coli .The study showed 13 mm of inhibition zone average at the concentration of 125 mg/ml [8]. Additionally, stated that pomegranate juice also had antibacterial effect with 20 mm against E.coli [13]. Since amikacin is a therapy option for urinary tract infections caused by E. coli, it was chosen as the antibiotic for this investigation and utilized as a control.

V. LIMITATIONS

To evaluate the antibacterial properties of punica granatum, additional research can be conducted utilizing various pomegranate parts and a variety of solvents at varying concentrations. These solvents include water, petroleum ether, acetone, and ethyl acetate extract.

VI. CONCLUSION

According to the current study, pomegranate juice may be used as a possible means of limiting the growth of E. coli in urinary tract infections. As additional research, the antibacterial properties of the punica grantum extracts against other microbiological isolates, including Salmonella, Pseudomonas, Staphylococcus, and Klebsiella, might be assessed.

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CONFLICTS OF INTEREST

No conflicts of interest have been declared by the authors.

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