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Research Article

Antibacterial Effect of Herbal Extracts on *Escherichia coli* Isolates from Urinary Tract Infection

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Abstract

Escherichia coli is seen as an important bacterium that causes diarrhea in humans and animals. In addition to being associated with intestinal infections, it is commonly associated with urinary tract infections. As a treatment, several antibiotics are used to control these infections, but over time the development of microorganisms resistant to their active principles has been observed, causing medicine to seek new treatment alternatives. Among the most widely used is herbal medicines, compounds made from the extract of medicinal plants. *Punica granatum* is an herbal medicine well known for its anti-inflammatory action and high antimicrobial content. Thus, it is important to investigate whether *Punica granatum* extract has antimicrobial action on strains of the bacterium *Escherichia coli*, inhibiting its growth. This study aimed to analyze the inhibition of *Punica granatum* extract on the growth of an isolate of *Escherichia coli* from a patient with urinary tract infection. The isolates of *Escherichia coli* were donated by the Laboratory of Clinical Analysis San Francisco. The method used for the distribution of the extracts in the plates was the diffusion of discs in agar. The experiment was carried out in triplicate. It was observed that there was no inhibition of the growth of the bacterium *Escherichia coli* in any concentration of the tested extracts. Between the treatments designed and applied, there was a significant difference, the positive control presented efficiency in the inhibition of *Escherichia coli*, while the negative control did not present antimicrobial activity, as expected. In view of the results obtained, it was concluded that the aqueous extracts of the fruit of *Punica granatum* did not inhibit the growth of the bacterium *Escherichia coli*, but there was a reduction of bacterial growth in the treatments with the peel and mesocarp of the fruit, showing that in both there is antimicrobial activity on isolates of *Escherichia coli*.

Keywords: Antibiogram; *Punica granatum*; Inhibition Halos; Uroculture

Introduction

Escherichia coli is a facultative anaerobic bacterium belonging to the family Enterobacteriaceae. Found in the normal bacterial flora of humans, it was considered harmless for a long time, however, it has been seen as causing diarrhea in humans and animals. In addition to being associated with intestinal infections, this bacterium has its name linked to urinary infections that, according to research previously conducted by Murray [1], this microorganism is the most common agent, being present in about 80% to 90% of acute bacterial infections. These urinary tract infections can affect the urethra causing urethritis, the bladder causing cystitis and the kidneys causing pyelonephritis [2].

Urinary Tract Infections (UTIs) are the invasion and multiplication of bacteria from the human and animal intestines in the urinary tract. The colonization of urinary tissue occurs due to the rise of intestinal bacteria from the anus to the urinary orifice, causing invasion of the urethra, bladder and ureters, and may even compromise the kidneys. These infections are evidenced in different phases, from urethritis to severe pyelonephritis [2].

According to Brandino., *et al*. [3] this type of infection occurs at all ages, affecting mainly males in the first year of life, due to congenital malformations. However, at preschool age there is a higher frequency in girls, which extends until adulthood. This is because, the woman has some sites that are more susceptible to UTIs, such as the larger bladder, thus being able to store urine for longer, in addition to having the urethra shorter, there is a greater proximity of the anus to the vagina and absence of antimicrobial properties, such as those found in the prostatic fluid.

To control UTIs and other types of bacterial infections, several antibiotics are used in order to inhibit the growth of these bacteria, but over time, most of these drugs have been shown to be ineffective, since the microorganisms become resistant to their chemical compounds [4].

Due to the resistance of bacteria to antibiotics and the side effects they cause to the human body, man has sought new treatment alternatives, among them is herbal medicines, remedies made with medicinal plant extract, which according to Veiga Jr., *et al*. [5]. In this context, pomegranate is a plant well known for its anti-inflammatory action and a high antimicrobial content.

Punica granatum, commonly known as pomegranate, is a woody, branched shrub belonging to the Punicaceae family, popularly known as pomegranate, widely cultivated throughout Brazil, but originated in Asia. It has small, hard, bright and membranous leaves, red-orange flowers arranged at the ends of the branches, originating spherical fruits, with many angular seeds in layers that are wrapped in pulpy aril [6].

According to Navarro., *et al*. [7], in Mexico, pomegranate is used for the cure of diarrhea, vomiting, thrush, parasitism, abscesses, cough, angina, pharyngitis, urinary tract infections and skin injuries. According to Menezes., *et al*. [8], it is also used as an astringent, hemostatic, antidiabetic, anthelmintic, antiseptic and antiviral.

Faced with numerous health disorders caused by the actions of bacteria, it was questioned whether the aqueous extract of the fruit of *Punica granatum* would have antimicrobial action on isolates of the bacterium *Escherichia coli*, inhibiting its growth. It was expected that the extract of the bark of *Punica granatum* would inhibit the growth of the bacterium in question, since, according to Negi., *et al*. [9], the extract of the bark of the pomegranate is rich in polyphenols and tannins, which have shown a strong antiseptic effect and also antimicrobial activity against gram-negative and gram-positive bacteria.

In view of the above, the general objective of this study was to investigate and describe the inhibition of the growth of *Escherichia coli*, using the aqueous extract of the *Punica granatum* fruit. The specific objectives were to verify the existence of the antimicrobial content from all parts of the *Punica granatum* fruit, pointing out which part of the fruit has the greatest potential for antimicrobial action on the colonies of *Escherichia coli*.

Methodology

Five fruits of *Punica granatum* were used to produce the extracts, and all parts of the fruit, peel, mesocarp, pulp and seed were used. For the experiment, four extracts were made, one for each part of the fruit, being them, peel, mesocarp, pulp and seed. The fruits were washed in running water, sanitized for 15 minutes in 0.5% sodium hypochlorite solution and dried in paper towels [8].

The isolate of *Escherichia coli* was provided by the São Francisco Laboratory of Goiatuba-GO, from a urine culture from an elderly female patient with a clinical picture of urinary tract infection. The inoculums were prepared by taking three to four colonies of the bacterium isolated in Müller-Hinton agar and diluted in 0.85% saline solution [10].

For the evaluation of antibiotics that inhibit the growth of the bacterium, as well as their concentrations, an antibiogram was performed using 25 antibiotic discs, as shown in Chart 1. Among the active principles, the Ceftriaxone disc was chosen to be used as a positive control because it presents a higher halo of inhibition.

To investigate which of the extracts would inhibit bacterial growth, the diffusion disc test described by Kirby-Bauer was

Antibiotics	Concentrations	Antibiotics	Concentrations
Nalidix Acid	30 MCG	Ciprofloxacin	5 MCG
Pipemidic Acid	20 MCG	Clindamycin	2 MCG
Amicacina	30 MCG	Co-Trimoxazole	25 MCG
Amoxicillin + Ac. Clavulânico	30 MCG	Gentamicin	10 MCG
Amoxacilin	10 MCG	Imipenem	10 MCG
Ampicillin + Sulbactram	20 MCG	Levofloxacin	5 MCG
Ampicillin	20 MCG	Nitrofurantoína	300 MCG
Cephalexin	30 MCG	Norfloxacina	10 MCG
Cephalothin	30 MCG	Penicillin	10 UI
Cefotaxime	30 MCG	Perfloxacina	5 MCG
Cefoxitina	30 MCG	Tetracycline	30 MCG
Ceftadizima	30 MCG	Tobramycin	10 MCG
Ceftriaxone	30 MCG		

Table 1: Antibiotics used in antibiogram and their respective concentrations used in test.

performed, according to the protocol of the Clinical and Laboratory Standards Institute [10]. Each suspension of microorganism was sown with the aid of a disposable sterile swab, on the entire surface of Muller Hinton agar medium. Then, filter paper discs (Whatman®, type 3), 6 mm in diameter and impregnated with 20 µL of each extract were added [11].

In the experiment, 30 petri dishes were used, in 05 replications per group. We used 06 experimental groups, being a negative control with distilled water, another positive control with the antibiotic Ceftriaxone and 04 groups of different aqueous extracts of *Punica granatum*, bark, mesocarp, pulp and seed. The experiment was carried out in triplicate, according to the sampling design presented in figure 1.

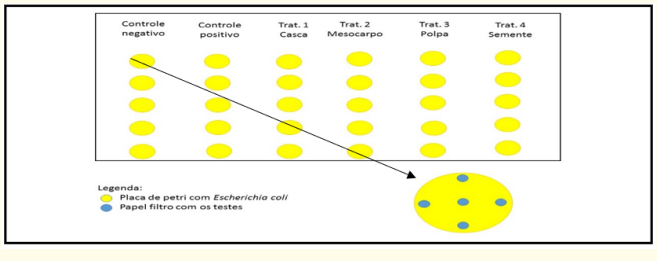

Figure 1: Sampling design for the performance of the antibiogram.

After placing the discs on the inoculum, the plates were inverted and incubated in the bacteriological greenhouse at 35°C for 24 hours. After that, the plates were read individually, measuring the diameter of the halos formed with the aid of a halometer, comparing tests and controls. The extracts that showed antimicrobial action were statistically analyzed using analysis of variance (ANOVA), with the aid of the MYSTATE program.

Results and Discussion

The results showed that there was no inhibition of the growth of the bacterium *Escherichia coli* in any of the extracts tested, because they did not present the formation of the halo of inhibition around the colonies. However, when observing the antibiogram plates, one can notice indications of antimicrobial activity represented by less intense bacterial growth or confluence in the vicinity of the discs impregnated with extracts from the bark and mesocarp of *Punica granatum*, as shown in figures 2, 3 and 4.

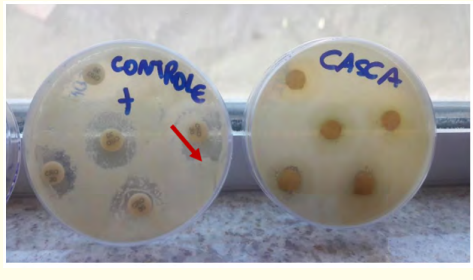

Figure 2: Petri dishes with positive control and bark extract, indicating with a red arrow where there was the less intense growth of *Escherichia coli*.

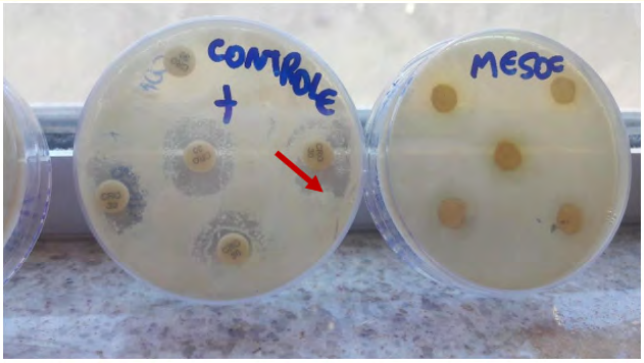

Figure 3: Petri plates with positive control and mesocarp extract, indicating with a red arrow where there was the less intense growth of *Escherichia coli*.

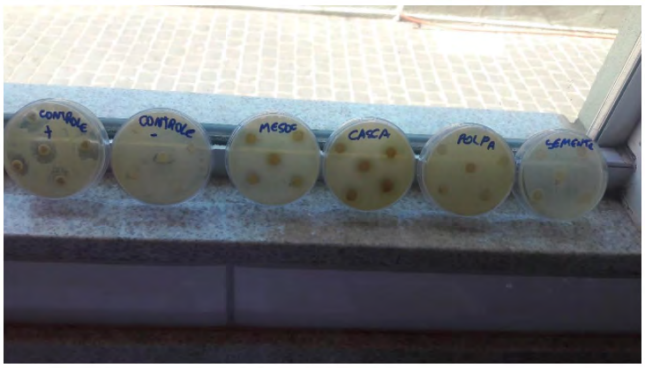

Figure 4: Petri dishes with positive control, negative control and treatments.

The discs of Ceftriaxone, a chemical antibiotic used as a positive control, showed expected efficacy of this active ingredient, with inhibition of the confluent growth of the bacterium and formation of halos ranging from 16 to 30 mm. The negative control also did not present any antimicrobial activity, which was already expected, since the discs of the same were impregnated with distilled water, figure 5.

Commonly, urinary tract infections are caused by bacteria, mainly Gram-negative ones, with *Escherichia coli* accounting for more than 70% of cases [12]. According to studies conducted, many plants have been tested and evaluated for antibacterial

Figure 5: Petri plates with positive and negative control.

activity against pathogens causing urinary tract infection Sharma., *et al*. [13]. In the present study, we chose to test the antibacterial potential of *Punica granatum*, given the description of previous scientific studies that showed strong antibacterial capacity of this fruit against *Escherichia coli* [11].

According to the results obtained in the analyses, it was found that although the treatments are not effective in fighting the bacteria, they do not rule out the antimicrobial activity of the bark and mesocarp extracts, because both presented in the vicinity of their discs, less intense bacterial growth. Similar results were found by Teles., *et al*. [11], when they reported the antimicrobial action of two extracts, among them the extract of the bark of *Punica granatum* using the same dilution (50%), on the bacteria *Escherichia coli* and *Staphylococcus aureus*.

The isolate of the bacterium *Escherichia coli* donated by the laboratory was removed from the urine culture examination of an elderly patient and according to Pompeo., *et al*. [14], the bacteria that cause urinary infections in elderly women are more resistant than those found in the younger population, due to the cycle of antibiotics ingested over the years, which would justify the fact that the extracts tested did not inhibit the growth of this bacterium.

Different inhibitory profiles of the extract of the bark and mesocarp against microorganisms may be, in part, due to the different methodologies of extraction, ripening of the fruit used and variations in the time and region of cultivation [15]. Many studies have opted for other types of extract, such as hydro-alcoholic,

stanolic, and glycoalcoholic extracts, and have been able to obtain satisfactory results in the inhibition of *Escherichia coli*.

The antibiotic Ceftriaxone used as a positive control is highly recommended in the treatment of urinary tract infections caused by Escherichia coli, as well as in other infections caused by other bacteria, because this antibiotic inhibits the synthesis of the cell wall of these microorganisms [16].

All extracts prepared in this study did not show inhibition halo. Thus, it is evident that their dilution was not effective in inhibiting the growth of the bacterium. According to Pereira., *et al*. [17], herbal extracts are able to inhibit bacterial growth when they present a halo of inhibition greater than 15 mm.

Voravuthikunchai., *et al*. [18] tested 58 aqueous and alcoholic extracts from 38 plant species used to treat gastrointestinal and urinary infections in Thailand. They verified antibacterial activity of 08 species on different colonies of *Escherichia coli*, among them *Punica granatum*. According to Telles., *et al*. (2014), the polycalic tannin is responsible for the antimicrobial activity of *Punica granatum*, and the microorganisms are affected by this tannin, through a reaction that occurs between this compound and sulfhydryl, a component present in bacterial proteins.

Conclusion

For the control of urinary infections, chemotherapy or antibiotics are prescribed and, alternatively, herbal medicines have shown potential antibacterial action for therapeutic purposes. In view of the results obtained, it is concluded that the aqueous extracts of the fruit of *Punica granatum* did not inhibit the growth of the bacterium *Escherichia coli*, however, there was a reduction in the confluence of bacterial growth with extracts based on peel and mesocarp of the fruit, showing that in both there is antimicrobial activity. The use of alternative and appropriate antimicrobials in the performance of an antibiogram provides a better adaptation of treatments, since it assists medical professionals in the prescriptions of effective and alternative antibacterial in the fight against pathogens that have importance in health.

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