Cranberry Juice Inhibit Bacterial Pathogens Associated To Urinary Tract Infection

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Authors’ contributions

This work was carried out in collaboration among all authors. Author CC led laboratory analysis interpretation of results and wrote the draft of the manuscript. Author JMLNG designed the study and revised the discussion of data. Author MF revised the manuscript and references. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To evaluate in vitro antimicrobial potential of 35% reconstituted juice (RJCr) against bacterial pathogen related to urinary tract infections (UTIs).

Place and Duration of Study: Food and Biotechnology Laboratory, Brazil.

Study Design: Cranberry juice for in vitro evaluation by agar well diffusion assay and direct in vitro assay.

Methodology: Cranberry fruits were used to produce RJCr pH 3. Five bacterial pathogens were tested: Escherichia coli ATCC 35218, Klebsiella pneumoniae ATCC 700603, Enterococcus faecalis ATCC 29212, Pseudomonas aeruginosa ATCC 27853 and Proteus mirabilis, an isolate of clinical origin. Two methods were used to assess antimicrobial activity. In the agar well diffusion (AWD) assay, each pathogen was inoculated on agar plates and the juice was added in wells drilled on this agar by incubation at 35°C/24hours. Then, the diameter of the inhibition zone was measured (mm). Based on dilutions methods, a direct in vitro assay (DA) was also performed. In test tubes 4.5 ml of RJCr was added an inoculum of each pathogen for a final concentration of >10⁶ and <10⁷ CFU.mL⁻¹. The performance was evaluated based on CFU.mL⁻¹ resulting on agar plates (35°C / 24 hours).

Results: By using AWD the RJCr inhibited E. coli and the average size of the diameter of inhibition halo reached 23.3 mm, that is, greater when compared to the group with Chloramphenicol (11.6

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mm). However, for the other strains the RJCr was not inhibiting with this method. But, by using DA, the action of RJCr was inhibitory for all strains here tested, with an average of 5.1 Log cycles of reduction in relation to initial concentration. For *E. coli* and *P. mirabilis* the reduction reached six Log cycles.

**Conclusion:** The inhibitory effect of RJCr was evident to *E. coli* by both types of inhibitory methods, a relevant result since it is the most recurrent microorganism in UTIs. Cranberry juice was stronger in inhibiting *E. coli* than antibiotic chloramphenicol as observed by AWD. Thus, the study reinforces the importance of Cranberry, even in the form of juice, in inhibiting *E. coli*.

**Keywords:** Proanthocyanidins; urinary infection; *Vaccinium macrocarpon*; antimicrobial methods.

### 1. INTRODUCTION

Urinary Tract infections (UTIs) are the main causes of consultations in medical practice [1]. About 150 million ITUs per year are reported worldwide [2]. Studies indicate that 48% of women, 12% of men and 7% of children will have at least one episode of UTI throughout their lives, and about 20% of women will have recurrent cases [3,4,5].

The UTIs are classified based on location in the urinary tract. As recently pointed by Medina & Castillo-Pino [6] the German guidelines consider the following main types of UTIs: Uncomplicated UTI - without relevant functional or anatomical abnormalities in the urinary tract; Acute uncomplicated cystitis - acute symptoms involving only the lower urinary tract; Uncomplicated acute - acute pyelonephritis with symptoms, flank pain, fever; Asymptomatic bacteriuria - positive urine culture in the absence of urinary symptoms; Recurrent uncomplicated UTIs - occurrence of 2 or more symptomatic episodes in 6 months or 3 or more symptomatic episodes in 12 months.

Women are more susceptible to urinary tract infection due to the use of contraceptives; antimicrobial resistance, menopause, sexual intercourse, genetic factors, and because they have a shorter urethra [4]. Although more common in women, urinary infections increase in male sex over 50 years of age and in bedridden due to the use of bladder catheterization, disease prostatic and the presence of comorbidities [7].

The urinary tract infection occurs when the bacteria pass through the urethra, adhere to the bladder wall, and proliferate [8]. Adhesion can occur through pil or fimbriae, which are structures responsible for the adhesion of the bacteria to the tissue [9].

The etiological agents most responsible for UTI are bacteria: *Escherichia coli*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Enterococcus faecalis*, *Staphylococcus aureus* and *Proteus mirabilis* [10,11]. But *E. coli* is the main responsible in 75 to 90% of the cases [12].

The most used treatment for UTI consists of antibiotics, since the discovery of Penicillin in 1928, they have been used on large scales [13]. However, with the technological advances of these drugs and their routine use they have become a gradual problem for the population, causing adverse reactions, gastrointestinal problems and resistant bacterial strains that have also developed causing morbidity and mortality in the population [13,14].

Cranberry (*Vaccinium macrocarpon*) belongs to the Ericaceae family and is a red colored fruit, with a sour taste; it is native to North America, grows in acid swamps and humid forests [15]. In the past, Cranberry was widely used by Indians to treat urinary infections, wounds caused by arrows, as a flavoring, antimicrobial for meat, and by sailors to prevent scurvy [16,17].

Cranberry is also known to have several benefits, such as: anti-cancer, antiplatelet, protective agent against chronic diseases, cardiovascular diseases, prevention of urinary infections, gums, and stomach ulcers [18,19].

In 1984, it was discovered that Cranberry interferes with the adhesion of bacteria to uroepithelial cells, and in 1989 proanthocyanidins (PACs) were identified, a compound capable of inhibiting the bacterium's adherence to the urogenital mucosa [16,20].

In the composition of Cranberry there is about 88% water, in addition to other constituents, such as vitamin C, organic acid, flavonoids, catechins, anthocyanins and PACs and the carbohydrate D mannose [21].
PACs are condensed tannins, constituents of catechin and epicatechin monomers that they can reduce biofilm production, preventing the bacterium from adhering to the urogenital mucosa and impairing the motility of its flagella [22,23]. PACs are well absorbed and when they fall into the blood circulation, they start to have effects on other parts of the body, functioning as anti-adhesion and antioxidant [24].

Cranberry’s inhibitory potential on UTI-related pathogenic bacteria has been indicated in several studies [20,21,24], but little has been said about the use of Cranberry juice to inhibit these bacteria. Natural methods are a prophylaxis option to be studied, since, with the decrease in the use of antimicrobials, they may reduce the risk of bacterial resistance. Therefore, the aim was to evaluate in vitro antimicrobial potential of 35% reconstituted Cranberry juice on bacterial pathogens related to urinary tract infections (UTIs).

2. MATERIALS AND METHODS

2.1 Cranberry Juice

The dehydrated fruit was commercially obtained from a store specializing in natural products from Osasco, São Paulo, Brazil. To prepare the juice, the fruits were mashed in a power mixer blender (400 W) with the addition of sterile distilled water to compose a 35% reconstituted juice of Cranberry (RJCr) pH 3.0.

2.2 Strains

Four standard bacterial strains ATCC (American Type Culture Collection) and one strain of clinical origin were used: Escherichia coli ATCC 35218, Klebsiella pneumoniae ATCC 700603 Enterococcus faecalis ATCC 29212, Pseudomonas aeruginosa ATCC 27853 Proteus mirabilis (clinical origin).

2.2.1 Bacterial culture for testing

For each test, bacterial strains previously stored at -20°C were reactivated using Brain Heart Infusion Broth (BHI, HiMedia Laboratories, India), incubating at 35°C for 24 hours. Subsequently, the cultures were grown in BHI agar plates or BHI broth / 24h at 35°C before the tests were carried out.

2.3 Methods for Assessing Antimicrobial Activity

Antimicrobial activity of 35% reconstituted Cranberry juice (RJCr) was evaluated using well diffusion method on Tryptic Soy Broth - with 1% agar-agar -TSA (standard agar) and a direct in vitro assay based on dilution method in which the diluent is the inhibiting substance, here the RJCr (+ bacterial pathogen).

2.3.1 Agar well diffusion assay

The microorganisms were reactivated in BHI broth and incubated at 35°C/24h. When necessary each microorganism was grown on standard agar. For each experiment, was used the MacFarland 0.5 scale corresponding to 1.5 x 10^8 CFU / mL.

Each strain was inoculated in sterile Petri dishes and added of TSA. After solidification of the agar, wells of about 5mm were drilled, and the following substances were added to each well: 40 µL of RJCr; 40 µL of Chloramphenicol solution (30 µg) as a control group 1, and 40 µL of 0.85% saline as a control group 2.

The agar plates were prepared in duplicates and incubated at 35°C / 24 h. After 24 h, the measurements of the inhibition halos around the wells (mm) were recorded, which correspond to the inhibition caused by the action of the 35% reconstituted Cranberry juice.

2.3.2 Direct in vitro assay with Cranberry juice

The direct in vitro assay was realized based on direct action of RJCr for each bacterial strain. Initially each pathogen was reactivated in BHI broth and incubated at 35°C / 24h. Then the samples were taken twice by centrifugation and the CFU/mL of each one was later confirmed by inoculum in BHI agar plates for 35°C / 24 h. Thus, in test tubes 4.5 ml of RJCr and an inoculum of each strain was added whose final concentration was >10^6 and <10^7 CFU/mL.

The control group without adding any antimicrobials was prepared only in saline solution (0.85%) with the same initial bacterial concentration. The tubes were incubated in a water bath at 30°C for 3 hours under agitation (110 rpm). Aliquots obtained from each test to assess the antimicrobial potential were defined based on the total number of colonies grown on standard agar after 24 hours of incubation at 35°C. The logarithmic reduction cycles were also registered.

All tests were performed in duplicates with two replications.
3. RESULTS AND DISCUSSION

3.1 Antimicrobial Activity by Well Diffusion Assay

In the first part of this study, each bacterial strain was subjected to the well diffusion assay using SRCr as an inhibitory agent. This method is one of the most used in practice to assess the antimicrobial potential of natural substances [25]. The result was obtained by averaging the size of the diameter of the inhibition zone in millimeters (mm). The mean of inhibition halos for control 1 with Chloramphenicol was: \( P. \) aeruginosa = 21.4 mm, \( E. \) faecalis = 20.3 mm, \( E. \) coli = 11.6, \( P. \) mirabilis = 23.6 mm and for \( K. \) pneumoniae = 18.5 mm. Regarding RJCr, the mean of the halo of inhibition for \( E. \) coli was 23.3 mm, that is, higher than the Chloramphenicol control group (11.6 mm). For the other strains tested, the RJCr was not an inhibitor (Fig. 1).

In the present study, by using agar well diffusion assay, \( E. \) coli was the only pathogen inhibited. \( E. \) coli is the most frequent in urinary infections, that is, it occurs in 50-60% in adult women [6]. Studies have evaluated the antimicrobial activity of dehydrated cranberry against multi-resistant strains of \( E. \) coli based on the well diffusion method and found that the phenolic compounds present in Cranberry are related to antimicrobial action against pathogenic bacteria that cause UTIs [26,27].

In a study on the inhibitory potential of cranberry extract it was found that the inhibition of different \( E. \) coli strains was dose dependent related to concentration of proanthocyanidins [28].

In a study [29] was analysed human urine after consuming products containing type A proanthocyanidins, found in Cranberry juice and with B-type linkages proanthocyanidins anti-adhesion activity, found in apple juice, green tea, and black chocolate. The anti-adhesion activity was verified, suggesting that the presence of binding to proanthocyanidins type A is related to bacteria inhibition. \( E. \) coli has pil and fimbriae that are responsible for cell adhesion and genetic transmission to other bacteria [9]. According to [30] pil 1 is sensitive to D-mannose and proanthocyanins present in cranberry and fimbriae are inhibited by fructose present in several fruits.

A study carried out with 65 women aged 19 to 28 years evaluated the effectiveness of consuming of dried Cranberry juice and it was concluded that cranberry fruits are effective in preventing UTIs as well as preventing oxidative stress [31]. Other authors [32] analysed a cranberry-based food supplement using the disk diffusion method, showing that it had antimicrobial activity for different strains of \( E. \) coli. However, another study published in 2012 concluded that there was still no statistically significant data so that Cranberry juice could be used as a preventive for UTIs [33].

3.2 Antimicrobial Activity by Direct \textit{in vitro} Action in 35% Reconstituted Juice of Cranberry (RJCr)

The bacterial strains \textit{Pseudomonas aeruginosa, Enterococcus faecalis, E. coli, Proteus mirabilis and Klebsiella pneumoniae} were also subjected to the direct action of the reconstituted cranberry juice. Table 1 shows the direct action of the RJCr on bacterial strains whose initial concentration was \( >10^6 \) and \( <10^7 \) CFU/mL.

Regarding the two methods used here to assess the antimicrobial activity of Cranberry juice, it was found that by using agar well diffusion assay the mean of the inhibition halo for \( E. \) coli due to RJCr was higher (23.3 mm) compared to chloramphenicol control group (11.6 mm). However, RJCr was not inhibitory for other strains. By using direct \textit{in vitro} assay based on dilution methods, the action of RJCr was relevant for all strains, especially for \( E. \) coli and \textit{Proteus mirabilis}, in which the reduction reached six Log cycles.

The results obtained in the present study based on the method of direct action of the reconstituted juice are similar to that performed \textit{in vitro} by Cesoniene et al. [34], who showed that Cranberry extract had antimicrobial activity against some Gram positive and Gram negative bacteria, including \textit{E. coli} and \textit{Enterococcus faecalis}.

Based on the literature, Cranberry is a rich source of bioactive compounds [22,34,35,36]. The methods used here to assess the antimicrobial activity of cranberry juice have made it possible to reinforce the potential of the Cranberry in inhibiting pathogens that are often associated with urinary infections.
Fig. 1. Antimicrobial inhibition of 35% reconstituted juice of Cranberry (RJCr) against bacterial pathogens in agar well diffusion method

Table 1. Antimicrobial activity of 35% reconstituted juice of Cranberry (RJCr) against bacterial pathogens by using direct in vitro assay

<table>
<thead>
<tr>
<th>Bacterial Load</th>
<th>Klebsiella pneumoniae</th>
<th>Proteus mirabilis</th>
<th>Escherichia coli</th>
<th>Enterococcus faecalis</th>
<th>Pseudomonas aeruginosa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial concentration</td>
<td>7.9 x 10^6 a</td>
<td>4.3 x 10^6 a</td>
<td>7.3 x 10^6 a</td>
<td>1.5 x 10^6 a</td>
<td>4.3 x 10^6 a</td>
</tr>
<tr>
<td>Final concentration CFU/mL after action of RJCr</td>
<td>2.5 x 10^6 b</td>
<td>0 c</td>
<td>0 c</td>
<td>2.5 x 10^6 b</td>
<td>2.5 x 10^6 b</td>
</tr>
</tbody>
</table>

* Control 1: 0.85% saline solution /no RJCr

** Equal letters do not indicate statistical difference (P = 0.05)

4. CONCLUSION

Cranberry juice at 35% had an inhibitory effect on the pathogenic bacteria tested here. It was inhibitory, especially for Escherichia coli, the most recurrent pathogen in UTI.

Cranberry juice was stronger in inhibiting E. coli than antibiotic chloramphenicol as observed by well diffusion assay. Thus, the study reinforces the importance of Cranberry, even in the form of juice, in inhibiting E. coli.

Cranberry juice can be incorporated for a healthy diet, so it can contribute to beneficial effects on human health. However, further studies must be carried out so that Cranberry can be considered a prophylactic use for urinary tract infections.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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23. Maisuria VB, Los Santos YL, Tufenkji N, Déziel E. Cranberry-derived proanthocyanidins impair virulence and


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