

***In vitro* Inhibitory Effect of Cranberry (*Vaccinium macrocarpum* Ait.) Juice on Pathogenic Microorganisms¹**

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Abstract—The purpose of this study was to determine the inhibitory effects of cranberry juice on pathogenic microorganisms. The microorganisms analyzed were *Escherichia coli* from patients with urinary infections, *Salmonella* spp., *Listeria monocytogenes*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*. The disc method was used to determine the sensitivity of bacteria to cranberry juice (CJ, both concentrated and diluted). A lawn of 10⁶ cfu/ml was grown on agar surfaces in Petri dishes and on Whatman discs that had been previously saturated with CJ and CJ : water. 1 : 1 to 1 : 50 juice solutions had been placed on the discs, which were cultured and incubated. The results indicated that *S. aureus* was more susceptible to cranberry juice inhibition than the other microorganisms. *L. monocytogenes* was the most resistant to the inhibitory action of cranberry juice, showing a significant difference from the inhibition of *P. aeruginosa*, uropathogenic *E. coli*, *Salmonella* spp., and *S. aureus*. This study also demonstrated that the inhibitory activity of cranberry juice for *E. coli* took place up to a dilution of 1 : 20.

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The determination of cranberry juice's inhibitory and antimicrobial activity against pathogenic microorganisms is important due to the human health benefits it provides, since these microorganisms are becoming increasingly resistant to pharmacological and antibiotic treatments. Cranberry juice has been widely used to treat and prevent urinary tract diseases, because it inhibits the adherence of *E. coli* to the uroepithelial cells of the bladder wall. It is important to determine whether cranberry juice shows a broader spectrum of antibacterial activity [1–6].

Cranberry fruit is low in calories and high in vitamins, minerals, and fibre. This fruit also contains various bioactive components, including tocotrienols, anthocyanin, flavones, and proanthocyanidins [7–8]. Phenolic and benzoic antioxidant components of cranberry have been identified and quantified. This fruit has a high content of benzoic and phenolic acids (5.7 and 4.7 g/kg of fresh fruit), benzoic acid being the most abundant, followed by *p*-coumaric and synapctic acid (0.25 and 0.21g / kg of fresh fruit) 9.

The inhibitory effects of cranberry juice on *E. coli* have been demonstrated by several authors, who report human health benefits, especially the prevention of infectious diseases of the urinary tract [1–4, 9, 10].

The *in vitro* inhibition of bacterial adherence was investigated using strains of uropathogenic *E. coli* isolated from 77 clinical cases. This study showed that

cranberry juice inhibited the *E. coli* bacteria 75% as much as other inhibited bacterial species, including *Proteus*, *Klebsiella*, *Enterobacter*, and *Pseudomonas* [11]. Cranberry juice consumption has been shown to prevent the development of bacteria. Bacteria was also inhibited in elderly people, mainly because of the action of hipuric acid, which is produced in large amounts after the consumption of cranberry juice and is later excreted in the urine 1. It is also said that cranberries' quinic acid is a precursor of hipuric acid, which is a strong antibacterial agent. To obtain the acid's bacteriostatic effect against *E. coli*, urine pH has to be reduced to a minimum of 5.0 with an acid concentration of 0.02 M, but in order to reach those levels, a person would have to drink at least 1500 ml of cranberry juice daily, a high level consumption. Nevertheless, acidification of urine is not the main factor responsible for the inhibitory effect of cranberry juice. Previous research indicates that condensed tannins of cranberries, called proanthocyanidins, can inhibit the adherence of *E. coli* 5. In addition, it is indicated that proanthocyanidins show other kinds of activities, such as anticarcinogenic activity and the inhibition of superoxide radicals [12, 13].

The antiadhesion activity of cranberry juice on non-urinary bacterial species has been verified, especially against buccal bacteria. This activity is caused by a nondialyzable material (NDM) with a high molecular weight that has been isolated from cranberry juice at concentrations of 0.6 to 2.5 mg/ml. At the same time, it has been observed that formation of cavity causing den-

¹ The text was submitted by the authors in English.

tal plaque involves two processes: addition of the bacterium to the saliva film which covers the teeth and the coaggregation of two different types of bacteria, generally from the genera *Streptococcus* and *Actinomyces* [14]. It has been concluded that a low concentration of NDM is needed to inhibit formation of those coaggregates, indicating that NDM acts on buccal bacteria pairs, one of these being a gram-negative anaerobe. Preliminary studies showed that NDM reduced counts of *Streptococcus mutans*, indicating that the antiadherence activity of cranberry juice is greatly effective in changing the subgingival microbial population, controlling periodontal diseases [15].

The general purpose of this study was to determine the in vitro inhibitory effects of cranberry (*Vaccinium macrocarpon* Ait.) juice on the following bacteria: uropathogenic *E. coli*, *S. aureus*, *Salmonella* spp., *P. aeruginosa*, and *L. monocytogenes*.

MATERIALS AND METHODS

Test strains used. Microorganisms used corresponded to strains of *E. coli* obtained from patients with urinary infectious diseases from the Main Hospital in Valdivia (Chile) and strains of *Staphylococcus aureus*, *Salmonella* spp., *Pseudomonas aeruginosa*, and *Listeria monocytogenes* obtained from the Institute of Food Science and Technology, Faculty of Agropecuarian Sciences Southern University of Chile.

Cranberry juice concentrate. Concentrated cranberry juice at 55° Brix was obtained from two processing plants in Chile's Lake District.

Preliminary trials. In order to determine the concentrations at which cranberry juice has an antibacterial effect, preliminary studies were carried out to identify bacterial strains by means of presumptive and confirmatory tests, following the methodology described for microorganisms like uropathogenic *E. coli*, *Salmonella* spp., *S. aureus*, *L. monocytogenes*, and *P. aeruginosa* [16].

Isolation of uropathogenic *E. coli*. Strains of uropathogenic *E. coli*, from patients with urinary infections that were not treated with antibiotics, were isolated on nutritive agar and later confirmed by presumptive and confirmatory tests. Presumptive tests consisted of growing suspected of *E. coli* colonies on VRB agar and incubating them at $30 \pm 1^\circ\text{C}$ for 24 ± 2 h. After the incubation period, red colonies with or without surrounding precipitates underwent a gram tinction, which makes it possible to observe characteristic gram-negative bacilli. Confirmatory tests were based on the identification of colonies on media. These colonies were replicated in tubes of lactosated brilliant green bile broth with Durham bubbles, incubated in a double boiler at $35 \pm 1^\circ\text{C}$ for 24 to 48 h and in an EC broth at 44.5°C for 24 to 48 h, taking note of gas production in the Durham bubbles. Finally, the following reaction tests of characteristic IMVIC were run for *E. coli*: indol, methyl red, Voges Proskauer, and citrate.

Isolation of *Salmonella* spp. Colonies were grown in stripes on *Salmonella Shigella* agar and sulfite agar plates, both incubated at 35 to 37°C for 24 to 48 h. Suspected *Salmonella* spp. colonies were isolated from these plates and then grown on selective differentiation media, like triple sugar iron agar (TSI), where H_2S production was observed at the bottoms of the tubes. On lysine iron agar, decarboxilation of lysine, with production of H_2S , and color change from medium to dark violet, characteristic of *Salmonella* spp., was verified. Reaction tests for indol, methyl red, phenylalanine, sorbitol, and urea were conducted later.

Isolation of *P. aeruginosa*. The colonies, grown on the surface of brain heart agar and treated with inhibitors of accompanying flora, like cephaloridine, fusidic acid, and cefrimide, previously incubated in aerobiosis at 25°C for 48 h, were subjected to gram tinction, making it possible to observe the presence of small gram-negative bacilli. Oxidase and catalase confirmation tests were performed.

Isolation of *L. monocytogenes*. Presumptive identification was carried out by isolating dark brown or black colonies with halos, typical of *L. monocytogenes*, on OXA (Oxford Listeria selective agar) and LPM (PALCAM Listeria selective agar base). As a presumptive test, gram tinction was performed, in addition to a catalase confirmative test. Finally, biochemical tests for *L. monocytogenes* were conducted through the outlined methodology: motility at 25°C , oxygen requirement, growth at 35°C , H_2S production, reaction of methyl red, Voges-Proskauer reaction, indol production, citrate use, acid from glucose, esculina hydrolysis, maltose fermentation, dextrose fermentation, and activity of the urease [16].

Isolation of *S. aureus*. Colonies of suspected *S. aureus*, glossy black with or without halo, on Baird-Park agar as a selective medium (*Staphylococcus* selective agar base), underwent the coagulase test. As a means of verification, gram tinction was carried out.

Bacterial inhibition through filter disc method. Strain activation. The pure cultures of uropathogenic *E. coli*, *Salmonella* spp., *S. aureus*, *L. monocytogenes*, and *P. aeruginosa* was replicated in 5 ml of brain-heart-broth and incubated for 2 h at $35 \pm 1^\circ\text{C}$ for uropathogenic *E. coli*, *Salmonella* spp., *S. aureus*, and at $30 \pm 1^\circ\text{C}$ for *L. monocytogenes* and *P. aeruginosa*, until a concentration of 10^6 cfu/ml was obtained.

Trial of bacterial sensitivity to cranberry juice. The bacterial inhibition trial was carried out using a modification of the diffusion technique on agar. Plates were used on which a lawn of 10^6 cfu/ml concentration had previously been grown. At the same time, dilutions of 55° Brix concentrated cranberry juice at pH 2.7 were prepared with sterile distilled water. Preparations were as follows: CJ concentrate, dilutions at 1 : 1, 1 : 10, 1 : 15, 1 : 20, 1 : 25, 1 : 30, 1 : 35, 1 : 40, 1 : 45, 1 : 50, and one control corresponding to distilled water adjusted to 2.7 pH. The 9-mm-diameter sterile Whatman disc was

Halos of bacterial inhibition

Concentration	Halos of inhibition, mm					
	<i>L. monocytogenes</i>	<i>P. aeruginosa</i>	<i>E. coli</i>	<i>Salmonella</i> spp.	<i>S. aureus</i>	Mean \pm S.D.*
Concentrate	9.50	12.16	11.07	12.62	14.58	11.99 \pm 1.88 a
1 : 01	6.76	9.43	9.81	11.96	12.27	10.05 \pm 2.23 b
1 : 10	0.66	1.75	3.24	4.84	5.63	3.22 \pm 2.07 c
1 : 15	0.01	1.13	1.42	1.30	4.24	1.62 \pm 1.57 d
1 : 20	0.00	0.25	0.96	0.94	2.73	0.98 \pm 1.07 de
1 : 25	0.00	0.13	0.00	0.55	2.37	0.61 \pm 1.01 de
1 : 30	0.00	0.12	0.00	0.21	2.00	0.47 \pm 0.86 e
1 : 35	0.00	0.07	0.00	0.17	1.15	0.28 \pm 0.49 e
1 : 40	0.00	0.06	0.00	0.07	0.84	0.19 \pm 0.36 e
1 : 45	0.00	0.07	0.00	0.01	0.75	0.17 \pm 0.33 e
1 : 50	0.00	0.03	0.00	0.01	0.35	0.08 \pm 0.15 e
Control	0.00	0.03	0.00	0.00	0.03	0.01 \pm 0.02 e
Mean \pm S.D.**	1.41 \pm 3.20 d	2.10 \pm 4.14 c	2.21 \pm 3.97 bc	2.72 \pm 4.67 b	3.91 \pm 4.76 a	

Notes: * Differences in mean \pm S.D. for concentration values with different letters within the row are statistically significant ($P < 0.05$).

** Differences in mean \pm S.D. for microorganisms values with different letters within the column are statistically significant ($P < 0.05$).

manipulated with a sterile clamp. The disc had previously been saturated with cranberry juice at the corresponding dilution. As a control, a disc was saturated with sterile water adjusted to pH 2.7. The size of the inhibition halo, as the distance between the edge of the disc and the beginning of the culture in mm, was measured using a digital micrometer.

Experimental design for bacterial inhibition by means of discs. The experimental design consisted of a 5×12 multifactor design. The first factor corresponded to the species of bacteria at five levels (uropathogenic *E. coli*, *Salmonella* spp., *S. aureus*, *L. monocytogenes*, and *P. aeruginosa*), and the second factor corresponded to the concentration of cranberry juice (including con-

trol). All measurements were done in triplicate. Bacterial inhibition caused by cranberry juice was assessed through descriptive statistics and one-way analysis of variance (Anova). When a significant difference was found among results, a multiple range test was carried out (Tukey Hsd 95% confidence).

RESULTS AND DISCUSSION

Bacterial inhibition through filter discs. Table shows halos, in mm, of uropathogenic *E. coli*, *S. aureus*, *Salmonella* spp., *P. aeruginosa*, and *L. monocytogenes*, that developed when discs saturated with the different cranberry juice dilutions were applied. Statistical analysis indicated significant differences ($P < 0.05$) between the concentrations of cranberry juice and the microorganisms, as shown in Table.

According to the results, no microbial inhibition was caused by the acid agent (control disc), considering that a minimal, almost nonexistent inhibition was observed in the studied microorganisms. Consequently, the inhibitory effect of cranberry juice cannot be attributed to its low pH. These results were similar to those of research carried out by several authors, who pointed out that cranberry juice contains a number of inhibitory bioactive components, mainly flavones and proanthocyanidines [3, 5, 8].

As seen in Fig.1, it is possible to conclude that, among the microorganisms involved in this study, *S. aureus* showed the greatest sensitivity to treatments with cranberry juice, both concentrated and diluted. Results from the concentrated treatments on a lawn of 1.27×10^6 cfu/ml showed an inhibition with an average halo of 14.58 ± 1.03 mm. This effect was extended up to the 1 : 50 dilution, with a 0.35 ± 0.3 -mm halo. In the case of *S. aureus* inhibition, it is necessary to emphasize

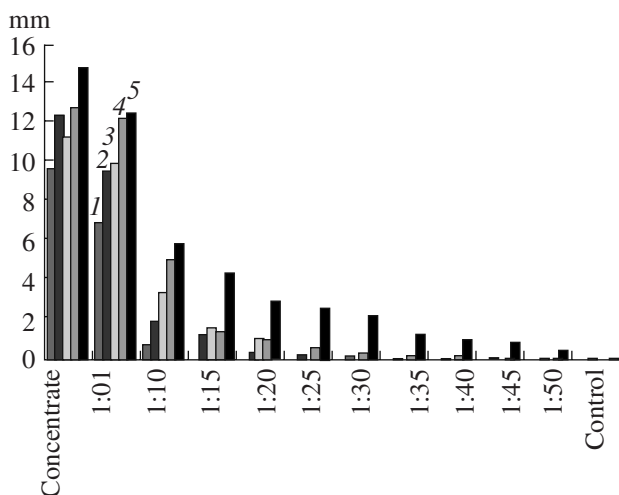


Fig. 1. Inhibition of uropathogenic *L. monocytogenes* (1), *P. aeruginosa* (2), *E. coli* (3), *Salmonella* spp. (4), and *S. aureus* (5) caused by different cranberry juice dilutions (1 : 1–1 : 50).

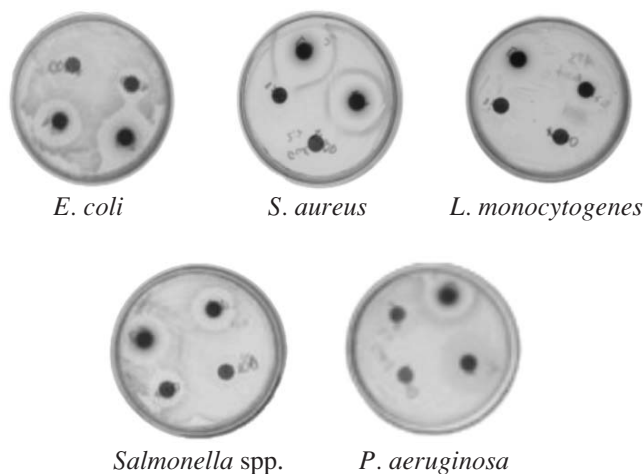


Fig. 2. Morphology of cranberry juice inhibition halos on different microorganisms.

the inhibitive properties of cranberry juice against gram-positive bacteria, which could cause the reduction in counts of Streptococcus mutants, a decline in the subgingival microbial population, and the control of periodontal diseases [15].

According to the results obtained when applying the disc with cranberry juice concentrate, *P. aeruginosa* showed an inhibition of 12.16 ± 2.97 mm, less than that of *S. aureus* (Table). In turn, from dilutions of 1 : 35 to 1 : 50, *P. aeruginosa* inhibition proved to be lower than that of *S. aureus*. Likewise, *P. aeruginosa* ATCC27853 was reduced in 8.3 Log₁₀ cycles in a culture with cranberry juice 3.

These results differ from those showed by Habash et al. [17], who added water, ascorbic acid, and cranberry juice in an in vivo study which demonstrated that count and adherence of *E. coli* and *Enterococcus faecalis* in urine were reduced, but count and adherence of *P. aeruginosa*, *Staphylococcus epidermis*, and *Candida albicans* were not reduced.

Regarding *Salmonella* spp., it was determined that, when using a lawn of 5.3×10^6 cfu/ml, a wider inhibition halo with the concentrate, of 12.62 ± 2.18 mm, and a narrower inhibition halo with the 1 : 50 dilution of 0.01 ± 0.01 mm, was observed. It was demonstrated that, with a cranberry juice dilution of 1 : 1 and an inoculation level of 10^4 cfu/ml, *Salmonella enteritidis* ATCC 14028, after 24 h of incubation at 35°C, showed a count of 2.3×10^1 cfu/ml, 7.1 Log₁₀ cycles. This was lower than that of the culture without cranberry juice, where the count reached 2.8×10^8 cfu/ml [3].

For uropathogenic *E. coli* on a lawn of 9.2×10^6 cfu/ml, the highest inhibition was recorded in the culture with cranberry juice concentrate with a 11.06 ± 0.48 -mm halo and a smaller inhibition in the 1 : 20 dilution with a 0.96 ± 0.32 -mm halo.

This agrees with what has been reported by Fleet [18], who suggested that, in order to reduce urinary tract infections caused by *E. coli*, one must drink cranberry juice that has been diluted to a concentration of 1 : 10 (100 ml concentrate in 1 l water) in daily quantities of 300 to 500 ml for 6 months. However, it was possible to conclude from this study that cranberry juice inhibits *E. coli* at a dilution of 1 : 20.

This research showed that the least inhibited microorganism was *L. monocytogenes* compared with all of the others used in this study (Table). This species displayed its highest level of inhibition, 9.50 ± 1.01 mm, with the disc saturated with cranberry juice concentrate and its lowest inhibition 0.01 ± 0.01 mm at a dilution of 1 : 15 (Fig. 1).

Although most investigations of microbial inhibition with cranberry juice focus on *E. coli* [1–4, 10, 19], our conclusions indicate there is also inhibition of some pathogenic microorganisms, such as *Salmonella* spp. and *P. aeruginosa*.

Effect of cranberry juice concentration upon bacterial inhibition. As shown in Table, for the five bacteria considered in this study, the higher inhibition halos were observed in treatments with concentrate and dilutions of 1 : 1, 1 : 10, and 1 : 15 (Fig. 1). The morphology of these inhibition halos caused by cranberry juice on the different microorganisms can be observed in Fig. 2.

This study demonstrated that cranberry juice inhibits uropathogenic *E. coli* bacteria at a dilution 1 : 20, which was greater than that indicated by other authors. Furthermore, it was shown that antibacterial activity against microorganisms like *Salmonella* spp., *P. aeruginosa*, *S. aureus*, and *L. monocytogenes* occurs at a dilution of 1 : 10.

REFERENCES

1. Avorn, J., Moname, M., Gurwitz, J.H., Glynn, R., Choodnovskiy, J., and Lipsitz, I., *J. Am. Med. Assoc.*, 1994, vol. 1, no. 271, 751–754.
2. Hamilton-Miller, M.J., *J. Am. Med. Assoc.*, 1994, vol. 8, no. 272, pp. 588–590.
3. Lee, L., Owens, Y., Thrupp, J.L., and Cesario, T., *J. Am. Med. Assoc.*, 2000, vol. 13, no. 283, p. 1691.
4. Kontiokari, T., Sundqvist, K., Nuutinen, M., Pokka, T., Koskela, M., and Uhari, M., *Br. Med. J.*, 2001, vol. 322, pp. 1571–1573.
5. Howell, A.B., *Crit. Rev. Food Sci. Nutr.*, 2002, vol. 42 (suppl.), pp. 273–278.
6. Ofek, I. and Foo, I., *N. England J. Med.*, 2003, vol. 324, no. 22, p. 1599.
7. Buzeta, A., *Berries para el 2000. Departamento Agroindustrial Fundacion Chile*, 1997, pp. 15–19.
8. Milner, J.A., *Crit. Rev. Food Sci. Nutr.*, 2002, vol. 42, pp. 265–266.
9. Zuo, Y., Wang, C., and Zhan, J., *J. Agric. Food Chem.*, 2002, vol. 50, no. 13, pp. 3789–3794.

10. Ofek, I., Goldhar, J., Zafiriri, D., Lis, H., Adar, R., and Sharon, N., *New England J. Med.*, 1991, vol. 324, no. 22, p. 1599.
11. Sobota, A., *J. Urol.*, 1984, vol. 131, pp. 1013–1016.
12. Zheng, W. and Wang, S.Y., *J. Agric. Food Chem.*, 2003, vol. 51, no. 2, pp. 502–509.
13. Howell, A.B., Vorsa, N., Maderosian, A., and Foo, L., *New England J. Med.*, 1998, vol. 339, no. 15, pp. 1085–1086.
14. Haffajee, A. and Socransky, S., *Periodontology*, 1994, vol. 5, pp. 78–111.
15. Burger, O., Weiss, E., Sharon, N., Tabak, M., Neeman, I., and Ofek, I., *Crit. Rev. Food Sci. Nutr.*, 2002, vol. 42, pp. 279–284.
16. IDF/FIL, *International Dairy Federation*, 1995, Bulletin 93A–143B: 2–7.
17. Habash, M.B. and Van Der Mei, H.C., *Can. J. Microbiol.*, 1999, vol. 45, no. 8, pp. 691–694.
18. Fleet, J.C., *Nutr. Rev.*, 1994, vol. 52, no. 5, pp. 168–170.
19. Ofek, I., Hasty, D.L., and Sharon, N., *FEMS. Immunol. Med. Microbiol.*, 2003, vol. 38, no. 3, pp. 181–191.