The Effect of Cranberry, Strawberry and Blueberry Juices on the Viability of Cariogenic Bacteria: An in Vitro Study

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Abstract

The purpose of this study was to examine the inhibitory effect of fresh cranberry, strawberry and blueberry juices on the viability of oral Streptococcus mutans, Streptococcus pyogenes and Streptococcus viridans.

Modified agar-well diffusion method was used to determine the antimicrobial activity of the fruit juices. A 100µl volume of the test solution was added into wells of the agar plates and zones of inhibition were measured following incubation.

The fresh cranberry juice, the three powders, and the mixed juices and juice powders had an antibacterial effect on S.mutans, S.pyogenes and S.viridans. The greatest zone of inhibition was observed with the fresh cranberry juice and juice powder of the same. The fresh strawberry juice showed an antibacterial effect on S.viridans. However, the fresh blueberry juice failed to provide an antibacterial effect on any of the tested pathogens.

This study concludes that the cranberry juices have a good inhibitory effect on the colonization of oral S.mutans, S.pyogenes and S.viridans.

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Introduction

Dental caries is a localized, post-eruptive, pathological process of external origin involving softening of the hard tooth structure and proceeding to the formation of a cavity.[1] The etiology of dental caries is multifactorial.[2] Although several studies have been conducted in the field of cariology, the accurate mechanism by which dental caries occur is not yet fully understood. The most acceptable, however, states that caries is a result of changes in the environment due to acid production by bacteria from the fermentation of dietary carbohydrates, initiating the demineralization process.[3-5]

The existence of microorganisms as a polyspecies consortium has profound implications for the etiology of dental caries,[1] which happens to be one of the most prevalent diseases in the oral cavity. The occurrence of dental caries has been attributed to a number of microbes that include *Streptococcus sobrinus, Streptococcus rattus, Streptococcus cricetus, Streptococcus downeii, Streptococcus ferus, Streptococcus macacae* and *Streptococcus mutans*; *S. mutans* and *S. sobrinus* being the predominant microorganisms involved in caries initiation. *S. mutans*, the most virulent Streptococci, has a variety of virulence factors contributing to its cariogenicity; these include acidogenicity, aciduricity and adherence.[6, 7] The adhesion of streptococci to the tooth surface is the first step in the formation of dental plaque[8] and it has been observed that controlling their number can prove beneficial in reducing the incidence of oral infections.

Currently, the anti-microbial and plaque removal strategies that exist to prevent the development of dental caries such as chlorhexidine mouthwashes and fluoride-containing toothpaste possess drawbacks including bacterial resistance, staining, fluorosis, and toxicity. The approach of including a high

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consumption of fresh fruits as a part of one's dietary lifestyle has many general and oral advantages, as almost no bacterial resistance is encountered as seen in other antibacterial or antibiotic treatments[9-11]. One such family of fruits, the berries native to North America, have demonstrated distinct bio-protective and antimicrobial properties.[12]

Cranberries (*Vaccinium macrocarpon*), strawberries (*Fragaria virginiana*) and blueberries (*Vaccinium corymbosum*) are a good source of vitamins (particularly vitamin C), minerals (manganese) and polyphenols including flavonols, anthocyanins and proanthocyanidins and are being actively researched for possible effects on the cardiovascular system, immune system and cancer.[10] A study investigating the anti-adhesion effect of cranberry and blueberry juices on *Escherichia coli* found the juices to affect the adhesion of uropathogenic micro-organisms to uroepithelial cells by interfering with specific modes of adhesion.[13-15] Other studies have reported that drinking cranberry juice decreased the frequency of bacteria in pyuria, particularly in women of age.[16] Additional studies have shown the cranberry, strawberry and blueberry juices to exhibit an antimicrobial effect on several pathogenic micro-organisms including *Staphylococcus epidermidis, Klebsiella pneumonia, Listeria monocytogenes* and *Salmonella enteritidis*.[17-19]

This study aimed to investigate the inhibitory effect of fresh cranberry, strawberry and blueberry juices on the viability of clinical isolates of the cariogenic pathogen *S. mutans* as well as two non-cariogenic strains of *S. pyogenes* and *S. viridans*.. The null hypotheses tested in this study were 1) fresh cranberry, strawberry and blueberry juices have no effect on the viability of *S. mutans*, *S. pyogenes* and *S. viridans* 2) there is no difference in the antibacterial effect between the fresh juices and the organic freeze-dried powders of the same fruits and 3) there is no synergistic activity of the three juices when mixed against the three pathogens.

Materials and methods

Microbial strains and culture conditions

Saliva samples were collected according to the approved protocol by the research & ethics committee. The diluted saliva samples were centrifuged and 1 µl of the solution was manually seeded on the pre-prepared agar plates of Tryptone Yeast Extract Cystine (TYCSB) with 2 units Bacitracin TYCSB supplement from HiMedia® [HiMedia Laboratories Pvt.Ltd, Mumbai, India] for the selective isolation of *S. mutans* as devised by Gold.[20] *S. pyogenes* and *S. viridans* were grown on sheep blood agar plates. The inoculums were spread across the plates and incubated in candle jars for 24-48 hours at 35°C to get the final bacterial cell concentration of 1.5×10^8 CFU/ml using a spectrophotometer at 625.0nm. [UV-1800, SHIMADZU, Kyoto, Japan]

Preparation of fresh juices

Organically grown cranberry, strawberry and blueberry fruits were prepared undiluted by direct squeezing in a commercial juicer. All the juices were filtered using a Whatman Grade 4 filter paper with a pore size of 20-25 µm. The filtered juices were then prepared to concentrations ranging from 10-100% by adding an appropriate volume of sterile distilled water.

Preparation of organic freeze-dried powders

Different concentrations of the freeze-dried cranberry, strawberry and blueberry organic powders were dissolved in sterile distilled water and mixed until a homogenous solution was obtained to achieve concentrations ranging from 10-80%. The synergistic fresh juices and solutions of freeze-dried powders were prepared by adding previously diluted juices/organic powders into sterile distilled water to achieve concentrations ranging from 10-100% and 10-80% respectively.

Assessment of antimicrobial activity

Antimicrobial activity of the three fruit juices, the three organic powders, and their mix was tested using the agar well-diffusion method. The blood agar plates were first swabbed with each of the prepared bacterial inoculums using a disposable cotton swab stick. Wells of 6mm diameter each were made using a sterile borer into the agar plates containing the inoculums. 100µl volumes of the test solution were poured into each well of the inoculated plates and were left at room temperature for 10 minutes, allowing the diffusion of the test solution into the agar. Following overnight incubation at 35°C, antibacterial activity was determined by measuring the corresponding zones of inhibition using a standard ruler in millimeter. Sterile distilled water poured into a well of each of the inoculated plates was utilized as a negative control and Listerine zero was used as a positive control. Each experiment for both the fresh filtered juices and the organic powders was carried out three times independently.

Statistical analysis

The quantitative data collected were analyzed using the Statistical Package for the Social Sciences (SPSS version 20) by three-way ANOVA and Tukey's post hoc tests.

Mean±SD Zone of Inhibition (mm) - S mutans										
	Cranberry		Strawberry		Blueberry		Mixed		Control	
(%)	Fresh	Powder	Fresh	Powder	Fresh	Powder	Fresh	Powder	Positive	Negative
100	18±0.5		0±0		0±0		12±0.5		11±0	0±0
90	17±0.5		0±0		0±0		11±0.5		-	-
80	16±0.5	17.67±4.04	0±0	12.33±3.51	0±0	13.67±2.89	10±0.5	17±2.65	-	-
70	15±0.5	17.67±2.52	0±0	12±4	0±0	13±3.61	9±0.5	16.33±3.21	-	-
60	13±0.5	17±2	0±0	10.67±3.06	0±0	11.67±4.04	9±0.5	13.67±3.79	-	-
50	12±0.5	15.67±2.08	0±0	10.33±3.21	0±0	10.67±5.03	7±0.5	13.67±3.79	-	-
40	10±0.5	14±3.61	0±0	9±2.65	0±0	8±7.21	0±0	11.67±3.79	-	-
30	9±0.5	12.33±2.31	0±0	3.33±5.77	0±0	7±6.25	0±0	10.33±3.21	-	-
20	8±0.5	6.67±6.11	0±0	0±0	0±0	0±0	0±0	7.33±0.58	-	-
10	7±0.5	2.33±4.04	0±0	0±0	0±0	0±0	0±0	0±0	-	-

Table 1: Antibacterial activities of the fresh and the organic freeze-dried powders of the three berries and their mix against *S.mutans.*

Mean±SD Zone of Inhibition (mm) - S pyogenes										
	Cranberry		Strawberry		Blueberry		Mixed		Control	
(%)	Fresh	Powder	Fresh	Powder	Fresh	Powder	Fresh	Powder	Positive	Negative
100	17±0.5		0±0		0±0		15±0.5		13±0	0±0
90	17±0.5		0±0		0±0		13±0.5		-	-
80	15±0.5	20±0.5	0±0	17±0.5	0±0	17±0.5	12±0.5	18±0.5	-	-
70	13±0.5	20±0.5	0±0	16±0.5	0±0	16±0.5	11±0.5	17±0.5	-	-
60	12±0.5	17±0.5	0±0	15±0.5	0±0	16±0.5	10±0.5	16±0.5	-	-
50	11±0.5	17±0.5	0±0	13±0.5	0±0	16±0.5	8±0.5	16±0.5	-	-
40	10±0.5	16±0.5	0±0	11±0.5	0±0	13±0.5	7±0.5	12±0.5	-	-
30	9±0.5	16±0.5	0±0	11±0.5	0±0	11±0.5	0±0	11±0.5	-	-
20	7±0.5	13±0.5	0±0	7±0.5	0±0	9±0.5	0±0	7±0.5	-	-
10	0±0	7±0.5	0±0	0±0	0±0	0±0	0±0	0±0	-	-

Table 2. Antibacterial activities of the fresh and the organic freeze-dried powders of the three berries and their mix against *S.pyogenes.*

Mean±SD Zone of Inhibition (mm) - S viridans										
	Cranberry		Strawberry		Blueberry		Mixed		Control	
(%)	Fresh	Powder	Fresh	Powder	Fresh	Powder	Fresh	Powder	Positive	Negative
100	18±0.5		13±0.5		0±0		15±0.5		11±0	0±0
90	16±0.5		10±0.5		0±0		14±0.5		-	-
80	16±0.5	21±0.5	9±0.5	16±0.5	0±0	18±0.5	14±0.5	17±0.5	-	-
70	16±0.5	19±0.5	8±0.5	15±0.5	0±0	16±0.5	12±0.5	16±0.5	-	-
60	15±0.5	18±0.5	7±0.5	14±0.5	0±0	16±0.5	12±0.5	15±0.5	-	-
50	13±0.5	18±0.5	0±0	13±0.5	0±0	15±0.5	11±0.5	13±0.5	-	-
40	13±0.5	17±0.5	0±0	11±0.5	0±0	13±0.5	9±0.5	8±0.5	-	-
30	11±0.5	15±0.5	0±0	10±0.5	0±0	12±0.5	8±0.5	0±0	-	-
20	8±0.5	13±0.5	0±0	8±0.5	0±0	9±0.5	0±0	0±0	-	-
10	7±0.5	9±0.5	0±0	0±0	0±0	8±0.5	0±0	0±0	-	-

Table 3. Antibacterial activities of the fresh and the organic freeze-dried powders of the three berries and their mix against *S.viridans.*

Results

The measurement of the zones of inhibition revealed that the cranberry juice at 100% concentration had the highest antibacterial effect on *S. mutans*, *S. pyogenes* and *S. viridans*. The fresh strawberry juice at 60-100% concentrations showed a bactericidal effect on *S. viridans* only. However, the fresh blueberry juice failed to provide an antibacterial effect on any of the tested pathogens.

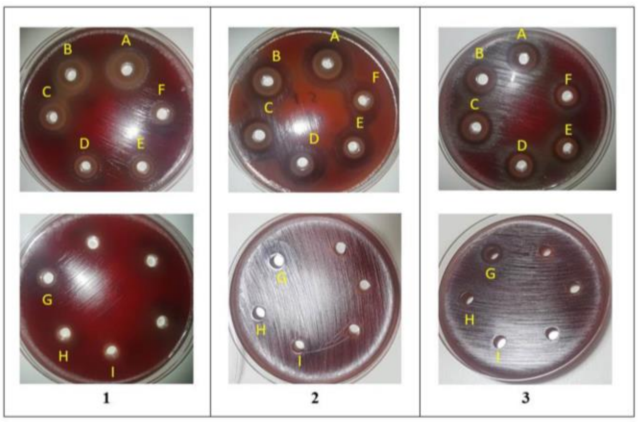

Figure 1. Representative images of zone of bacterial growth inhibition following overnight incubation with test solutions. 1(A-I): Fresh cranberry juice at concentrations of 90%-10% respectively with S. mutans, 2(A-I): Fresh cranberry juice at concentrations of 90%-10% respectively with S. pyogenes, 3(A-I): Fresh cranberry juice at concentrations of 90%-10% respectively with S. viridans.

Among the organic freeze-dried powders, all the fruit powders and their mix showed an antibacterial effect. The results with cranberry juice and powder were found to be statistically significantly different from strawberry, blueberry, the mixed juices/powders and the positive control at a P<0.01. The negative control presented with no inhibitory effect on the three tested pathogens.

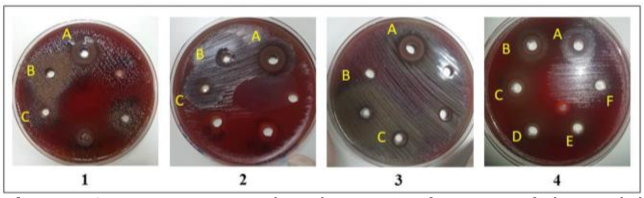

Figure 2. Representative images of zone of bacterial growth inhibition following overnight incubation with test solutions. 1A: 100% cranberry, 1B:100% blueberry and 1C: 100% strawberry with S. mutans. 2A: 100% cranberry, 2B:100% blueberry and 2C: 100% strawberry with S. pyogenes, 3A: 100% cranberry, 3B:100% blueberry and 3C: 100% strawberry with. viridans. 4 (A-E): Fresh Mixed juice at concentrations of 100-50% with S. mutans.

Multiple comparisons revealed the antibacterial effect by 70-100% juices and 70-80% powders to be statistically significantly different from the effect of 10-50% juices and powders at a P<0.05.

However, the fresh cranberry and mixed juices were statistically insignificant compared with the organic freeze-dried powders of the same (P>0.05).

Figures 1, 2, and 3, Tables 1, 2 and 3 present the antibacterial activities of the fresh as well as the organic freeze-dried powders of the three berries and their mix screened against *S. mutans. S. pyogenes* and *S. viridans.*

Discussion

In the present study, the fresh cranberry juice showed good efficacy to inhibit the growth of *S. mutans, S. pyogenes* and *S. viridans*. The fresh strawberry juice showed an antibacterial effect on *S. viridans* only. However, the fresh blueberry juice failed to inhibit the growth of the tested microbes so the first null hypothesis was partially accepted. The organic freeze-dried powders showed better growth inhibition than the fresh juices on all the tested microbes. Thus the second null hypothesis was rejected. Additionally, the three juices when mixed presented with a synergistic effect on the test pathogens, rejecting the third null hypothesis.

Among all the fruits, the highest antibacterial activity was recorded with the fresh cranberry juice as well as the organic powder of the same. In a study led by Yamanaka *et al*, lyophilized non-dialyzable high molecular weight constituents of cranberry juice powder were tested against many strains of oral streptococci, including *S. mutans.* He proposed these components to have an inhibitory effect on the colonization by oral streptococci to the tooth surface, promoting dental plaque deceleration.[8] This is in consistent with the findings of our study, as an antibacterial effect by the fresh cranberry juice on *S. mutans* was readily observed. This was further supported by another in vitro study done by Ragini Sethi *et al* where the author explain that the inhibitory effect of cranberry fruit extract on Streptococci species is concentration dependent.[10]

The exact mechanism of action of the cranberry juice is yet to be identified. However, previous reports proposed three plausible explanations. Howell *et al* found that the proanthocyanidins from cranberry juice prevent the expression of the fimbria of certain bacteria, inhibiting its adherence activity.[21] In addition, Weiss *et al* reported a cranberry constituent to inhibit the adhesion and co-aggregation activities of oral streptococci.[22, 23] Cell surface hydrophobicity plays a vital role in the adhesion of oral bacteria to the tooth surface.[24] The use of cranberry juice can bond to or mask the hydrophobic proteins on the cell surface of oral streptococci. This aids in reducing their cell surface hydrophobicity resulting in significant reduction of their adsorption to the hydroxyapatite of the tooth structure.[8]

The strawberry extract was proven to have an antibacterial effect on several bacteria[25] including *S. mutans* which can aid in inhibiting biofilm formation.[26] These results do not agree with the findings of this study. However, we observed the fresh strawberry juice to have a bactericidal effect on *S. viridans*. This could be beneficial in reducing the risk of infective endocarditis, if any, following dental procedures. Ben Lagha *et al* evaluated the action of blueberry proanthocyanidins on *Aggregatibacter actionomycetecomitans* and documented them to be novel therapeutic agents for periodontal disease.[27] In our present work, the fresh blueberry juice failed to provide an antibacterial effect on any of the tested pathogens. The difference in the antibacterial activity of the cranberry, strawberry and blueberry juices may be attributed to the variations in the nutrient profiles of the berries. Cranberries outrank the other berries with the highest antioxidant content, while strawberries are well known for their high vitamin C content.[28]

Conclusions

The results obtained in this study clearly demonstrate a significant antibacterial effect of fresh cranberry juice on the colonization of oral streptococci. The positive synergistic activity of the three juices can be readily observed but this is less than the effect of the fresh cranberry juice alone. Further studies are warranted to verify which major ingredient(s) of these berries are responsible for the observed antibacterial effect.

Declaration of Interest

The authors report no conflict of interest.

References

1. Featherstone JD. Dental caries: a dynamic disease process. Aust Dent J 2008;53(3):286-91.

2. Yildiz G, Ermis RB, Calapoglu NS, Celik EU, Turel GY. Gene-environment Interactions in the Etiology of Dental Caries. J Dent Res 2016;95(1):74-9.
3. Featherstone JD. The continuum of dental caries--evidence for a dynamic disease process. J Dent Res 2004;83 Spec No C:C39-42.
4. Featherstone JD. The science and practice of caries prevention. J Am Dent Assoc 2000;131(7):887-99.
5. Asty Samiaty Setiawan RRD, Sri Susilawati, Diah Ayu Maharani, Ariadna Adisattya Djais. Biological Factors in 2 – 3 years old Children in Determining Risk Factors of Early Childhood Caries: Pilot Study. Journal of International Dental and Medical Research 2019;12(2):655-71.
6. Forssten SD, Bjorklund M, Ouwehand AC. Streptococcus mutans, caries and simulation models. Nutrients 2010;2(3):290-8.
7. Matsui R, Cvitkovitch D. Acid tolerance mechanisms utilized by Streptococcus mutans. Future Microbiol 2010;5(3):403-17.
8. Yamanaka A, Kimizuka R, Kato T, Okuda K. Inhibitory effects of cranberry juice on attachment of oral streptococci and biofilm formation. Oral Microbiol Immunol 2004;19(3):150-4.
9. Puspa Dwi Pratiwi SBB, Eva Fauziah, Mochamad Fahlevi Rizal, Margaretha Suharsini, Heriandi Sutadi, Ike Siti Indiarti Garlic Extract Effectivity Against the Viability of Biofilms Produced by Streptococcus mutans Serotypes C and F in Pediatric Patients with Early Childhood Caries. Journal of International Dental and Medical Research 2019;12(3):894-99.
10. Sethi R, Govila V. Inhibitory effect of cranberry juice on the colonization of Streptococci species: An in vitro study. J Indian Soc Periodontol 2011;15(1):46-50.
11. Nikita Syahrussiami Firdaus EF, Heriandi Sutadi. Antibacterial Effectiveness of Virgin Coconut Oil Mousse against Streptococcus mutans Biofilm in Early Childhood Caries. Journal of International Dental and Medical Research 2019;12(2):429-33.
12. Cavanagh HM, Hipwell M, Wilkinson JM. Antibacterial activity of berry fruits used for culinary purposes. Journal of medicinal food 2003;6(1):57-61.
13. Ermel G, Georgeault S, Inisan C, Besnard M. Inhibition of adhesion of uropathogenic Escherichia coli bacteria to uroepithelial cells by extracts from cranberry. J Med Food 2012;15(2):126-34.
14. Hisano M, Bruschini H, Nicodemo AC, Srougi M. Cranberries and lower urinary tract infection prevention. Clinics (Sao Paulo) 2012;67(6):661-8.
15. Kessler T, Jansen B, Hesse A. Effect of blackcurrant-, cranberry- and plum juice consumption on risk factors associated with kidney stone formation. Eur J Clin Nutr 2002;56(10):1020-3.
16. Raz R. Urinary tract infection in postmenopausal women. Korean J Urol 2011;52(12):801-8.
17. Lee YL, Cesario T, Wang Y, Shanbrom E, Thrupp L. Antibacterial activity of vegetables and juices. Nutrition 2003;19(11-12):994-6.
18. Flessa S, Lusk DM, Harris LJ. Survival of Listeria monocytogenes on fresh and frozen strawberries. Int J Food Microbiol 2005;101(3):255-62.
19. Xu W, Zhou Q, Yao Y, et al. Inhibitory effect of Gardenblue blueberry (Vaccinium ashei Reade) anthocyanin extracts on lipopolysaccharide-stimulated inflammatory response in RAW 264.7 cells. J Zhejiang Univ Sci B 2016;17(6):425-36.
20. Gold OG, Jordan HV, Van Houte J. A selective medium for Streptococcus mutans. Arch Oral Biol 1973;18(11):1357-64.
21. Gupta K, Chou MY, Howell A, et al. Cranberry products inhibit adherence of p-fimbriated Escherichia coli to primary cultured bladder and vaginal epithelial cells. J Urol 2007;177(6):2357-60.
22. Weiss EL, Lev-Dor R, Sharon N, Ofek I. Inhibitory effect of a high-molecular-weight constituent of cranberry on adhesion of oral bacteria. Crit Rev Food Sci Nutr 2002;42(3 Suppl):285-92.
23. Weiss EI, Kozlovsky A, Steinberg D, et al. A high molecular mass cranberry constituent reduces mutans streptococci level in saliva and inhibits in vitro adhesion to hydroxyapatite. FEMS Microbiol Lett 2004;232(1):89-92.
24. Jalowiecki L, Zur J, Chojniak J, Ejhed H, Plaza G. Properties of Antibiotic-Resistant Bacteria Isolated from Onsite Wastewater Treatment Plant in Relation to Biofilm Formation. Curr Microbiol 2018;75(5):639-49.
25. Khalifa HO, Kamimoto M, Shimamoto T. Antimicrobial Effects of Blueberry, Raspberry, and Strawberry Aqueous Extracts and their Effects on Virulence Gene Expression in Vibrio cholerae. Phytotherapy research : PTR 2015;29(11):1791-7.
26. Philip N, Bandara H, Leishman SJ, Walsh LJ. Inhibitory effects of fruit berry extracts on Streptococcus mutans biofilms. European journal of oral sciences 2018.
27. Ben Lagha A, LeBel G, Grenier D. Dual action of highbush blueberry proanthocyanidins on Aggregatibacter actinomycetemcomitans and the host inflammatory response. BMC Complement Altern Med 2018;18(1):10.
28. Skrovankova S, Sumczynski D, Mlcek J, Jurikova T, Sochor J. Bioactive Compounds and Antioxidant Activity in Different Types of Berries. Int J Mol Sci 2015;16(10):24673-706.