Original Article

Assessment of the antimicrobial efficacy of hydroalcoholic fruit extract of cranberry against Socransky complexes and predominant cariogenic, mycotic and endodontic climax communities of the oral cavity: An extensive *in-vitro* study

Vaibhav Kumar[1], Anil V Ankola[2], Roopali Sankeshwari[2], Sagar Jalihal[2], V Deepak[3], Harshvardhan S Jois[4]

[1]Department of Public Health Dentistry, TPCT's Terna Dental College, Navi Mumbai, Maharashtra, [2]Department of Public Health Dentistry, KLE VK Institute of Dental Sciences, KLE Academy of Higher Education and Research, Belagavi, [3]Department of Dentistry, ESIC Medical College, PGIMSR and Model Hospital, Bengaluru, Karnataka, [4]Department of Oral Pathology, Army College of Dental Sciences, Secunderabad, Telangana, India

Abstract

Background and Objective: Contemporary global complementary and alternative practices in dentistry envisage to manipulate the molecules from traditionally used plant derivatives as adjuncts. The aim of this study was to assess the *in-vitro* antimicrobial efficacy of hydro-alcoholic fruit extract of cranberry against Socransky complexes and predominant cariogenic, mycotic and endodontic climax communities of the oral cavity.

Materials and Methods: An extract of *Vaccinium macrocarpon* was prepared using a hydro-ethanolic solvent (water – 30%: ethanol – 70%) using the standardized maceration protocol. Standard American Type Cell Culture and Microbial Type Culture Collection and Gene Bank strains of *Streptococcus mutans*, *Lactobacillus acidophilus*, *Candida albicans*, *Enterococcus faecalis*, *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans* were used. The minimum inhibitory concentration was determined by the serial broth dilution. The minimum bactericidal concentration was obtained by subculturing method.

Results: The extract showed satisfactory inhibitory and bactericidal effects against all test pathogens.

Conclusion: *V. macrocarpon* can prove to be an adjunct to the existing antimicrobial complexes being routinely used to combat pathogenic oral climax community.

Keywords: Antimicrobials, climax communities, cranberry, phytodentistry

Address for correspondence: Dr. Vaibhav Kumar, Department of Public Health Dentistry, TPCT's Terna Dental College, Nerul, Navi Mumbai, Maharashtra, India.
E-mail: webs.k@hotmail.com
Received: 08.01.2018, **Accepted:** 27.06.2019

INTRODUCTION

The modern-day dental science aims at aiding and guiding the masses to gain maximum oral health from the womb to the tomb. The major challenge for achieving this goal is the widespread nature of the dental disease. The key elements related to the ecology of the oral microbiota include the habitat and the stability of the "climax" community.[1]

Access this article online

Quick Response Code:

Website: www.jomfp.in

DOI: 10.4103/jomfp.JOMFP_3_18

How to cite this article: Kumar V, Ankola AV, Sankeshwari R, Jalihal S, Deepak V, Jois HS. Assessment of the antimicrobial efficacy of hydroalcoholic fruit extract of cranberry against Socransky complexes and predominant cariogenic, mycotic and endodontic climax communities of the oral cavity: An extensive *in-vitro* study. J Oral Maxillofac Pathol 2019;23:407-11.

Dental caries, periodontal diseases, endodontic enigmas and candidal colonization constitute significantly to the oral core concerns. Successful attempts have been made to combat the high incidence of these oral diseases in developed countries, but their incidence is still a great burden in developing countries.[2,3]

A growing interest has developed to identify the dietary components and botanical or nutritional supplements owing to their therapeutic potentials. In the search for new antimicrobials for the prevention of diseases, it has stated that plant extracts could be a better source for a variety of drugs and active molecules.[3] Scientific interest in medicinal plants has grown due to increased efficiency of new plant-derived drugs, growing interest in natural products and rising concerns about the side effects of conventional medicine. The use of various herbal remedies and preparations is described throughout the human history representing the origin of modern medicine.[4] Dental medicine has become especially amenable to plant-derived products, driven by evidence that shows that population which regularly incorporates food or beverages-containing certain phytochemicals into their diet have better oral health.[5]

The perpetual search is on to find botanical complementary adjuncts to the conventional therapies used, that is, cost-effective and reduces the side effects associated with conventional synthetic drugs available in the market and tackle the issue of antimicrobial resistance.

Cranberry (*Vaccinium macrocarpon*) the "Wonder Fruit" has recently come into limelight owing to its significant therapeutic potential. Cranberry itself is a unique and rich source of several classes of bioactive flavonoids, including flavanols, anthocyanins and proanthocyanidins, which confer it the significant therapeutic potential.[6]

The medicinal use of cranberry dates way back to the 17th century when it was a popular treatment for scurvy and gastric problems. They have proven to prevent adhesion of *Helicobacter pylori* to the gastric mucosa, thus interrupting a critical stage in the development of gastric ulcers in humans.[7] They have also shown remarkable effects in the prevention of urinary tract infections by acting against *Escherichia coli*.[8]

Limited studies are available in literature pertinent to cranberry fruit extract in dental medicine.

However, these studies suffer from a number of limitations. One of the most important limitations was the wide range of cranberry products used in various studies, and the second was their dosage regimes,[8] which has created confusion to the existing knowledge. Further, the organic solvent used in most of the studies is methanol, which has its own limitations to be used as a vehicle for *in vivo* studies.[6] The polyphenolic fraction of the fruit reportedly repressed the formation of biofilms and acid production by cariogenic Streptococci[9] and inhibits the docking of *Porphyromonas gingivalis* to various adhesion proteins, including type I collagen and may reduce bacterial coaggregation involving periodontopathogenic bacteria.[1,10]

To the best of our knowledge, literature lacks evidence about the activity of *V. macrocarpon* extract on *Enterococcus faecalis* and *Candida albicans*. Further, high dextrose and fructose of the commercially available cranberry products make it unsuitable for oral use, and the beneficial effect of high-molecular weight constituent requires further research.[11]

Thus, there is an impending need for assessing and standardizing the antimicrobial properties of a novel Cranberry extract on the oral microenvironment. The current *in vitro* research aims at establishing a standardized hydroethanolic extract of *Vaccinium macrocaropon* and assessing its antimicrobial activity on standard strains of six oral pathogenic microorganisms under the cariogenic, periodontic, endodontic and candidal domain, namely *Streptococcus mutans, Lactobacillus acidophilus, P. gingivalis, Aggregagibacter actinomycetemcomitans, E. faecalis and C. albicans.*

MATERIALS AND METHODS

Plant materials

Fresh, preidentified individually quick frozen cranberry fruits (*V. macrocarpon*) (FSSAI FSSL 11214331000541) were procured from Very Berry Fruits Pvt. Ltd., Bengaluru, Karnataka, India. The fruits were authenticated by a botanist and pharmacist. The fruits were weighed, rinsed under running tap water and transversely sectioned into two halves. The water/fluid content from the specimens was removed by tissue paper blotting. The fresh fruits were dried under the shed (25°C) for 7 days till constant weight was achieved.

Preparation of crude extract

The dried samples of *V. macrocarpon* were subjected to the maceration method to extract the alkaloids from it. Twenty gram of the dried fruit was macerated with 200 ml of hydro-alcoholic solvent, with a solvent ratio of ethanol (70%): water (30%) in a conical flask, plugged with cotton and kept occasionally on a rotary shaker at 190–220 rpm for 48 h at room temperature. The macerated liquid was filtered through Whatman Filter Paper No. 1 (Sigma Aldrich Chemicals Pvt. Ltd., Bengaluru, India).

The extraction processes were carried out for 3 days to obtain crude extracts which were evaporated and concentrated under reduced pressure using a vacuum rotary evaporator (Sigma Aldrich Chemicals Pvt. Ltd., Bangalore, India). The residue left was semi-solid, reddish-pink in color, which was soluble in aqueous solution. It was stored at 4°C in air-tight sterile container till further use.[12,13]

Preparation of bacterial suspension

A total of six oral pathogens were used in this study. Four MTCC (Microbial Type Culture Collection and Gene Bank, Chandigarh, India) aerobic Gram-positive organisms, which included *S. mutans* (MTCC 25175), *E. faecalis* (MTCC 35550), *L. acidophilus* (MTCC 8129), *C. albicans* (MTCC 2091) and two American Type Cell Culture (ATCC) obligate anaerobic Gram-negative periodontopathic bacteria, *A. actinomycetemcomitans* (ATCC 29522) and *P. gingivalis* (ATCC 33277) [Table 1].

Biochemical tests

Conventional bacteriological methods such as colony morphology, Gram staining and biochemical tests were used for the identification of the isolates.

Microbial subculturing

Isolated microorganisms were kept on glycerol broth at − 80°C. Before testing antibacterial activity, stored anaerobic microorganism was subcultured on blood agar supplemented with hemin, and Vitamin K (HiMedia, Mumbai, India) and aerobic microorganism subcultured on brain–heart infusion (BHI) agar plate and stored in 2°C–4°C and it used for further testing. The bacterial stock cultures (stored at −80°C freezer) were obtained from Basic Science Research Laboratory, KLE University, Belagavi, Karnataka, India. The bacterial stock cultures were thawed and placed in BHI (HiMedia, Mumbai, India) broth for Gram-positive bacteria and supplement-BHI (S-BHI) (Thermo Fisher Scientific India Pvt. Ltd., Mumbai, India) which was supplemented with yeast extract (Scharlau, Spain) for Gram-negative bacteria. All the broths with bacterial growth were incubated at 37°C, 5% CO_2 (Shel Lab, Oregon, USA) for 24 h (faculative anaerobic) or 48 h (obligate anaerobes). The bacteria were then standardized using Mac Farland standard 0.5 for aerobic culture and 1 for anaerobic culture.

Determination of minimum inhibitory concentration and minimum bactericidal concentration

Minimum inhibitory concentration: Serial broth dilution method

Two hundred microliter of the following media was added from the 2nd to 10th tube by using a micropipette. BHI Broth was added to *S. mutans* (MTCC 25175), *E. faecalis* (MTCC 35550), *L. acidophilus* (MTCC 8129) and *C. albicans* (MTCC 2091). For *P. gingivalis* (ATCC 33277) and *A. actinomycetemcomitans* (ATCC 29523), 200 µl of BHI broth supplemented with 2% horse serum. 200 µl of the fruit extract was added to the 1st and 2nd tube. Broth and fruit extract combination solution was roughly mixed using a micropipette. Further, 200 µl of the diluted extract is pipetted and added to the 3rd tube, and the serial dilution was done till the 9th tube. Hence, the first tube is positive control, and the 10th tube is negative control. Finally, 200 µl of inoculums of respective microorganisms (culture) were added to all the ten tubes. All the microcentrifuge tubes were incubated in a bacteriological incubator (aerobic: 24 h and anaerobic: 48 h). The minimum concentration that showed no turbidity was considered to be the MIC value of the test compound against the tested organism.

Minimum bactericidal concentration: Agar plate subculture streaking method

The minimum bactericidal concentration (MBC) value was determined by subculturing on BHI or S-BHI agar plates of each well component that was not showing visible indicator changes. The least concentration which showed no visible growth on the agar plate after incubation period was considered as the MBC value. Gram-positive bacteria or S-BHI broth for Gram-negative bacteria and serially diluted by the twofold method. From the 10th well, 50 µL of solution was pipetted out and disposed of. Subsequently, 50 µL of bacterial suspension containing 1–2 × 10^8 CFU/mL was added into the 1st–11th well. The 11th well served as the positive control, containing 50 µL BHI (for Gram-positive bacteria) or S-BHI broth (for Gram-negative bacteria) and 50 µL of bacterial suspension. The 12th well contained 50 µL broth (BHI or S-BHI) and 50 µL of tested extract with no bacterial inoculation and served as negative control.

Table 1: Test microorganisms used for *in vitro* analysis

	Cariogenic pathogens	Socransky complex periodonto-pathogens		Endodontic pathogen	Mycotic pathogen
		Colour complex	Micro-organism		
Gram positive aerobic	*Streptococcus mutans* *Lactobacillus acidophilus*			*Enterococcus faecalis*	*Candida albicans*
Gram negative anaerobic		Red Green	*Porphyromonasgingivalis* *Aggregatibacteractinomycetemcomitans*		

RESULTS

The minimum inhibitory concentrations (MICs) and MBCs of the hydroethanolic extract of the fruit of *V. macrocarpon* against oral pathogens are specified in Table 2, Figures 1 and 2. The relatively lower levels of concentration of the extract indicate a high level of antimicrobial properties. Growth inhibitory effect was seen for all the test microorganisms. *S. mutans*, *E. faecalis* and *C. albicans* showed MIC values of 50 μg/ml. *A. actinomycetemcomitans* (ATCC 29523) showed a significant antimicrobial efficacy at the lowest concentration of 12.5 μg/ml (MIC) and 25 μg/ml (MBC).

Table 3 elucidates the antimicrobial efficacy of the extract after inoculation into various tubes during the serial dilution method for MIC determination.

The ratio of the MIC and MBC concentrations indicated that the extract is bacteriostatic at lower concentrations and bactericidal at relatively high concentrations. The overall results revealed a wide spectrum of antimicrobial efficacy for the *V. macrocarpon* extract, targeting a versatile range of cariogenic, mycotic, periopathogenic and endodontic oral climax communities.

Table 2: Total minimum inhibitory concentration and minimum bactericidal concentration values of test microorganisms

Test microorganism	Total MIC value±0.00 SD (μg/ml)	Total MBC value±0.00 SD (μg/ml)
Streptococcus mutans	50	100
Enterococcus faecalis	50	100
Lactobacillus acidophilus	25	50
Candida albicans	50	100
Porphyromonas gingivalis	25	50
Aggregatibacter actinomycetemcomitans	12.5	25

The lowest MIC value indicates the highest inhibitory effect. The lowest MBC value indicates highest bactericidal effect. MIC: Minimum inhibitory concentration, MBC: Minimum bactericidal concentration, SD: Standard deviation

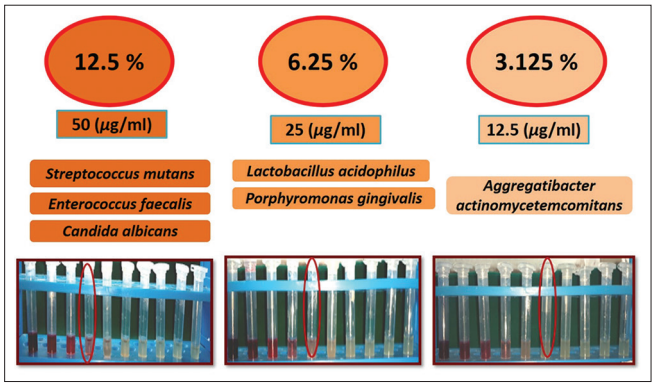

Figure 1: Determination of minimum inhibitory concentration (MIC) by serial broth dilution method

DISCUSSION

The present study utilizes a novel extract preparation from a fruit. Previous studies have used a pure alcoholic extract. However, incorporating water in the extract preparation could contributory to the dissolution of the water-soluble active components in the fruit, rendering the wide therapeutic range to the test compound. Anti-adhesion property of the extract against *S. mutans* was reported by Yamanaka *et al*.[9] and Weiss *et al*.,[11] which showed similar results when compared to our present study. Labrecque *et al*.[10] reported similar antimicrobial efficacy with a pure alcoholic extract of *V. macrocarpon* against *P. gingivalis* (MIC = 62.5 μg/ml and MBC = 125 μg/ml). *A. actinomycetemcomitans* and *Fusobacterium nucleatum* were also inhibited at concentrations of 12.5 μg/ml and 25 μg/ml. In an attempt to elucidate the anti-periopathogenicity of the *V. macrocarpon* extract, it was hypothesized that a pro-inflammatory cytokine response of macrophages induced by lipopolysaccharides was responsible for the inhibition of interleukin 6, interleukin 1β and tumor necrosis factor-α.[14]

The significant antimicrobial activities of the *V. macrocarpon* fruit extract indicate its medicinal properties that could be effectively used against oral pathogens. To the best of our knowledge, the present study is the first of its nature, which aimed to assess the antimicrobial efficacy of a novel extract preparation against six different oral pathogens that are responsible for the various dental pathogen such as cariogenic, periopathogenic, endodontic and mycotic oral climax communities. The vehicle of the application was also modified from methanol to a water-based ethanol solvent, which negates the toxicity. All the previous studies have used a methanolic extract, with no evidence of gas chromatography analysis employed to elucidate 100% elimination of methanol vapors from the extract.

With its proven antimicrobial efficacy and high medicinal properties, the *V. macrocarpon* extract promised to be of

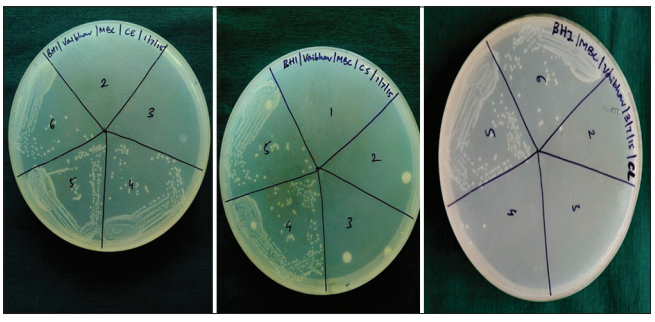

Figure 2: Demonstration of minimum bactericidal concentration (MBC) by agar plate sub-culturestreaking metho

Table 3: Antimicrobial efficacy of Vaccinium macrocarpon extract against predominant oral climax communities

Concentration of *Vaccinium macrocarpon* extract.(μg/ml)	*Streptococcus mutans*	*Lactobacillus acidophilus*	*Porphyromonas gingivalis*	*Aggregagib acteractinomycetemcomitans*	*Enterococcus faecalis*	*Candida albicans*
400.000	-	-	-	-	-	-
200.000	-	-	-	-	-	-
100.000	-	-	-	-	-	-
50.000	-	-	-	-	-	-
25.000	+	-	-	-	+	+
12.500	+	+	+	-	+	+
6.250	+	+	+	+	+	+
3.125	+	+	+	+	+	+
1.562	+	+	+	+	+	+
0.000	+	+	+	+	+	+

tremendous public health and clinical potential. This novel extract preparation could be self-administered by incorporating in the formulation of a mouthwash that could be used for independent or supervised rinsing. It could be given to children too as a swish and swallow procedure since small amounts of accidental swallowing would be noncontributory to side effects, owing to the multiple systemic medicinal effects. It could be professionally administered in the form of local drug delivery systems such as irrigants (endodontic infections), gels (periodontal disarray), microspheres, nanoparticles or buccal patches (candida colonization).

We further encourage *in vitro* studies to be performed using this extract to isolate individual components of the extract, time-kill assays, DNA fragmentation procedures and to discern the actual mechanism of the target on cancer molecules through the gene expression or proteomic studies.

CONCLUSION

The study forms as a baseline for extensive research to be carried out with the *V. macrocarpon* extract. Clinical trials would further consolidate the findings of this study. For the manifold menaces of the oral cavity, i.e., dental caries, periodontitis, endodontic and candidal lesions, the authors have strived to present one universal, versatile and standardized solution.

Acknowledgments

The authors would like to acknowledge KLE University's Basic Science Research Center, Belagavi and Mr. Bolmal, College of Pharmacy, KLE University Belagavi, for all their help and support.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Marsh PD. Role of the oral microflora in health Microb Ecol Health Dis 2000;12:130-7.
2. Petersen PE, Bourgeois D, Ogawa H, Estupinan-Day S, Ndiaye C. The global burden of oral diseases and risks to oral health. Bull World Health Organ 2005;83:661-9.
3. Cowan MM. Plant products as antimicrobial agents. Clin Microbiol Rev 1999;12:564-82.
4. Suk Lee J. Medicinal plants: A powerful health aid? Sci Creat Q 2013;8:34-8.
5. Yoo S, Murata RM, Duarte S. Antimicrobial traits of tea-and cranberry-derived polyphenols against *Streptococcus mutans*. Caries Res 2011;45:327-35.
6. Pappas E, Schaich KM. Phytochemicals of cranberries and cranberry products: Characterization, potential health effects, and processing stability. Crit Rev Food Sci Nutr 2009;49:741-81.
7. Shmuely H, Ofek I, Weiss EI, Rones Z, Houri-Haddad Y. Cranberry components for the therapy of infectious disease. Curr Opin Biotechnol 2012;23:148-52.
8. Rahbar M, Diba K. *In vitro* activity of cranberry extract against etiological agents of urinary tract infections. Afr J Pharm Pharmacol 2010;4:286-8.
9. 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;19:150-4.
10. Labrecque J, Bodet C, Chandad F, Grenier D. Effects of a high-molecular-weight cranberry fraction on growth, biofilm formation and adherence of *Porphyromonas gingivalis*. J Antimicrob Chemother 2006;58:439-43.
11. Weiss EI, Lev-Dor R, Kashamn Y, Goldhar J, Sharon N, Ofek I. Inhibiting interspecies coaggregation of plaque bacteria with a cranberry juice constituent J Am Dent Assoc 1998;129:1719-23.
12. Mbata TI, Debiao LU, Saikia A. Antibacterial activity of the crude extract of Chinese green tea (Camellia sinensis) on Listeria monocytogenes. Afr J Biotechnol 2008;7:1571-3.
13. Nair R, Kalariya T, Chanda S. Antibacterial activity of some selected Indian medicinal flora. Turkish J Biol 2005;29:41-7.
14. Bodet C, Chandad F, Grenier D. Anti-inflammatory activity of a high-molecular-weight cranberry fraction on macrophages stimulated by lipopolysaccharides from periodontopathogens. J Dent Res 2006;85:235-9.