

Effect Of Vaccinium Macrocarpon On Candida Albicans Adhesion To Denture Base Resin

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Abstract

Objective

This study aimed to assess the effectiveness of Vaccinium macrocarpon (cranberry) extract on Candida albicans adhesion to cranberry added denture base biomaterial.

Method

Different proportions of cranberry extract were incorporated into polymer of polymethyl methacrylate (PMMA) self-cure resin material for fabrication of 4 mm x 4 mm x 2 mm samples. Group I contained 0 wt/wt% cranberry was used as control group. 0.5 wt/wt%, 1.0 wt/wt%, 1.5 wt/wt% and 2 wt/wt% was graded as Group II to V in order. Addition of 0.1 mL of Candida albicans suspension was done in each well of tissue culture plate and incubated for 48 hours. 2% cranberry was used as disinfectant solution in four serial dilutions at undiluted, 1:10, 1:100, 1:1000 ratio. Immersion of resin samples in disinfectant solution was done for 10 minutes. A colony forming unit was measured after 0.1 mL of the solution was plated on Sabouraud dextrose agar (SDA) plate and cultured for 72 hours. Data were subjected to statistical analysis.

Results

The mean colony forming units (CFU) per mL showed that there were several confluent colonies with all proportions of cranberry extract added poly methyl methacrylate (CA-PMMA) resin samples in undiluted and 1:10 dilution state with both distilled water and cranberry disinfectants. While immersion in distilled water at 1:100 dilution, mean CFU/mL was 2.99 ± 0.29 in all groups of CA-PMMA. In 1:1000 dilution state, the mean \pm SD was 117 ± 0.42 . Immersion in cranberry solution at 1:100 and 1:1000 showed 2.65 ± 0.66 and 1.26 ± 0.59 for all groups of CA-PMMA. On comparison between distilled water and cranberry as disinfectants, a statistically significant difference was noted with all groups as $p < 0.043$. Insignificant variation was observed within the groups with both disinfectants as $p > 0.05$.

Conclusion

Adherence of Candida albicans to cranberry added self-cure poly methyl methacrylate biomaterial was evidenced to be less irrespective of extract proportions of 1%, 1.5% and 2% in PMMA. Cranberry's effectiveness as disinfectant showed less Candida growth when compared to distilled water immersion. This infers that cranberry has anti-adherence property when used both as a polymer constituent material as well as disinfectant solution.

Keywords: Denture stomatitis, Edentulism, Geriatrics, Cranberry, Polymethyl methacrylate

Introduction

Increase in geriatric population in developed and developing countries is prevalent with high life expectancy. While natural teeth are lost, mastication, speech and facial appearance is compromised. Restoration of oral functions with dental prosthesis becomes subject imperative for well-being of edentulous individuals.¹ Till date, the preferred material in removable denture fabrication is polymethyl methacrylate resin, attributed to its superior mechanical, physical and biological properties. Maintenance of proper oral and or denture hygiene mandates patient compliance and follow-up of adequate practices.²

Infection of dental prosthesis, called as denture stomatitis is a common clinical condition which occurs because of multifactorial reasons. *Candida albicans*, is a distinct oral fungus that can turn opportunistic and infect the dentures leading to complications. Irregular denture surface attracts these microbes to form a concrete biofilm. Chronic persistence of oral candida infections subject to serious detrimental issues such as aspiration pneumonia and systemic candidiasis.³⁻⁵ Several therapeutic methods such as antifungal treatment with synthetic drugs, use of mouth rinses, lozenges, immersion in disinfectant solutions, physical denture brushing, photodynamic therapy are attempted over the years. Though, systemic drugs are proven to be effective in mucosal lesions, they do not exert direct benefit when it comes to tissue surface of dentures.⁶

In recent years, many medicinal plants are researched for their antimicrobial properties against denture infections.⁷ One such is *Vaccinium macrocarpon*, commonly called as cranberry has shown promising anti-infective potential owing to its high and enriched phytochemical profile.⁸ In medical health sciences, it is commonly used for treatment of urinary tract infections.⁹ It has also shown documented evidence in reduction of oral infections in dental caries, periodontal disease, has antioxidant, and anticancer property.¹⁰ In this study, pure cranberry powder extract was prepared from cranberry fruit and was added into different proportions into polymethyl methacrylate denture base resin material. This new biomaterial was then checked for *Candida albicans* adhesion when immersed in distilled water and 2% cranberry juice solution.

Methods

Extraction process

Frozen cranberry (*Vaccinium macrocarpon*) fruits were purchased (Maple Leaf Exports Co., India) and stored at -20°C in deep freezer. Before usage, it was thawed at 5°C and subjected to cold press in a hydraulic domestic juice extractor. Berry press residue was got after removal of seeds and skin. This primary residue was then lyophilized at -45°C for 3 days. Lyophilized preliminary extract was then subjected to hot extraction process using a Soxhlet apparatus (Singhla Scientific Industries, India). Hot extraction process was facilitated with aid of 96% ethanol and 0.5% trifluoroacetic acid (TFA) (v/v). Reagents were purchased from Sigma-Aldrich. Processing was done at 80°C for 48 hours with a condenser. Ethanol and TFA extract was concentrated in a rotary evaporator and collected extract was stored at 4°C. Acquired extract was filtered in a Whatman No.1 filter paper to remove insoluble particles. Filtrate were then lyophilized to obtain final extract in powder form. Cranberry powder extract was loaded in planetary ball milling unit (PM 100 CM, Retsch GmbH, India) and was programmed at 300 RPM for 90 minutes to achieve uniform homogenization of particles. Final extract was stored in vacuum desiccator until further use.¹¹

Preparation of samples

Addition of cranberry extract into polymer of polymethyl methacrylate resin material was done in varying proportions as 0 wt./wt.%, 0.5 wt./wt.%, 1.0 wt./wt.%, 1.5 wt./wt.%, and 2 wt./wt.%. A digital weighing balance (Wensar MAB 250, digital micro balance, India) was used for appropriate measurement. The study comprised of five groups from Group I to V based on the incorporation of extract proportions, in order as shown in Table 1. For achievement of a new composite biomaterial constituting self-cure PMMA polymer and cranberry

extract powder was ball milled for 30 minutes at 300RPM. This cranberry added biomaterial was stored in five different vacuum desiccator based on proportions, until utilised for acrylic block preparation.

A standardized metal die of 4mm x 4mm x 2mm was prepared for making of study samples. It was duplicated in addition silicone putty material (Aquasil, Dentsply, India). A total of 50 wax samples were first obtained after filling the putty index with modelling wax (Hindustan modelling wax, India). These wax blocks were invested in Type III dental stone (Ultrastone, Kalabhai, India) in dental flask for making of mould. On setting of investment material, the flask was placed in dewaxing bath unit for boil out of wax. Once all the wax was eliminated the mould was ready for packing with all new cranberry added self-cure PMMA (CA-PMMA) biomaterial. Mixing of CA-PMMA polymer and monomer was done in ration of 3:1. When the material reached the dough stage, it was packed into the mould and flask was placed in hydraulic bench press for 30 minutes until the material was set to harden. A waiting period of additional 15 minutes was given to ensure near polymerization. After it was set, samples were retrieved for final finishing and polishing. Gross trimming was done with various grades of acrylic trimmers followed by sandpapering till 400 grit size paper. This ensured no excess material and gross roughness was removed. Wet polishing was done using pumice slurry loaded on a cotton buff in dental lathe (Suguna lathe, India). Dry polishing was done with woollen buff in lathe. Samples were disinfected with 70% alcohol and washed under sterile distilled water prior to adherence assay.

Adherence testing

Of the five groups, comprising of 10 samples in each, five were immersed in 2% cranberry juice and five in distilled water for evaluation of *Candida albicans* ATCC 3153 strain adherence testing. For adherence assay, a 10mL suspension of fresh growth was formulated in a test tube by incorporating *Candida* cells grown from Sabouraud dextrose agar (SDA) medium. It was facilitated with aid of bacteriological inoculation loop to sterile saline solution and was kept in cyclomixer (Remi CM-101 Plus cyclomixer, India) for 60 seconds. A standardized suspension containing 1.5×10^8 colony forming unit (CFU) was prepared corresponding to 0.5 McFarland standard turbidity. Each specimen was placed in a well of tissue culture plate. To the culture plate, 1.5 mL of Sabouraud's dextrose broth (SDB) and 0.1 mL of *Candida albicans* suspension was added with the help of a micropipette. The procedure was repeated for all the culture plates, sealing was done and was incubated for 48 hours at 37° C.

Distilled water and 2% cranberry juice solution was opted as disinfectant test solutions in this study. Five samples from each group was immersed in distilled water and five other was immersed in 2% cranberry solution. Out of fifty, twenty five samples were immersed separately in two different disinfectants. For this, 10mL of disinfectant was taken in 20 mL test tube with pipette, and specimens were immersed in the corresponding solution for 10 minutes according to the group mentioned. Following this, each specimen was shifted to tubes with 1 mL of sterile physiological solution, where the tubes were agitated in cyclomixer for 60 seconds to allow dispersion of adhered cells. Direct subculture from the suspension was not done as it could lead to confluent growth of *Candida albicans*, making colony counting difficult. To avoid this, serial dilution of initial suspension was done at 10, 100, and 1,000 times in the physiological solution. By this way, totally four plates for each sample was prepared at undiluted (UN) solution, 1:10, 1:100, and 1:1000 dilutions. Each culture plate was incubated for 72 hours at 37° C and the number of colony forming unit in each plate of each disinfectant at various dilutions were measured using a digital colony counter (10DCC01 DBK digital colony counter, India). The values were expressed as number of colonies forming units per mL (CFU/mL).¹²

Results

According to Table 2, descriptive statistics, in undiluted and 1:10 dilution when immersed in distilled water and cranberry juice disinfectants, in all the groups the colony forming units were confluent and too numerous to be counted. In distilled water immersion, mean CFU/mL was 2.99 ± 0.29 and 1.17 ± 0.42 in 1:100 and 1:1000 respectively. When soaked in cranberry it was 2.65 ± 0.66 and 1.26 ± 0.59 with 1:100 and 1:1000 dilutions correspondingly. Intragroup comparison between the disinfectants at various dilutions was statistically significant as p values was less than 0.05 (Table 3). Intergroup comparison amongst the groups proved to be statistically

insignificant as $p > 0.05$ (Table 4). Numerical mean CFU/mL showed definitive reduction in *Candida* adhesion with both distilled water and cranberry disinfectants in 1:100 and 1:1000 dilutions compared to undiluted and 1:10 series. Culture plates with colony forming units for five groups when immersed in disinfectants are shown in Figures 1 – 5.

Table 1: Different groups based on proportions of extract added into denture base resin

S.no	Proportion of extract	Group
1	0 %	I
2	0.5 %	II
3	1.0 %	III
4	1.5 %	IV
5	2.0 %	V

Table 2: Descriptive statistics of the mean colony forming units (CFU/mL) in each group

Disinfectants	Subgroup	Minimum	Maximum	Mean	Standard Deviation (SD)
Distilled water	1:100	2.74	3.50	2.99	0.29
	1:1000	.61	1.65	1.17	0.42
Cranberry juice	1:100	1.89	3.54	2.65	0.66
	1:1000	.71	1.96	1.26	0.59

Table 3: Wilcoxon signed rank test for intragroup comparison between the two disinfectants

Disinfectants	Rank	Mean Rank	Sum of Ranks	Z	P value
Distilled water	Negative Ranks	3.00	15.00	-0.194604	0.043
	Positive Ranks	0.00	0.00		
Cranberry juice	Negative Ranks	3.00	15.00	-0.194604	0.043
	Positive Ranks	0.00	0.00		

Table 4 : Mann Whitney U test for intergroup comparison between the disinfectants

Subgroup	Group	Mean Rank	Sum of Ranks	U	p value
1:100	Distilled water	6.20	31.00	9.000	0.465
	Cranberry juice	4.80	24.00		
1:1000	Distilled water	5.20	26.00	11.000	0.754
	Cranberry juice	5.80	29.00		

U statistic and p value obtained from Mann Whitney U test. $p \text{ value} \leq 0.05$ is significant

Discussion

Vaccinium macrocarpon, commonly called as cranberry is a fruit native to North America. It is consumed in varied forms as fruit, juice, sauce, medicated powders, or tablets. Extract obtained from cranberry is rich in flavonoids, flavanols, quercetin, anthocyanins, polyphenols, to prove its health benefits.¹³ In medical field, it is widely used in prevention of kidney and bladder infections.¹⁴ Diseases associated with blood, gastrointestinal disorders, scurvy, and cancer have been treated with cranberry.¹⁵ According to Howell et al., proanthocyanidins present in cranberry inhibited the adhesion of uropathogenic microbes.¹⁶ Shmueli et al., stated, cranberry limited adherence of *Helicobacter pylori* to gastric mucosa thereby prevented gastric ulcer development.¹⁷ Cranberry showed anti-infective property against bacteria, virus and fungus as per research work of Weiss et al.¹⁸ Sun and Hai Liu¹⁹ observed cranberry's prospect in inhibition of human oral, colon, prostate and breast tumor cells.

When definitive evidence is perceived from previous research work, its effect in prevention of oral infections was attempted in this study. Denture stomatitis is a common oral infection affecting denture wearers causing inflammation, redness and discomfort. Based on Gendreau L et al., denture stomatitis affects 15% to 70% of denture wearers. Several therapeutic strategies are attempted such as denture cleaning, photodynamic therapy, synthetic antifungal drugs. Limitations such as drug resistance, drug-to-drug interaction, side effects, high cost provoked to search for a more natural medicament to combat denture stomatitis.²⁰ Of the many microorganisms that infect dentures, *Candida albicans* is an opportunistic yeast that has a potential to form filament and biofilm on both biotic and abiotic surfaces. Its biofilm architecture permits it to stay on denture surface for prolonged time leading to recurrent infections.²¹

Incorporation of cranberry extract in varying proportions into self-cure polymethyl methacrylate denture base resin was done to study its effect on *Candida* adherence when immersed in distilled water and cranberry solution. Pure extract from cranberry was obtained with ethanol and TFA. To achieve bioactive polyphenols and anthocyanins in cranberry extract, ethanol and TFA which is readily used in food industry, and has negligible toxicity compared to methanol was used in the study. Denev et al. stated that the use of acids in anthocyanin extraction stabilised these molecules in the flavylium cation form. The choice of acid influenced the stability of anthocyanins.²² Di Martino et al., in his double blinded randomized trial proved that administration of cranberry juice was dose dependent and decreased bacterial adhesion to human epithelial cells to 45% when 250mL was given and 72% decline was noticed when 750mL was given.²³

Results of this study showed that, all samples of cranberry added PMMA in undiluted and 1:10 dilution when immersed in distilled water and cranberry juice showed confluent growth of *Candida* that were too numerous to count. Least colony forming units per mL was observed at 1:1000 dilution in Group V where 2% of CA-PMMA while immersed in distilled water and cranberry juice. On comparison between 1:100 and 1:1000 dilutions, a significant reduction in CFU/mL was noted in all groups at 1:1000 dilution while immersion in both distilled water and cranberry juice ($p < 0.05$). Intergroup differentiation between distilled water and cranberry juice for all proportions of CA-PMMA showed numerical variation which was statistically insignificant ($p > 0.05$). Intragroup comparison revealed least growth in Group V where 2% of cranberry extract was added into PMMA samples. It is evident that anti-adhesion effect was dose dependent, as higher the concentration of cranberry extract lower was the colony forming units per milli litre. Less numerical CFU/mL was determined in higher dilutions demonstrating the anti-adhesion potential of cranberry.

The results of current study was supported by research done by Marion Girardot et al who showed promising role of cranberry extract against oral candida biofilm at concentrations $> 1.25 \text{ mg mL}^{-1}$. It was also demonstrated in his study that pre-treated surface of cranberry showed higher anti-adhesion property towards *Candida glabrata* species rather than *Candida albicans*. Anti-adhesion property of cranberry was concentration dependent, species and strain specific.²⁴ Fungistatic activity of cranberry was evidenced by antimetabolic action on *Candida* as furnished by Swartz & Medrek in 1968.²⁵ Yamanaka et al²⁶ and Wojnicz et al.,²⁷ reported a decrease in hydrophobicity of bacteria such as *E. coli* and cariogenic when cranberry was given as extract or juice form. The main bioactive phytoconstituents present in cranberry responsible for anti-adhesion property was low molecular proanthocyanidins (PAC), flavonoids, flavanols. The present results are further in accordance with previous research done by Feldman et al.,²⁸ and Rane et al⁸ who proved anti-adherence property of cranberry against

C. albicans. Possible mechanism was attributed to surface hydrophobicity and changes in surface composition on exposure to cranberry juice.

Conclusion

This in-vitro study which was first of its kind, by addition of cranberry extract into polymethyl methacrylate denture base resin material, must be inferred within its limitations. Promising results were obtained to show its anti-adherence property against *Candida albicans* species. Inclusion of sweeteners to limit its acidity in juices, less crude component of cranberry must not be overlooked as no difference was elicited when compared with distilled water, as disinfectants. Though cranberry is assumed to be “wonder fruit” its exact mechanism for its efficacy mandates more research in this domain. Its drug bioavailability, in-vivo metabolism demands extensive clinical research in field of dentistry. From the inference of present study, it is understood that addition of cranberry extract is more effective than juice form in producing anti-adherence property. As part of preliminary research, less samples were utilized in this study. Further studies with large samples, use of heat or light activated polymethyl methacrylate denture base resins, can be performed for better understanding of its integration with cranberry. Within the shortcomings of this study, it can be concluded that 2% cranberry extract when added into self-cure polymethyl methacrylate showed anti-adherence property by means of least colony forming units of *Candida albicans* per mL than its effect while immersed in cranberry juice as disinfectants.

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Figurelegends

Figure 1: Group I: Colonies on undiluted, 10, 100, 1000 dilution of 0% cranberry added PMMA samples

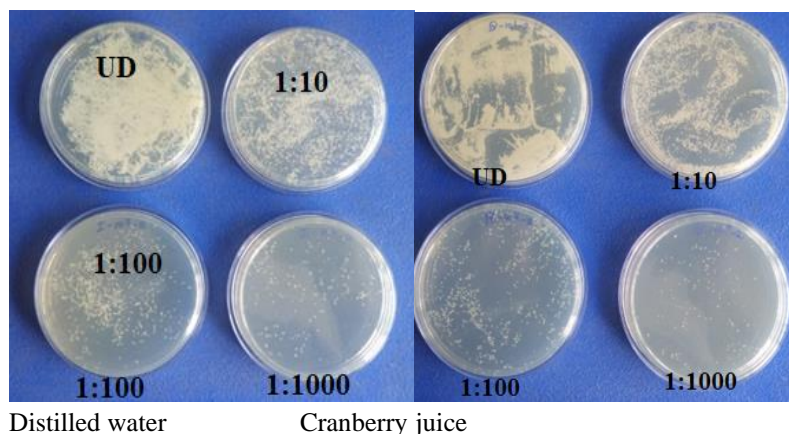


Figure 2: Group II: Colonies on undiluted, 10, 100, 1000 dilution of 0.5% cranberry added PMMA samples

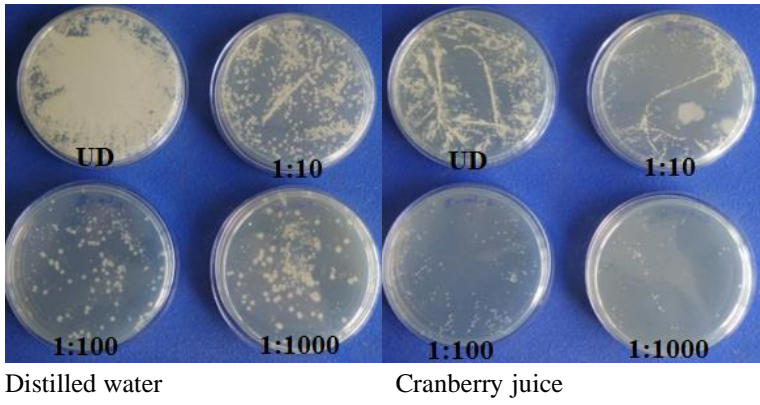


Figure 3: Group III: Colonies on undiluted, 10, 100, 1000 dilution of 1% cranberry added PMMA samples

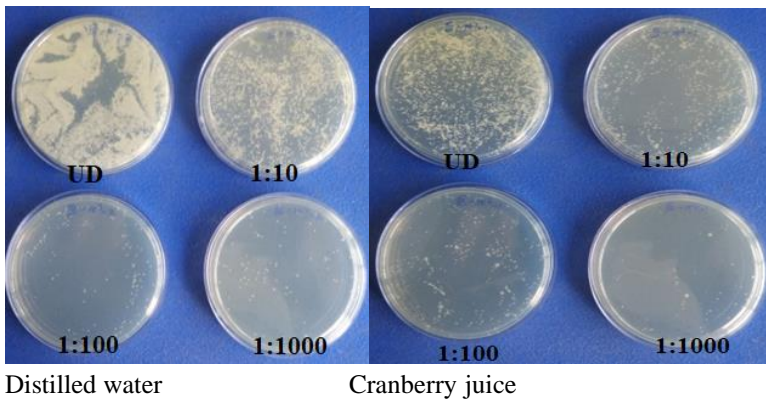


Figure 4: Group IV: Colonies on undiluted, 10, 100, 1000 dilution of 1.5% cranberry added PMMA samples

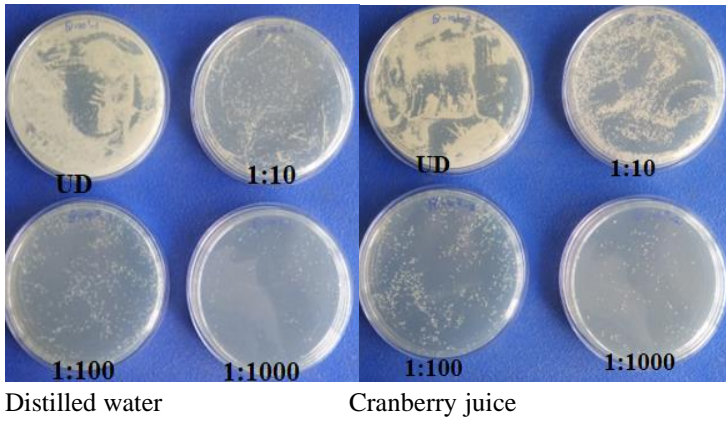


Figure 5: Group V:Colonies on undiluted, 10, 100, 1000 dilution of 2% cranberry added PMMA samples

