Original Article

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Dentistry Section

Effect of Bamboo Salt on Inhibition of Adhesion of *Candida albicans* to Denture Acrylic Resin: An in vitro Study

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ABSTRACT

Introduction: Adhesion of *Candida albicans* on denture surfaces is one of the main aetiology of denture stomatitis. Currently, chemical disinfectant agents have been employed to prevent its adhesion on the denture surface. However, due to its harmful effects on the physical properties of the denture material there has been a lookout for a natural product as a replacement.

Aim: Investigate the effects of Bamboo Salt Solution (BSS) on inhibition of adhesion of *Candida albicans* (*C. albicans*) to denture acrylic surface.

Materials and Methods: Transparent acrylic strips were pretreated with bamboo salt at concentration of 5, 10, 20 w/v %, normal saline (negative control) and 0.2% chlorhexidine gluconate (positive control) for 30 minutes followed by inoculation with *C. albicans* (10^7 cells/mL). Adherent yeast cells were then counted under microscope at 40X magnification in 20 randomly selected fields on each strip after staining with crystal violet. The statistical significance was calculated by Kruskal-Wallis and Mann-Whitney non-parametric tests at a significance level of $p<0.05$.

Results: The mean percentage of inhibition after pretreatment with 5%, 10% and 20% of the extract were 43.9%, 65.7% and 86.7% respectively. Pretreatment with BSS significantly reduced the adhesion of *C. albicans* to acrylic surfaces in a dose dependent manner.

Conclusion: This observation indicates that BSS has an inhibitory effect on the ability of *C. albicans* to adhere to denture acrylic and could be employed as a denture disinfecting agent for preventing denture stomatitis.

Keywords: Chlorhexidine gluconate, Denture disinfection, Denture stomatitis

INTRODUCTION

Acrylic dentures, complete or partial, have been one of the most common treatment option for edentulism. These dentures being placed in the mouth for a considerable amount of time act as a reservoir for a plethora of microorganisms existing in the mouth [1]. Amongst all the microorganisms present, *C. albicans*, an opportunistic fungal pathogen is responsible for causing *Candida* associated denture stomatitis in 65% of denture wearers [2]. The ability of *Candida* species to adhere to inert polymeric surface of the denture gives the organisms direct access to the human host by being in contact with the mucosal tissues [3]. One possible way to directly control the development of the infection is by management of the denture surface. A simple management strategy is the cleaning of dentures. One such method is the use of chemical solutions, like sodium hypochlorite and chlorhexidine gluconate, which have been successfully used to disinfect dentures [4]. The control of the adhesion of microorganisms on denture surfaces remains the main objective.

Several studies demonstrated that various disinfectants affect the physical properties of denture base resins such as hardness transverse strength, roughness and deterioration on the surface of the denture resin [5-7]. Due to the harmful effects of the chemicals, there has been a considerable interest in the possible use of natural products either to delay the growth of pathogens or to prevent the colonisation or infection.

Bamboo Salt has recently been introduced as a miracle salt that has anti-cancerous and anti-microbial effects [8]. It has antiseptic power that can be used to treat mouth sores and has found to have anti-plaque effects [9]. Bamboo salt is produced from solar salt by packing them into bamboo trunks sealed with clay, and roasting them at high temperatures ranging from 1000°C to 1500°C [10]. The process of roasting infuses essential minerals into the salt that gets absorbed through the bamboo and clay [11]. Research is being conducted on the possible uses of the bamboo salt for the cure of various illness [12-14]. Thus, the study aimed to investigate the effect of bamboo salt on the inhibition of adhesion of *C. albicans* to denture acrylic resin.

MATERIALS AND METHODS

An in vitro study was conducted at the Department of Microbiology, SRM Dental College, Chennai, India betwen 16 November 2016 to 23 November 2016 to evaluate the effect on the inhibition of adhesion of *C. albicans* to the denture acrylic resin.

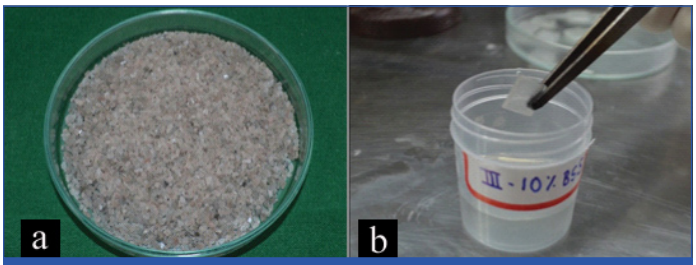
Preparation of Acrylic Strips

The acrylic strips for the adhesion assay were prepared as described by Samaranayake LP and MacFarlane TW [15]. Fifty heat polymerised clear acrylic resin (DPI Heat Cure P/L- Clear) strips of 10×10 mm dimension and 0.5 mm thickness were prepared and immersed in distilled water for one week to leach out the residual monomer. Following which, all the samples were washed with fresh distilled water, dried and sterilised in a Ultra Violet (UV) chamber (Kleanzone devices Pvt. Ltd.,).

Preparation of Bamboo Salt Solution and Pretreatment of Acrylic Strips

The bamboo salt used in the study was nine times roasted purple bamboo salt. Purple bamboo salt crystals (9x roasted, Koreasalt Co. Ltd.) [Table/Fig-1a] were weighed and dissolved in the quantity of 5 gm, 10 gm and 20 gm with 100 mL of distilled water

in to obtain 5%, 10% and 20% concentration of BSS respectively. Chlorhexidine gluconate 0.2% (ICPA Health Products Ltd.) served as a positive control, whereas Normal Saline (NS) solution 0.9% w/v (Claris Otsuka Pvt. Ltd.,) served as a negative control in the present study. The study groups were divided as follows: Group I: NS solution, Group II: 5% BSS, Group III: 10% BSS, Group IV: 20% BSS, Group V: 0.2% Chlorhexidine solution. The sterilised acrylic strips were then placed [Table/Fig-1b] in 10 mL solution of each group and pretreated for 30 minutes at room temperature prior to the adhesion assay.



[Table/Fig-1]: (a) Purple bamboo salt crystals, (b) pretreatment of acrylic strips.

Preparation of *C. albicans* Suspension

C. albicans (ATCC® 10231TM) were cultured in Sabouraud dextrose broth (MM1067: HiMedia Pvt. Ltd.,) at 37°C for 18 hours, harvested by centrifugation at 120 rpm (R4C, REMI) and washed three times in Phosphate Buffered Saline (PBS) (0.01 M, pH 7.2). Yeast cells were counted with a hemocytometer and resuspended in PBS at 10^7 cells/mL.

Adhesion Assay

The pretreated strips were washed with PBS, and placed in sterile containers. Approximately 400 µL of the yeast-cell suspension was added to each container which completely soaked the acrylic strips. The strips were then placed in a shaker incubator (SciGenics biotech Ltd.,) for one hour at 37°C with gentle agitation at 75 rpm [16]. After incubation, the acrylic strips were removed carefully and washed three times by dipping in PBS to dislodge the loosely attached yeast cells. Subsequently, the strips were stained with crystal violet. After air-drying at room temperature, they were mounted on glass slides with glycerol and the adherent yeasts were quantified under microscope (Nikon YS-100). Twenty-fields at 40X magnification were randomly counted for each strip. The mean number of adherent *Candida* cells per 20 fields was finally expressed as counts per square millimeter. The percentage inhibition of adhesion in comparison to the negative control was calculated as: (Quantity of adherent cells in control–Quantity of adherent cells in sample/Quantity of adherent cells in control) ×100 (i.e., multiplied by 100) [17].

Groups	mean±SD*	Sum of Ranks (R)	Test Statistic (H)
Group I: Normal Saline Solution	9.99±0.33	455	
Group II: 5% Bamboo salt solution	5.60±0.25†	355	
Group III: 10% Bamboo salt solution	3.43±0.19†	255	H=47.012 p<0.001
Group IV: 20% Bamboo salt solution	1.33±0.12†	154.5	
Group V: 0.2% Chlorhexidine	0.84±0.04†	55.5	

[Table/Fig-2]: Comparison of *Candida* adhering to acrylic surface after pretreatment–Results of Kruskal-Wallis and Mann-Whitney tests.
*Data are expressed as mean number of adherent candida /mm²
†significant difference when compared to Group I at p<0.05

STATISTICAL ANALYSIS

The data obtained from the adhesion assay were analysed using Kruskal-Wallis and Mann-Whitney tests with STATA Statistical Software: Release 11. In all comparisons, statistical significance was declared if the p-value<0.05. [Table/Fig-2].

RESULTS

The statistical analysis of the groups using Kruskal-Wallis test, reveals that the pretreatment of the acrylic strips using 0.2% chlorhexidine, BSS in 5%, 10% and 20% concentration had significantly reduced the number of *Candida* adhering to acrylic surface when compared to NS solution at p <0.05. On comparison between the groups using Mann-Whitney test, all the three different concentrations of bamboo salt showed statistically significant difference in the reduction of adhesion. The mean number of adherent *Candida* cells on the acrylic strips after pretreatment with 5%, 10%, 20% BSS and 0.2% chlorhexidine were compared to the mean number of adherent cells on the strips pretreated with NS to obtain the percentage inhibition of the cells in each group. The percentage inhibition achieved in different concentrations of the extract, showed greatest inhibition at 20% extract where the adherent yeasts were reduced by 86.86 % which was comparatively less than 0.2% chlorhexidine gluconate, a positive control, which produced more than 90% inhibitory effect [Table/Fig-3].

Groups	II	III	IV	V
Percentage Inhibition (%)	43.9	65.7	86.7	91.6

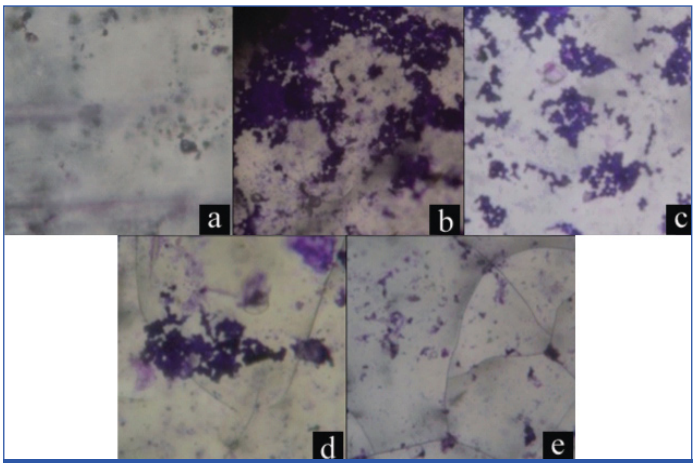
[Table/Fig-3]: The mean percentage inhibition of *C. albicans* adhesion to acrylic surfaces compared to Group I.

DISCUSSION

The initial adhesion of *C. albicans* begins with the transport of the organism to the surface either through diffusion or active movement where the hydrophobicity and free surface energy of the host surface play a key role [18]. The successful adherence of the organism to the surface is an essential prerequisite for its colonisation. Thus, prevention of the initial adhesion can prevent the colonisation and subsequent development of the infection [19].

This study was conducted to investigate the effects of BSS on the inhibition of adhesion of *C. albicans* on heat cured denture acrylic resin. The microscopic visual method was used to quantify the adhesion of *Candida* in vitro. Based on the interpretation of results, it has been inferred that 30 minutes pretreatment of acrylic surfaces with BSS significantly reduced the ability of *C. albicans* to adhere to these surfaces compared with those treated with NS, the negative control, and this effect was found to be dose-dependent. Bamboo salt has shown deleterious effects on *C. albicans*.

When assessed under the microscope, the samples pretreated by 0.2% chlorhexidine shows almost complete non-adherence of



[Table/Fig-4]: a) The microscopic image (40X) of the acrylic strips after pretreatment with 0.2% chlorhexidine, b) The microscopic image (40X) of the acrylic strips after pretreatment with Normal Saline Solution c) The microscopic image (40X) of the acrylic strips after pretreatment with 5% bamboo salt solution d) The microscopic image (40X) of the acrylic strips after pretreatment with 10% bamboo salt solution e) The microscopic image (40X) of the acrylic strips after pretreatment with 20% bamboo salt solution.

Candida on the acrylic resin [Table/Fig-4a]. When compared to the samples treated with NS [Table/Fig-4b], the samples treated by BSS at 5% [Table/Fig-4c] and 10% [Table/Fig-4d] concentration have diminished the number of adherent cells. In the samples treated with 20% concentration of BSS, the adherent cells have significantly reduced in size [Table/Fig-4e], which might affect its viability and its colonisation.

Treatment of acrylic surfaces with chlorhexidine gluconate, an antiseptic and a disinfectant agent, has proven to have substantially reduced the adherence of *C. albicans* [20-22]. The percentage inhibitory effect of 0.2% chlorhexidine, in the present study was found to be above 91.5%. Similar results were obtained by Sookto T et al., with 96-98% of inhibition of adherent *Candida* cells [23]. Sroisiri T and Boonyanit T exhibited more than 90% inhibitory effect of 0.2% chlorhexidine on both epithelial cells and denture acrylic surfaces [24].

In the present study, the mean percentage of inhibition of *Candida* adhesion on the acrylic surface after pretreatment with 5%, 10% and 20% concentration of BSS was found to be 43.9%, 65.6% and 86.8%, respectively.

The properties of the bamboo salt are said to be enhanced by the number of times it is roasted within the sealed bamboo trunks/shoots [25]. In comparison with crude salt, the contents of iron, silicon, potassium, and phosphate in the purple bamboo salt are higher, while the sulfate content is lower [26]. The presence of these essential minerals known to have various therapeutic effects on inflammations, fungal and viral diseases, diabetes, circulation organ disorder and cancer [27,28]. According to a study, nine times baked Bamboo salt showed a greater increase in anti-mutagenic activity than salts baked once or three times [29].

The reduction of *C. albicans* adhesion on acrylic surfaces by bamboo may be explained in several ways. The surface free energy of acrylic, which is an important factor of *Candida* adhesion on it, may be interfered with the adsorption of the components of the bamboo salt [30]. It may also be speculated that the salt solution might establish changes in the cell surface properties of the *Candida* cells and thereby modulating the cell surface hydrophobicity. Although chlorhexidine showed better results in comparison, the decrease in cell size and visible clumping of cells suggest the positive action of bamboo salt on the *Candida* cells. Thus, there may be a potential for its use in treatment of *Candida* infections. Additionally, the use would depend on cost considerations, odour and flavour. However, further studies are necessary for elucidating the mechanism of action of bamboo salt at the molecular level as well as the actual effect in clinical tests.

LIMITATION

In the present study, adhesion was evaluated with the aid of in vitro assay, this however does not take into account of other factors such as saliva and the presence other *Candida* species and bacteria. The concentration of the BSS used was limited only to three, more information on its minimum inhibitory concentration of the bamboo salt needs to be evaluated.

CONCLUSION

The results indicate that soaking acrylic dentures in bamboo salt solution for 30 minutes would have potential to reduce *Candida* cell adhesion and may be a useful adjunct to treat *Candida*-associated denture stomatitis and help to prevent recurrence of the infection. Further studies are warranted to study the effects of bamboo salt on *Candida* and its possible side effects in humans.

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