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Effect of an Indigenous Cleanser on the Microbial Biofilm on Acrylic Denture Base – A Pilot Study

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ABSTRACT

Introduction: Edentulous patients wearing dentures may develop Candidiasis or denture sore mouth due to improper cleaning of the dentures. Denture cleansers remove not only the bio film, but also stains and other forms of debris of food on dentures. Since people prefer natural substances for daily use, natural indigenous cleanser was prepared to replace the chemical denture cleansing solution.

Aim: The aim of this study was to check the effect of soapnut on *Candida* and *Streptococcus mutans* bio film formed on dentures compared with a control and commercially available Clanden tablet.

Materials and Methods: 18 heat cure acrylic blocks were fabricated and (DPI heat cure) and divided into two groups for biofilm formation of *C.albicans and S.mutans* with 9 blocks in each group. Three solutions: distilled water(control), commercial cleanser (Clanden tablet- Global Dent Aids Pvt Ltd) and indigenous solution(soapnut) were used to check biofilm cleansing efficiency by crystal violet staining method and optical density (OD) analysis using spectrophotometry. 24 hours bacterial culture was carried out to check for zone of inhibition of the test solution. The biofilm cleansing ability tests was done thrice in triplicate. The statistical analysis was performed by Student T test

Results: The highest OD values were obtained in *S.mutans* group tested with control (Water). Least values were obtained in *C.albicans*group tested with Clanden tablet. But, similar result was seen with the indigenous solution as well. No significant difference was seen between the effects of the two test specimens on either group of microorganisms compared with that of the control group. A zone of inhibition was seen around the indigenous solution.

Conclusion: Indigenous denture cleanser is an effective solution for daily denture hygiene maintenance.

Key Words: Acrylic denture base, Denture cleansers, Indigenous solution, Soapnut (*Sapindusmukorossi*), Candida, *S.Mutansbiofilm*

INTRODUCTION

Improper cleaning of dentures may lead to denture sore mouth or candidiasis. Denture stomatitis is an oral inflammation related to denture wearing and poor oral hygiene, which occurs in 70% of the denture wearers.[1] Associations of denture stomatitis have been reported with mucosal trauma due to poor denture fit, prolonged duration of denture use, bacterial and fungal (primarily *Candida*) infection, and poor denture hygiene.[2] *Candida* cells in the oral cavity are associated with biofilms, which differ substantially from planktonic cells due to their higher antifungal resistance.[3]

In case of removable partial dentures the poor oral and denture hygiene can lead to colonization of Streptococci and that may cause inflammation of tissues and caries in abutment teeth where as in implant supported removable it can lead to peri implantitis and implant failure.

Microorganisms like *Candida albicans* are commensals of oral cavity which gets attached to the denture in the same way that dental plaque grows on natural teeth. As time progresses, the bio film that develops on dentures may harden and become difficult to remove. Various methods like mechanical aids, chemical methods, and microwave irradiation have been used to maintain denture hygiene. The common mechanical methods used are brushing, sonic vibrators, and ultrasonic cleaner[4]

Brushing the dentures is challenging tasks for old individuals.[5] Manyelderly patients suffering from dementia and poor

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dexterity cannot adequately brush their dentures because of disease. Various forms such as tablets, pastes and powders are available. Their relative efficacies have varied with different manufacturers, not only in removing the bio film, but also in staining food and other debris on dentures. The primary cleaning agent used is denture cleaners is sodium hypochlorite. Commercially available denture cleansers usually contain many chemical substances which could be harmful/allergic to the patients.[6]

Since nowadays people prefer natural products for day-to-day use, naturally available substances and traditionally used cleansers can be tested for cleaning ability. Soap nut (*Sapindusmukorossi*) also known as soapberry, ritha, aritha is an indigenous fruit found in upper Indo-Gangetic, Shivaliks and Sub Himalayan tracts. The fruit contains saponin due to which it is widely used as a cleanser for washing hair, removing freckles, psoriasis.[7]

Powdered seeds had been used for arthritis, common cold, nausea etc. The soapnut has anti-fungal and antibacterial properties.[8] Saponin from the saopnuts has been extracted by using solvents such as ethanol and water.[9] Traditionally it was used as a detergent for clothes and also to polish gold ornaments.[10] However there has been no study carried out to check the efficiency of soapnut on denture bio film. The aim of this study was to check the effect of soapnut on *Candida* and *Streptococcus mutans* bio film formed on dentures compared with a control and commercially available tablet.

MATERIALS AND METHODS

The experimental *invitro* **s**tudy was conducted after obtaining ethical clearance. The bacterial and fungal strains were procured from the Yenepoya Research Centre microbial repository. The sample size was calculated based on triplicate values i.e three samples for each group of microorganism and cleanser solution.

METHODOLOGY

Fabrication of acrylic blocks

A 2mm thickness of modelling wax sheet was flasked and dewaxed. The mould space obtained was packed with heat cure clear acrylic (DPI heat cure resin) and cured in a short curing cycle. The sheet of heat cure acrylic was polished to obtain smooth surfaces devoid of any roughness and irregularities and cut into blocks of 10mm x 10mm x 2mm each (Fig.1).

These were disinfected with surgical spirit and stored in distilled water. A total of 18 blocks were obtained and divided into group A (*C. albicans*) and group B (*S.mutans*) with 9 blocks in each group.

Preparation of cleansing solutions:

Ripe soap nuts collected from a tree in Mangalore were dried at room temperature for 10 days (fig 2). 10- 12 dry Soap nuts were boiled in 60 ml of water for 30 minutes at 270ºC. The nuts were strained from the liquid and the concentrated soap nut solution was stored in a sterilized container and refrigerated. (Fig 3) The solution was sterilized under germicidal Ultra Violet light prior to use.

One Clanden tablet (Global Dent Aids Pvt Ltd) was dissolved in 50 ml of water and this solution was used for testing.

Antimicrobial activity:

Antimicrobial assay was done by disc diffusion method. The 4hour microbial culture was uniformly spread on Muller-Hinton agar plates. The microorganism strains used were ATCC 90028 and ATCC 25175 for *C. albicans* and *S.mutans* respectively. Known concentration of test compounds were placed on the inoculated plates. The zone of inhibition was measured after incubating for 24hours at 37º C.A ruler was used to measure the diameter of the disk plus the surrounding clear area in millimetres (mm).

Biofilm assay by crystal violet stain method

Overnight bacterial culture was inoculated to sterile conical flasks containing potato dextrose AGAR growth media. After 8hour of shaking incubation, the acrylic denture resin squares were immersed in the culture and allowed to form biofilm under static condition in multiwall culture plates. (Fig 4)

After 48 hours the acrylic blocks were taken out and immersed in distilled water, soapnut and commercial denture cleanser for 8 hours. (Fig 5).They were washed and allowed to dry and the biofilm was fixed using 95 % methanol for ten minutes. The dried and fixed bio films were stained with crystal violet (0.1%). Acrylic blocks were rinsed with PBS and air dried again. The stained biofilm was solubilised in acetic acid solution (33%) and optical density (OD) of each sample was recorded at a wavelength of 580 nm using a spectrophotometer.

Analyses:

The experiments were performed thrice in triplicate. Statistical analysis was performed by student's t test using SPSS Version 22 software (SPSS Inc., Chicago, USA). p values <0.05 were regarded significant to compare the optical density values of 3 cl.24-hour inoculation on a culture plate was obtained. (Fig. 6)

The mean values of zone of inhibition were obtained and it was found to be highest around the commercial tablet(table1).

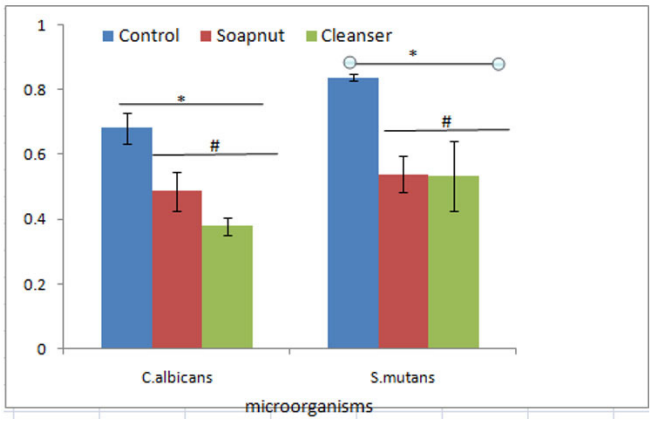
Table 1: Mean values of the zone of inhibition of the tested samples.

	Zone of inhibition (mm)		
	WATER	SOAPNUT	TABLET
C. *albicans (A)*	No zone	14	17
S. *mutans (B)*	No zone	13	15

The control group showed no zone of inhibition indication no antimicrobial activity. While the tablet group and soapnut group showed a mean zone of 17mm and 14 mm respectively against *C.albicans.* A mean of 13mm and 15mm was found against *S.mutans* for soapnut and tablet respectively.

Optical density after crystal violet staining:

The highest OD values were obtained in B group *(S.mutans)* tested with control (Water). Least values were obtained in A group *(C.albicans)* tested with Clanden tablet. No significant difference was seen between the effect of the two test specimens on either group of microorganisms compared with that of the control group. (graph.1)



Graph 1: Effectiveness of cleasing solution in terms of optical density. (x axis- Optical Density values, y axis – microorganisms).

* indicates significant difference between control and test groups. p values < 0.05

# Indicates no significant difference between cleanser and soapnut groups. p value > 0.05

DISCUSSION

Denture hygiene maintenance is the most important step to be followed by the patient. Denture cleaning methods used on daily basis may be mechanical or chemical. Studies show a positive effect of mechanical and chemical cleaning and of the combination of both on denture cleanliness.[12]

According to a study by Peracini et al. mechanical cleaning method was the most prevalent among various denture users.[13] Various pastes used to clean dentures contain abrasive agents that may lead to surface roughness of the denture. This surface roughness would increase the adherence of plaque and microorganisms on the abraded denture surface. Also, mechanical cleaning with pastes requires patients to manually clean the dentures, which becomes cumbersome in old age due to loss of dexterity and increased dependence.

Commercial denture cleaning products are based on chemicals like sodium hypochlorite, peroxides, neutral peroxides with enzymes, oracids.

We used 3 different solutions to check the efficiency on microbial biofilm growing on denture surface. Since in recent years people have adapted to naturally available substances for their daily use, a cleansing solution was extracted from an indigenous fruit and compared with that of a chemical cleansing tablet. The control solution used was water and it showed to have the least cleaning efficiency on biofilm formed on denture surface. This proved that use of water alone would not lead to any cleaning of dentures.

The denture cleansing tablet used was Clanden which is an effervescent tablet. Effervescent denture cleansing tablets contain alkaline peroxides; the tablet used in this study contains sodium perborate monohydrate. The tablets have an effervescent action when they come in contact with water by producing an alkaline solution of hydrogen peroxide containing active oxygen. This effervescence has a mechanical action of removing debris, and oxygen has antimicrobial and stain removing effects.[14]

The main disadvantage of conventional chemical cleansers, such as sodium hypochlorite, is their whitening properties on dentures when used for prolonged periods.[15,16] The increased resistance of pathogenic organisms to synthetic antifungal and antibacterial agents is drawing attention to plant extracts having antifungal efficiency. For geriatric patients cost and availability may be a factor while selecting a denture cleanser.[17]

Nowadays various plant materials having antifungal properties are being tested in research field. The main advantage of opting for naturally occurring substances over commercial chemical solutions is minimal chances of harmful effects as well as allergic reactions.

A study was conducted to check the efficiency of lemongrass extract on the *C. Albicans* denture biofilm found that there was reduction in cell counts.[18] Another study to check the effect of *Triphala* as a denture cleanser revealed that it had superior cleansing ability compared to chlorhexidine gluconate.[19]

In our study, an indigenous naturally occurring cleanser was

tested for antimicrobial activity and cleaning efficiency on acrylic denture material. *Sapindustrifoliatus Linn.,* a small deciduous tree belongs to the family Sapindaceae which is known as soapnut in English, *Ritha* in Bengali and *Ponnangottai* in Tamil[20] The major constituents of the fruits are saponins (10%-11.5%), sugars (10%) and mucilage[21] In a study conducted to evaluate the antimicrobial activity of soapnut against *S.mutans* and *C.albicans*found that soapnut had higher antifungal activity against *C.albicans*.[22]

But in our study, the antibacterial action of soapnut was higher than antifungal activity according to the biofilm assay test. The action of soapnut is mainly by saponins. Plant derived saponins are secondary metabolites and traditionally used as detergent. It has amphiphilic nature due to the presence of a lipid-soluble aglycone and water-soluble chains in its structure.[23] Saponins are divided into two groups which are triterpenoid and steroid glycosides. Glycosides structure characterizations are varied by the numbers of sugar units attached at different positions.[24]

Various methods for extraction of saponins have been used, but according to a study by Merve et al.**,** Ethanol water solution (50 % v/v) with 1:10 solid- liquid ratio was the best. Bioassay directed isolation and characterization of a new acetylated triterpene saponin, named hederagenin 3-O-(2,4-O-diacetyl-α-L--arabinopyranoside)-(1-3)-α-L-rhamnopyranosyl- (1-2)- α-L-arabinopyranoside (1), together with six known triterpenoidal hederagenin saponin (2-7) from *S. Mukorossi*.[25]

The indigenous soapnut solution had a significant effect against the denture biofilm but slightly lesser compared to the commercially available tablet cleanser. The antimicrobial activity test by disc diffusion method also showed a inhibitory zone formed around the well containing soapnut solution, thus proving it has antimicrobial action.

CONCLUSION

The indigenous soapnut solution had a significant effect against the denture biofilm but slightly lesser compared to the commercially available tablet cleanser. The antimicrobial activity test by disc diffusion method also showed a inhibitory zone formed around the well containing soapnut solution, thus proving it has antimicrobial action Thus naturally available sources could be an economic and chemical free alternative.

LIMITATIONS

Effects of the indigenous solution on the Physical and esthetic properties of the acrylic material were not evaluated in the present study.

Biofilm formation was not carried out in an environment simulating the oral cavity

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Authors' Contribution: Dr.Vidya Bhat –concept, guide and overall conduct of study, Dr.Mrinali- methodology and conduct of study. Mr.Vipin- Microbial assay

Dr. Sanath Shetty-approval of study design

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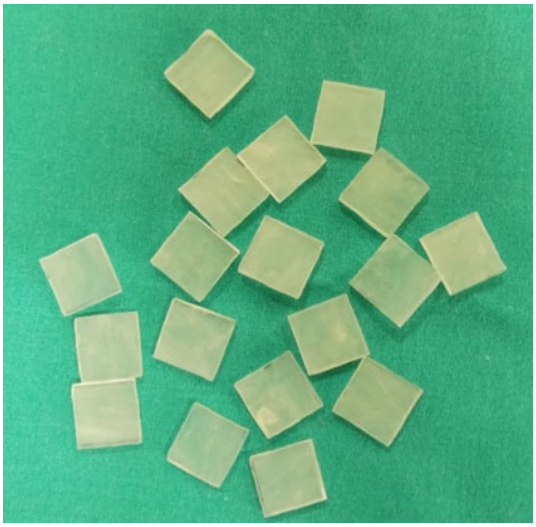

Figure 1: Acrylic blocks (1 x 1 cm).

Figure 2: Ripe and Dried soapnut.

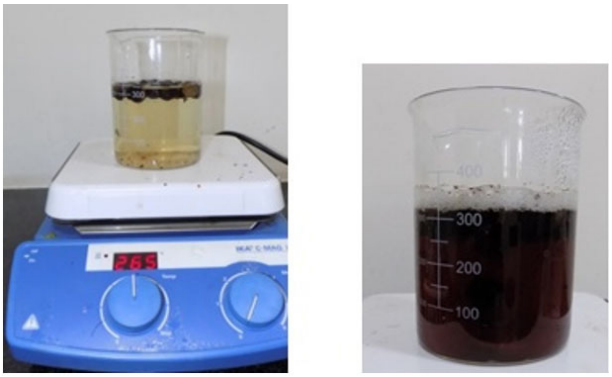

Figure 3: Process of extracting saponins.

Figure 4: Inoculated acrylic chips in culture media for biofilm formation.

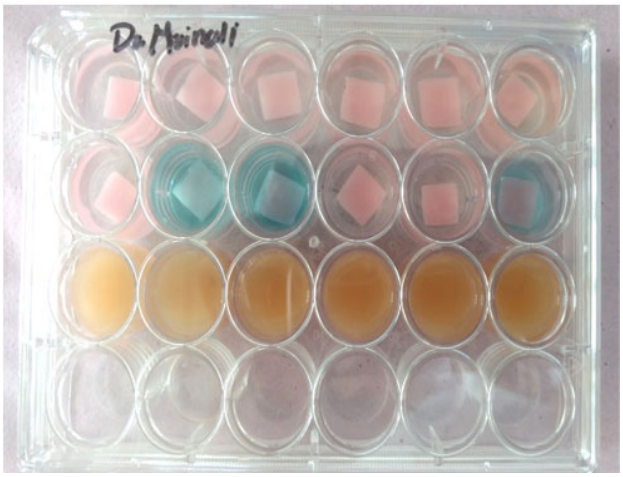

Figure 5: Samples immersed in cleansing solutions.

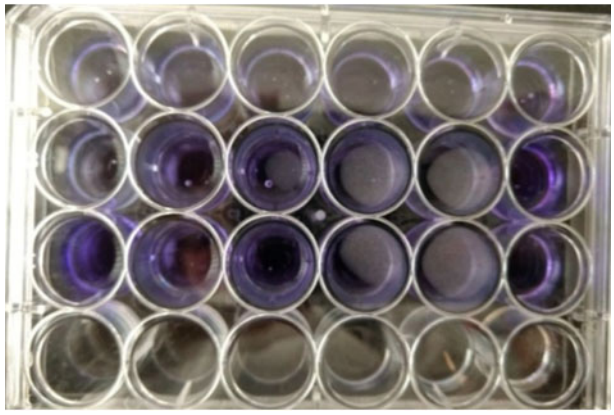

Figure 6: Solubilized biofilm in acetic acid.

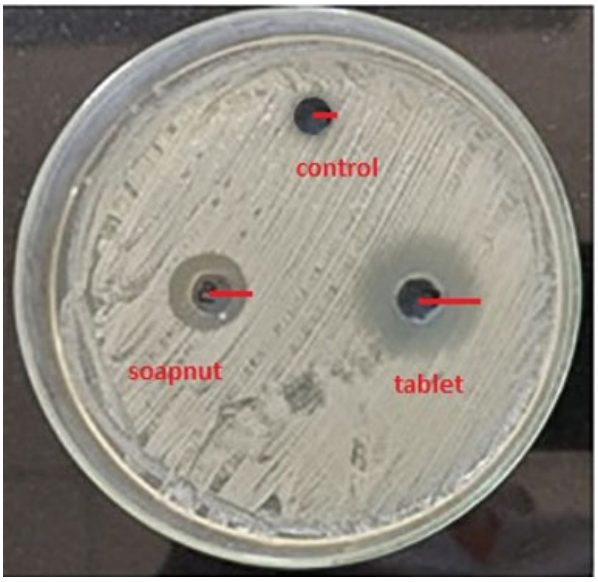

Figure 7: Representative image of the inoculated culture plate.