Original Article

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Dentistry Section

Commercial and Plant Extract Denture Cleansers in Prevention of *Candida albicans* Growth on Soft Denture Reliner: In Vitro Study

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ABSTRACT

Objective: To evaluate and compare the efficacy of two plant extracts and two commercially available denture cleansers against *candida albicans* adherent to soft denture reline material.

Materials and Methods: In this study 60 specimens of soft denture reliner material specimens were fabricated with dimensions 10x10x2 mm. The sterile specimens were inoculated by immersion in Sabourand broth containing *Candida albicans* for 16 hours at 37°C in an incubator. Then the specimens were washed and immersed in denture cleansers which were divided into group five groups from Group I-V for CD Clean®, Nigella sativa, thyme essential oil, Fittydent® and distilled water respectively, for 8 hours at room temperature. Then they were washed, fixed with methanol and stained with crystal violet. *Candida* cells adherent to the specimens were counted under microscope. The number of cells adherent to test samples were compared with that adherent to control.

Results: The effectiveness of Fittydent® was more than CD Clean® in reducing the adherent *candida albicans* and the difference was statically significant (p = <0.001). Both thyme essential oil and nigella sativa were almost same in effectiveness against *candida albicans* but the difference was not statically significant (p= 0.79). Post-hoc Tukey s test was performed which indicated that Fittydent® was the most effective amongst the denture cleansers tested in this study, followed by thyme essential oil, nigella sativa and CD Clean®.

Conclusion: The results of the study showed that all denture cleansers used in the study were significantly effective. The study indicated that Fittydent is more effective amongst the denture cleansers because of its mechanism of action; however the plant extracts used in this study were also significantly effective against *candida albicans*.

Keywords: Candidiasis, Nigella sativa, Sodium perborate, Thyme essential oil

INTRODUCTION

The pathogen yeast *Candida albicans* is a common component of oral bacterial flora and is present in >60% of healthy adult population. Although the presence of yeast is not, per se, indicative of oral infection, some local and systemic factors could be conversion from a commensal to a parasite form, releasing the yeast from biologic competition with the bacteria and allowing conversion into pathogens. The adherence of the microorganism is a pre requisite for the pathological process to be initiated. Many factors, such as the structure and composition of the surface, the chemical/physical properties of microorganisms, can influence the adhesion. Therefore, colonization depends on several factors related to the substrate morphology. For example, due to leaching processes, the soft liners will deteriorate as they lose plasticizers and mechanical cleaning becomes difficult because brushing damages the surface [1]. The proliferation and organization into a biofilm lead to a clinical condition defined as candidiasis. Candidiasis is one of the most common human oral infections and is expressed in several clinical manifestations. Many people wearing dental prostheses are affected by oral candidiasis in association with prosthesis stomatitis. Materials for the prostheses, such as acrylic resin and silicon based materials represent a perfect support for biofilm formation [2]. Silicone elastomers provide an ideal interface for microorganism colonization and aggregation in the oral cavity [3]. It is difficult to avoid pathogenic microorganism adhesion to the surface of the dental materials though some efforts have been made.

Denture cleansing is essential to maintain the service ability of the denture, because of aesthetic concerns and for prevention of denture related stomatitis. Adequate denture hygiene is believed to be the most effective preventive and curative treatment for the pathogens [4].

An ideal denture cleanser has to be biocompatible, bactericidal and fungicidal, harmless to the structure of denture, should effectively remove organic and inorganic deposits and should be easy to use effectively.

Amongst all methods, mechanical cleansing has shown to be an effective measure for providing denture cleanliness and maintaining a healthy mucosa as a denture bearing tissue surface. However, in geriatric or handicapped denture wearers, chemical denture cleansers can be an alternative, as their manual dexterity may be compromised [5].

MATERIALS AND METHODS

Preparation of Specimens

In this study, 60 specimens of soft denture reliner, were fabricated with dimensions 10x10x2 mm [6]. A stainless steel metal die having the dimensions 10x10x2 mm was used to prepare the wax pattern. The wax patterns were flasked according to conventional technique ensuring complete closure between the counter parts of the flask. Dewaxing was carried out. The moulds formed were immersed in hot water to remove any traces of petroleum or wax and also facilitate the application of separating medium. The mould cavities thus obtained were used for the fabrication of the specimens.

Soft reline material recommended powder and liquid ratio of 11g of powder to 8ml of liquid was taken and poured into a large mixing cup. The powder was added slowly into the liquid and stirred for 30 seconds. The soft liner was poured directly into the mould space and the two components of the flask were pressed against each other for 3 minutes. Then the flask was opened and the excess was trimmed and again the flask was closed for 5 minutes [Table/Fig-1].

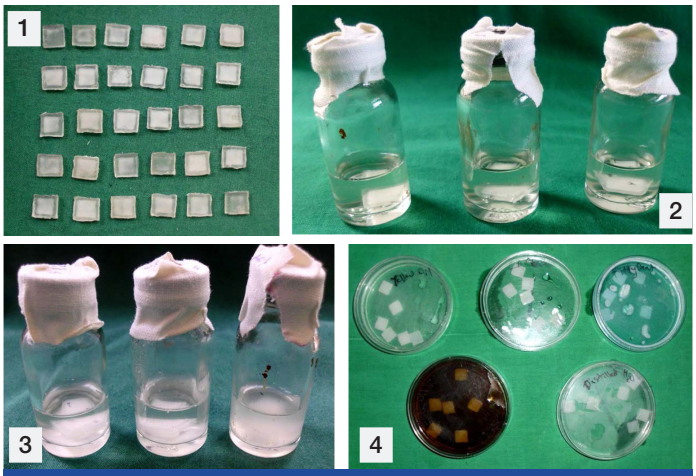
To sterilize the specimens an ultraviolet light chamber was used. The specimens were kept in the chamber for 5 minutes. As the ultraviolet light does not penetrate opaque materials, the specimens were over turned and the process was repeated [6]. Twelve specimens each were then placed in a zip lock plastic bag, sterilized in the same chamber.

Preparation of Culture Media for *Candida albicans*

The blood agar base was prepared as per manufacturer instruction. Then it was sterilized by autoclave at 121°C for 15 minutes. The prepared blood agar base was transferred to 50° C water bath. Then when the agar base was cooled to 50°C, it was poured aseptically and mixed gently. Formation of air bubbles was avoided. Then 15 ml was dispensed onto each sterile petridishes.

For Sabround's dextrose broth 65g of 50 ml medium was suspended in one liter of purified water and heated with frequent agitation and boiled for one minute to completely dissolve the medium. Then it was autoclaved at 121°C for 15 minutes. After which it was allowed to cool at room temperature.

Candida albicans were obtained from ATCC (American type culture collection) standard *Candida albicans* strains. A streak of *Candida albicans* were picked up with a sterile inoculator loop and inoculated on the blood agar which was kept for incubation in an incubator for 48 hours at 30°C. Then a Brain Heart Infusion (BHI) was prepared and candida colonies were introduced into it with an inoculator loop to make a suspension of 3 ml. This suspension of BHI with *Candida albicans* was introduced into the Sabround's dextrose broth (SDA) which was kept in a penicillin bottle of 5ml using a micropipette, and stirred slowly. All procedures performed in aseptic condition. This was incubated aerobically at 37°C for 24 hours [Table/Fig-2,3].

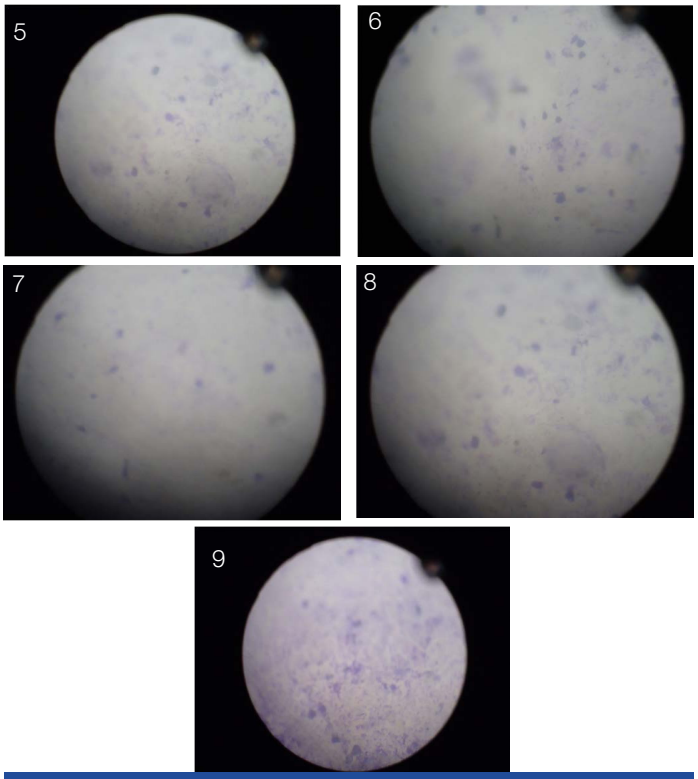


[Table/Fig-1]: Soft liner specimens. **[Table/Fig-2]:** Specimens inoculated in SDA broth containing candida colonies. **[Table/Fig-3]:** Specimens after inoculation with candida biofilm formation. **[Table/Fig-4]:** Specimen immersed in prepared denture cleansing agents.

Preparation of Denture Cleansers

One teaspoon full powder of peroxide cleanser, CD CLEAN® (Group I) was added to 100 ml of water as per the manufacturer's instructions. 40ml of nigella sativa (Group II) was taken in the petridishes. And also, 40 ml ofthyme essential oil (Group III) was poured in the petridishes. One tablet of peroxide cleanser, FittyDent® (Group IV) was added to 100ml of water and for the control group distilled water was taken (40ml) in a petridishes.

Then the specimens were introduced into the prepared denture cleansers contained in the petridishes [Table/Fig-4]. To mimic the overnight soaking of the denture by the patient, they were stored for 8 hours, as specified by the manufacturers. After which under running tap water, the specimens were washed. Then they were fixed with methanol and stained with crystal violet stain and were left to air dry. Then an inverted microscope was used to examine them [6].



[Table/Fig-5]: Microscopic image of specimens immersed inCD Clean (Group I). **[Table/Fig-6]:** Microscopic image of specimens immersed in Nigella Sativa (Group II). **[Table/Fig-7]:** Microscopic image of specimens immersed in Thyme Essential oil (Group III). **[Table/Fig-8]:** Microscopic image of specimens immersed in FittyDent (Group III). **[Table/Fig-9]:** Microscopic image of specimens immersed in Distilled water (Group IV).

Candida cells adherent to acrylic resin specimens were counted under the inverted microscope (x40 magnification) [6]. The candida cells on the specimens appeared as round to oval cells of about 4 to 8µm, the crystal violet stains were retained by the candida [Table/Fig-5-9]. The entire surface (10x10 mm) of the specimen was counted. Each field (1.1mm^2) was counted and totaled. The number of cells adherent on the test samples were compared with that adherent to control [6].

STATISTICAL ANALYSIS

Results were presented as Mean ± SD and percentages whenever required. Unpaired t-test was used for comparison between two groups followed by Kruskal Walli's ANOVA test which was used for multiple groups and followed by Tukey's test for overall comparison between main groups. For all tests a p-value of 0.05 or less was considered for statistical significance.

All the statistical calculations were done using SPSS® (statistical package for the social sciences) for Windows® version 14.0 (SPSS Inc., New York).

RESULTS

The candida biofilm was formed after inoculation of 16 hours. The four agents were used with distilled water as control. Again unpaired t-test was performed between different individual agents. The mean cell count for Group II was more than that for group III and the p-value was <0.001 which was highly significant [Table/Fig-10]. Group III had better efficacy than group II. Also the unpaired t-test was performed between Group IV and Group I, the mean cell count of group I was more than that of group IV. Group IV had better efficacy against *Candida albicans* than group I [Table/Fig-10].

Comparison between commercial and plant extract denture cleansers used for soft denture reliner. One-way Annova test was performed. The mean cell count for plant extracts was more compared to group IV (thyme essential oil), and less compared to Group I (CD Clean).

Finally Tukey's test was performed, according to which Group IV had the best efficacy against the *candida albicans* followed by Group III, Group II, Group I and followed by Group V which was the control [Table/Fig-10].

Group	Mean±Standard deviation	ANOVA
Group I	9.67±1.43	
Group II	8.83±1.33	
Group III	6.58±0.66	F= 153.08
Group IV	4.08±1.24	
Group V	42.5±9.65	p< 0.001

[Table/Fig-10]: Comparison between commercial and plant extract denture cleansers used for soft denture reliner.
Tukey's test: Group IV> Group III> Group II> Group I> Group V

DISCUSSION

The high demand for new antimicrobial and antifungal agents followed increased resistance shown by pathogenic micro-organisms against drugs has drawn attention to plant extracts as a new source of antimicrobial and antifungal agents. Some plant extracts have been reported to be suitable alternatives to synthetic medicines and may therefore be used as effective and safe therapeutic agents. For geriatric patients, cost and easy availability are important factors when selecting a denture cleanser [7]. Liu et al., have suggested that antifungal agents such as plant extracts and food preservative can be used as denture cleansers and have good efficacy against *Candida albicans*. Specially thyme essential oil possesses strong antifungal activity against *Candida albicans* in vitro and can be used as natural disinfectant for the prevention of denture stomatitis [8]. Sokovie and Van Griensven indicated that thyme essential oil, is composed of p-Cymene and thymol [9].

Plant extracts have a wide variety of application and for centuries have been a potential source of novel antimicrobial compounds. Natural products can be an alternative to synthetic chemical substances and the interest in medicinal plants as a source of antimicrobial agents has increased. A wide variety of plant extracts have been reported to have antifungal activity against *C. albicans*. Nigella sativa is an herb from ranunculaceae family which is used for the variety of therapeutic purpose like anticancer, antifungal, antibacterial, anti-parasites and anti-inflammatory [10]. Nigella sativa acts by inhibiting DNA synthesis of the pathogen by blocking the HDAC enzyme interacting with the chromosome [11]. Thymoquinione was found to be the active ingredient [10]. Therefore two plants extract thyme essential oil and nigella sativa were used in this study as denture cleansers and their efficacy against *candida albicans* was evaluated. Distilled water was used as control. Alkaline peroxides are the most commonly used denture cleansers. And also, Nikawa et al., stated that peroxide based denture cleansers were more efficient against *Candida albicans*. Therefore two alkaline peroxides cleaners- Fittydent® (tablets) and CD Clean® (powder), were chosen for this study. Alkaline peroxide mixed with water forms the solutions of hydrogen peroxide and liberates nascent oxygen, in the presence of organic material. The oxygen bubbles exert a mechanical cleansing effect. Thus, alkaline peroxide cleansers which act on the organic portion of plaque are able to remove Candida from soft liners due to its efferversant action [12]. An important part of denture cleansing is removal of adherent cells from the denture base [13]. In the present study the efficacy of denture cleaning agents was evaluated by Candida removal test. The present study, evaluates the effectiveness of different denture cleansers. The candida removal test revealed that the commercial denture cleanser, Fittydent was better than the rest of the denture cleansers used. Fittydent removed Candida better than other cleansers after immersion of the specimens for 8 hours which was consistent with the findings of Kumar et al., [6].

Though there was not much difference between the plant extracts and Fittydent, thyme essential oil was the second best, followed by nigella sativa. According to Liu et al., in his investigation thyme essential oil had the best efficacy against the *candida albicans* amongst the various essential oils used [8].

All the agents managed to effectively reduce the count of adhered candidal cell on the surface of the acrylic denture base and also on the surface of the soft denture reline material. The effectiveness of Thyme essential oil and Nigella Sativa on different denture base was evaluated using one-way Annova test, which was highly significant. And was consistent with the findings of Liu et al., Suthar et al., & Ahmad et al., respectively [8,10,11].

CONCLUSION

A simple method of candida removal test was employed to assess the efficacy of the denture cleansers against *Candida albicans* upon and within the limitations of this invitro study, it can be concluded that amongst the commercial denture cleansers Fittydent® was more effective than the CD Clean® and amongst the essential oil (plant extracts) thyme essential oil showed better efficacy than Nigella sativa however there was not much significant difference between them. When all the four denture cleansers are compared together and Annova test is applied, Fittydent® gives the best result followed by Thyme essential oil, Nigella sativa and CD Clean® when compared to the control which was distilled water. Also it can be concluded that the rough surface of the soft liners facilitate adhesion of *Candida Albicans* cells in large number hence the number of *Candida albicans* cells that remain viable on the surface is more after treatment as compared to the smooth surface of both the types of heat cure acrylic resin denture base. Within the limitation of this study, which was evaluated only for one day denture cleansing procedure, stimulating the patient's normal denture cleansing routine, and the entire Candida cells were not removed from the surface of the denture base, therefore reorganizing of the Candida cells is inevitable. Also, long term effectiveness of the Cleansing agents has to be evaluated. In further research the effect of these materials on the soft liner should be evaluated.

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