Evaluation of efficacy of removing Candida albicans from the orthodontic acrylic base material using Triphala solution and its comparison with different cleaning methods - An in-vitro study.

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INTRODUCTION:

The impact of using orthodontic appliances on intraoral bacterial and fungal colonization is crucial to understand because acrylic resin plates are frequently used as removable orthodontic appliances[1].A network of bacteria, fungi, and other microorganisms make up this intricate oral biofilm. As a result, fungi like Candida albicans and gram-positive bacteria like Streptococcus mutants and Lactobacillus may become more prevalent in the oral cavity[2]. It promotes colonisation and is the root of side effects of orthodontic treatment such caries, gingivitis, and candidiasis. More than 60% of healthy populations are colonized by this yeast without displaying any signs of infection, hence it is regarded as one of the normal oral flora's constituents[3]. Increased growth of candidiasis can become pathogenic both locally and systemically leading to various forms of candidiasis[4]

The hydrophobic effect and van der Waals forces allow C. albicans to adhere to the orthodontic acrylic's hydrophobic surface[5]. Combining these factors may tip the scales in favor of Candida colonization and proliferation in patients who use orthodontic appliances, boosting dentine demineralization by enhancing the cariogenic potential[6] of biofilms containing Streptococcus mutans. There are several conventional methods of cleaning removable orthodontic appliances like cleansing with a toothbrush, denture cleaning solution, and chlorohexidine

mouthwash using a toothbrush to clean surfaces is thought to be the most typical way to prevent plaque from forming on acrylic ones.[7]soaking samples of acrylic in 0.12% chlorhexidine causes a significant reduction in viable cells. This is because chlorhexidine can enhance the permeability of C. albicans cells, allowing chlorhexidine gluconate to enter the cell and cause cell wall destruction and cell death[8].

Soaking the orthodontic acrylic bars in a commercial denture cleaning solution results in penetrating through the cell wall and permeabilizes the cell membrane causing leakage of intracellular components and cell lysis.[9]

Triphala is an ayurvedic herbal rasayana consisting of three fruits Terminalis chebula, T. Belerica and phyllantus embelica in 1:1:1 proportion. It has antifungal, anti-inflammatory, antioxidant and immunomodulatory properties. It is widely used as an antifungal agent.[10]

Therefore the Aim of the study is to assess the effectiveness of cleaning using Triphala solution (4:1) to remove C. albicans from orthodontic acrylic resin and its comparison with conventional cleaning methods with a toothbrush, soaking in denture cleaning solution, chlorohexidine gluconate oral rinse solution and distilled water.

OBJECTIVE:

1) To evaluate the Colony-forming unit (CFU) of Candida albicans on an orthodontic acrylic base before and after cleansing with a toothbrush.

2) To evaluate the Colony-forming unit (CFU) of Candida albicans on an orthodontic acrylic base before and after soaking in a denture cleaning solution.

3)To evaluate the Colony-forming unit (CFU) of Candida albicans on an orthodontic acrylic base before and after soaking in a chlorohexidine mouthwash.

4) To evaluate the Colony-forming unit (CFU) of Candida albicans on an orthodontic acrylic base before and after cleansing with a toothbrush and soaking in a triphala solution (4:1).

5) To evaluate the Colony-forming unit (CFU) of Candida albicans on an orthodontic acrylic base before and after soaking in distilled water.

6) To compare the Colony -forming unit (CFU) of Candida albicans on an orthodontic acrylic base before and after cleansing with a toothbrush, soaking in a denture cleaning solution, triphala solution, chlorohexidine mouthwash, and distilled water.

MATERIAL AND METHODS:

The study was conducted on acrylic bars in the Department of Orthodontics and dentofacial orthopedics. Identical acrylic bars with a surface topology of clinical relevance were fabricated using duplicated dental stone of an orthodontic patient. An alginate impression of the patient's upper dental arch was taken as part of routine orthodontic treatment, and a cast was made using a dental stone.

In the dental cast's palate section, a 12X25X2-mm rectangle was created. The cold cure-polymerization process was used for all the samples according to the manufacturer's instructions. The acrylic bars were finished and polished on only one side to simulate the fabrication of a removable orthodontic appliance. Fresh Candida albicans clinical isolates were obtained by sampling the palatal surface of a patient's removable orthodontic appliance with a sterile swab,

plating on Sabouraud dextrose agar with chloramphenicol and incubating at 37 °C for 48 h.

Human saliva was applied to acrylic bars to replicate the in vivo condition for orthodontic appliance the saliva supernatant was immediately kept at 70 °C.

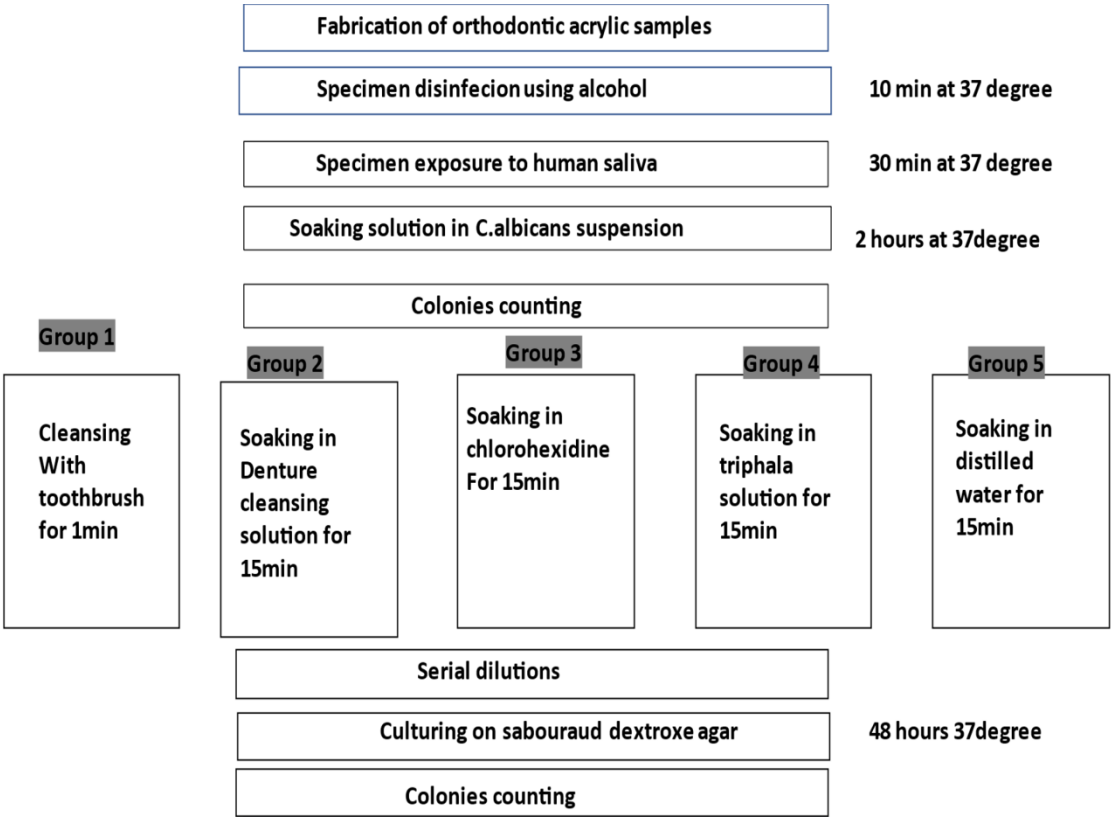
The acrylic bars were cleaned by submerging them in 70% v/v (volume per volume) alcohol for 10 minutes, followed by a 1-minute rinse with water. After that, each acrylic bar was put into a test tube with 8 mL of the C. albicans suspension and kept in an incubator at 37 ^{O}C.

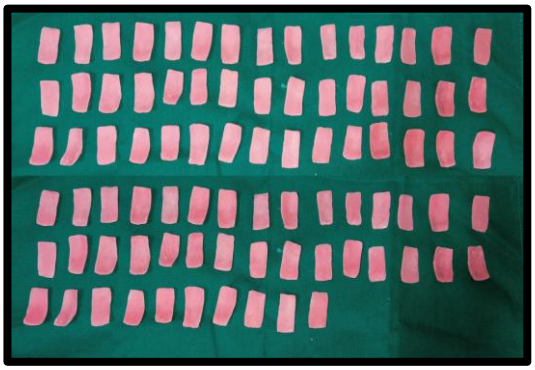
Out of 75 samples, 15 samples were randomly assigned to each of the five groups, and various cleaning techniques were employed. Group 1 with a toothbrush for 1 minute, Group 2 with denture cleaning solution for 15 minutes, Group 3 with chlorhexidine for 15 minutes, Group 4 with Triphala solution for 15 minutes, and Group 5 with distilled water for 15 minutes. Following the cleansing procedure, samples will be dipped into a broth containing 1 mL of Sabouraud dextrose and chloramphenicol and vortexed for 2 minutes. Each sample was made in tenfold serial dilutions ranging from 10^{2} to 10^{3} and then was distributed on a Sabouraud dextrose agar plate.

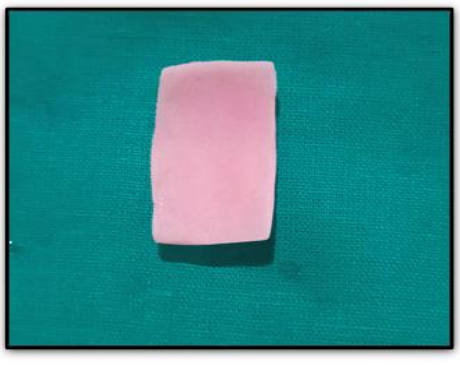
The agar plates were incubated at 37 °C for 48 h. The C. albicans colonies on the agar plates were counted manually (the total number of viable Candida albicans cells = the number of colonies x the dilution factor × 10) and expressed in numbers of colony-forming units per milliliter (CFU/mL) which is equivalent to the CFU/bar so accordingly 10^{2} dilutions were used to calculate the efficacy of Candida albicans on orthodontic acrylic bases using different cleaning methods.

The percentage reduction due to the cleaning was calculated using the following formula: percentage reduction =(CFU/barc - CFU/bart) CFU/barc 100, where CFU/barc is the CFU/bar before and after the therapy, respectively. comparing the number of colony-forming units (CFU) of Candida albicans on an orthodontic acrylic base before and after brushing, soaking in a denture cleaning solution, triphala solution, chlorohexidine mouthwash, and distilled water.

Flow chart of the method employed in the study







Colony formation on SDA after different cleaning methods.

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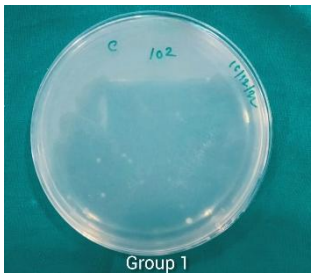

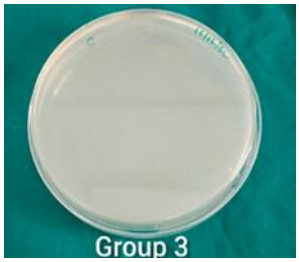

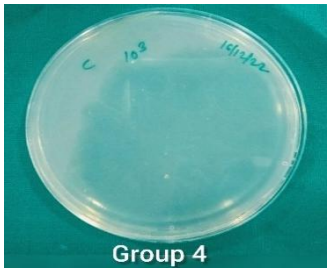

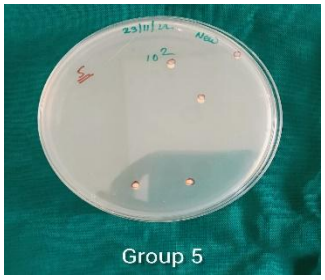

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Fig 1:group 1 - toothbrush

Fig 2:group 2 - tablets

Fig 3:group 3-chorohexidine

Fig 4:group 4-triphala solution

Fig 5:group 5-distilled water.

Statistical analysis

Data entries were done in Microsoft Office Excel 2010 and analyses of results were done using Statistical Product and service solution (SPSS)

version 22 software. Descriptive statistics such as mean and standard deviation was calculated for quantitative variables. The p-value was fixed at 0.05. Data normality was checked using the Shapiro-Wilk test. One-way ANOVA f-test was used for overall efficacy comparison (parametric) among five study groups. Tukeys post hoc test was used for pairwise comparison between groups in relation to efficacy.

Results:

- According to Table 1 and Graph 1,The amount of Candida albicans CFU attached to the bars was decreased by all cleaning techniques. During repeated actions, no differences were found in the three replicates of the Brushing group, Tablets group, Chlorhexidine group, Triphala group, or Control group, according to the ANOVA test. Chlorhexidine has a mean feasible reduction in candida albicans of 98%, Triphala is at 89%, brushing is at 81%, and denture cleaning tablets are at 75%.
- Table 1 and Graph 1 show an overall comparison of the efficacy of removing Candida albicans using different cleaning methods respectively using the one-way ANOVA F test. The p-value shows a high statistical difference.
- Table 2 compares the efficacy of removing Candida albicans from acrylic base material to fabricate removable orthodontic appliances using different cleaning methods by the Tukey range test. It shows high statistical differences in all the groups except for group 2.

Table 1. Overall comparison of the efficacy of removing C. albicans using different cleaning methods

10^2	Mean	SD	One-way Anova F test value	P value, Significance
Group 1 (Toothbrush)	81.13	4.18	F = 171.923	p< 0.001**
Group 2 (Tablets)	75.06	4.41		
Group 3 (Chlorohexidine)	98.06	2.15		
Group 4 (Triphala)	89.66	3.95		
Group 5 (Distilled Water)	64.4	4.03		

Graph 1 Overall comparison of efficacy of removing C. albicans using different cleaning methods

Table 2: Pairwise comparison of the efficacy of removing Candida albicans from acrylic base material using different cleaning methods

10^2 Dilution			
Group	Comparison Group	Mean Difference	P value
Group 1 (Toothbrush) vs	Group 2 (Tablets)	6.06	P< 0.001**
	Group 3 (Chlorohexidine)	16.93	P< 0.001**
	Group 4 (Triphala)	8.53	P< 0.001**
	Group 5 (Distilled Water)	16.73	P< 0.001**
Group 2 (Tablets) vs	Group 3 (Chlorohexidine)	23.0	P< 0.001**
	Group 4 (Triphala)	14.6	P< 0.001**
	Group 5 (Distilled Water)	10.66	p =0.021*
Group 3 (Chlorohexidine) vs	Group 4 (Triphala)	8.4	P< 0.001**
	Group 5 (Distilled Water)	33.66	P< 0.001**
Group 4 (Triphala) vs	Group 5 (Distilled Water)	25.26	P< 0.001**

Discussion:

The oral environment has the capacity to adjust to the presence of an orthodontic appliance. This has been shown by studies reporting an increase in stimulated flow rate, buffer capacity, and salivary pH that enhance the anticaries properties of saliva. These alterations are physiologic responses to maintain oral health in adverse situations by avoiding potentially pathogenic microorganism colonization, acidogenic bacteria products, and demineralization.[11]

Studies reveal that high colonization by the fungal pathogen Candida albicans is frequently detected in orthodontic patients[12]. Orthodontic appliances were reported to increase the oral candida carriage rate and alter candida counts during treatment. Recent research suggests that any foreign objects in the mouth, including both fixed and removable appliances, appear to alter the microbial habitat by providing Candida with the surfaces it needs to adhere to the mouth. A more cautious approach is warranted for immunocompromised orthodontic patients to avoid candidal infections[13]

The impact of using orthodontic appliances on intraoral bacterial and fungal colonization is crucial to comprehend because acrylic resin plates are frequently used as removable orthodontic appliances[14]. When treating a variety of malocclusions, removable orthodontic appliances are a useful tool in the orthodontist's toolbox[15]. It has been demonstrated that using such devices reduces oral hygiene, occludes surfaces, and promotes the growth of bacteria.

81% of Candida albicans cells were eliminated by brushing, which is thought to be the most typical means of preventing plaque formation on acrylic surfaces. Pellizzaro et al., who achieved a 96% reduction of Candida biofilm, reported a finding that was comparable to other studies done before. It is possible that the toothbrush is unable to dislodge cells within the pores of the fitting surface of the acrylic bars[16]. Brushing with a toothbrush is considered the most common method of controlling plaque development on acrylic surfaces.

Chlorhexidine can increase C. albicans cell permeability, allowing the chlorhexidine gluconate to enter the cell, leading to cell wall damage and cell death. Soaking acrylic samples in chlorhexidine causes a significant reduction in viable cells.

Tablets is one of the effective method of cleaning. The mechanism of cleansing acrylic resin with tablets starts once the tablet dissolves in water, as an alkaline peroxide[17] solution is formed. This solution then releases oxygen bubbles enabling a mechanical cleaning in addition to chemical cleaning enhanced by sodium lauryl sulfoacetate, a detergent that penetrates through the cell wall and permeabilizes the cell membrane causing leakage of intracellular components and cell lysis[18].

Contents in Triphala have antibacterial and antifungal properties. It has been demonstrated that the phenolic ring in these phytochemicals is poisonous to bacteria. Based on the data that higher hydroxylation causes increased toxicity, it is believed that the site(s), the number of hydroxyls, and the phenol group are related to their relative toxicity to microorganisms. The majority of the highly oxidized phenols, according to some writers, are also inhibiting. Enzyme inhibition by the oxidized chemicals, possibly by reactivity with sulfhydryl groups or through more unspecific interactions with the proteins, is one of the mechanisms hypothesized to be responsible for phenolic toxicity to microorganisms.[19]

When compared to Triphala extract in this investigation, chlorhexidine was found to be more efficient. However, the long-term use of chlorhexidine may be constrained by its well-known side effects, including discoloration of teeth and tooth repair, modification of taste perception, and growth of resistant microbes.Triphala is typically recognised, culturally acceptable, economically possible, and has less adverse effects than herbal remedies, making it suitable for long-term usage in a variety of formulations for oral care.[20]According to our study Chlorhexidine has a mean feasible reduction in candida albicans of 98%,

Triphala is at 89%, brushing is at 81%, and denture cleaning tablets are at 75%.

Limitations of the study:

- The main weakness of this study is that it was undertaken in a laboratory. It was an in vitro study, other oral parameters like temperature, stress were not taken into consideration.
- It was performed on an orthodontic acrylic base plate and not on an actual appliance.
- Shelf life of the Triphala solution is 3-4 days.

Conclusions:

- This study showed that brushing, denture cleaning tablets, chlorhexidine gluconate and triphala solution can remove Candida albicans from the surface of the orthodontic acrylic base material.
- Chlorohexidine mouthwash proved to be the most effective method in followed by the triphala solution (4:1), and least with denture cleaning tablets.

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