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The Impact of Propolis on Denture Hygiene: A Comparative Study with Commercial Alternatives

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ABSTRACT:
Introduction: Edentulism is a common problem among the elderly population that leads to a significant reduction in their quality of life. Most of the patients are rehabilitated with removable prosthesis for the replacement of their missing teeth. An ideal environment for the proliferation of microorganisms is created by removable complete dentures. Most of the commercial denture decontaminants contain chemicals that considerably reduce the mechanical properties of denture base resins. Biofilm present on dentures have become resistant to conventional cleaning methods. Newer strategies are required to overcome this issue. Propolis is a substance known to have potent antibacterial and antifungal effects. This study is aimed at exploring the prospective uses of propolis in modern-day dentistry and explores the possible uses of Indian propolis for decontaminating dentures.
Materials and methods: 15 complete denture wearers were selected as subjects for the study.
The dentures of each participant were decontaminated using 2 different products as per the groups. Dentures were soaked in the respective decontaminant overnight. Swabs were collected before and after decontamination for microbiological analysis. The microorganisms tested in the study were Candida albicans, Streptococcus mutans, and Staphylococcus aureus.
Results: Propolis has shown an overall efficacy of 99.9% and the commercial product has shown an equal overall efficacy, i.e 99.9%
Conclusion: Propolis has shown to be equally effective as the commercial product against the tested microorganisms.
KEYWORDS: Denture contamination, Propolis, Denture cleaning, Candida, Denture decontamination, Denture disinfection

I. INTRODUCTION

"Edentulism is a debilitating and irreversible condition and is described as the final marker of disease burden for oral health". [1] Within the domain of oral health, edentulism presents a multifaceted challenge. These individuals often rely on complete dentures for the restoration of essential functions such as speech, mastication and aesthetics, which significantly diminishes their quality of life and social interactions. However, despite their vital role in oral rehabilitation, dentures are susceptible to progressive contamination over time. This contamination comprises a diverse array of microorganisms, which varies significantly from the microflora observed in dentulous individuals.

According to a study done by Juliana et al, Candidal colonization was observed in 64.2% of denture wearers and this percentage was 19.4% among dentulous people. [2] Therefore, hygiene maintenance becomes more important among people wearing dentures.

The most frequently observed microorganisms on complete dentures are Candida albicans, Streptococcus mutans and Staphylococcus aureus. These organisms are responsible for the formation of resistant biofilms on denture surfaces. The development of biofilm not only affects the appearance of dentures but also establishes a complex relationship between the oral environment and the growth of microorganisms. Denture contamination, if left unaddressed may lead to systemic complications as well.

Numerous methodologies have been proposed to address denture contamination, ranging from chemical decontaminants to natural alternatives.

Chemical decontaminants, such as chlorine-based agents or quaternary ammonium compounds, have traditionally been utilized for their broad-spectrum antimicrobial properties. However, concerns regarding their potential cytotoxicity and adverse effects on denture materials, including acrylic resins have prompted a reevaluation of their suitability for long-term denture maintenance.

Microorganisms have also demonstrated resistance to these decontaminants.

Commercial products may contain certain components which may pose a health hazard to the operator if not properly handled according to manufacturer's instructions. Inhalation or ingestion of chemicals may cause toxicity, especially where there is misuse or overuse. These products in the long run may lead to an alteration in the physical properties of denture base materials, for example; discoloration, deterioration in strength, or any change in the surface texture.

In contrast, natural alternatives, such as herbal extracts and plant-derived compounds, offer promising prospects for denture decontamination with less chances of adverse effects. There has been no evidence of microbial resistance to Propolis till date.

Among these natural products, propolis, a resinous substance produced by honeybees, has garnered significant attention in recent times. Propolis, also known as "bee glue" is a sticky material used by honeybees to protect their hive. Honeybees collect resins from plant buds and mix them with their salivary enzymes and beeswax to make Propolis. [3,4]

While propolis, notably Indian propolis, is promising against oral infections, there is not as much data on its efficacy as a denture decontaminant. [5] However, there has been an in-vitro study on this topic done in Canada, which has shown favourable results.

This study is aimed at exploring the possible use of Indian propolis for decontaminating dentures.

II. MATERIALS AND METHODS

The study was conducted by the Departments of Prosthodontics at JSS Dental College and Hospital, Mysore, and the Department of Microbiology at JSS Medical College and Hospital, Mysore, over a period of 6 months. Approval from the Institutional Ethics Committee was obtained.

Fifteen healthy complete denture users were selected from the OPD of JSS Dental College and Hospital according to specific inclusion and exclusion criteria. Participants were informed about the study, and written consent was obtained. They were instructed on the importance of denture decontamination and advised to refrain from any decontamination other than routine brushing under running water.

Upper and lower complete dentures were collected, washed, and assigned to either the Propolis group (Group A) or a commercial decontaminant group (Group B). This division of dentures into these 2 boxes was done in an alternating manner, i.e the upper denture of the 1st participant was kept in the box labelled as Group A and the lower denture in the box labelled as Group B, the upper denture of the 2nd participant was kept in the box labelled as Group B and the lower denture in the box labelled as Group A. Contamination was assessed by collecting 3 microbial swabs per denture before and after the decontamination procedure as per the specific group protocols for decontamination. Dentures were immersed overnight (about 8 hours) in the respective decontaminants: Propolis extract mixed with distilled water (1:25 ratio) or a denture cleaning tablet (Clinsodent) in 200 ml of distilled water. After decontamination, dentures were rinsed under tap water for 20-30 seconds and post- decontamination swabs were collected. The swabs were stored in sterile containers, labelled and sent to the microbiology lab within two hours of collection for analysis.

Microbiological analysis-

Candida albicans was cultured using Sabouraud Dextrose Agar (SDA), samples were streaked onto the SDA plates using a sterile inoculation loop and a semi-quantitative streaking technique. The plates were incubated at 30-37°C for 24-48 hours. Colony morphology was described, and Gram staining revealed gram-positive budding yeast cells. Confirmatory tests included a positive germ tube test and Chlamydospore production on cornmeal agar.

Streptococcus mutans was cultured using Mitis-Salivarius Agar with Tellurite (MSAT), prepared per the manufacturer's instructions with added tellurite for selectivity. The culturing procedure followed for inoculation and incubation was the same as explained above. Post-incubation, S. mutans appeared as small, round blue-black colonies. These colonies underwent presumptive identification via catalase test, alpha hemolysis on blood agar, bile esculin test, and resistance to optochin and bacitracin.

Staphylococcus aureus was cultured using Mannitol Salt Agar (MSA), prepared by autoclaving MSA powder in distilled water. The culturing procedure followed was the same as explained above. Post-incubation, colonies resembling S. aureus (yellow or golden with yellow zones due to mannitol fermentation) were counted. Isolates were presumptively identified by their catalase and coagulase test positivity, and colony morphology was noted.

For all the tested microorganisms, post-incubation, colony growth was manually counted and recorded as CFU/ml.

The microbial load before and after decontamination was analyzed, tabulated, and compared. Results were statistically analyzed to evaluate and compare the microbial efficacy against Candida albicans, Streptococcus mutans, and Staphylococcus aureus.

Statistical methods-

The difference between microbial samples before and after decontamination was statistically analyzed using descriptive and inferential methods. Descriptive statistics included mean and standard deviation, while inferential statistics involved paired samples t-test, ANOVA for repeated measures, and tests for normality (Kolmogorov-Smirnov and Shapiro-Wilk). Chi-Square tests (Pearson and Fisher's Exact) and independent t-tests for independent samples were also used. Non-parametric analysis was conducted with the Wilcoxon Signed Ranks Test. SPSS Software, Version 28 was used for analysis.

III. RESULTS

Normality tests revealed that the sample data does not follow a normal distribution, as all p-values were less than 0.05. Consequently, non-parametric tests were utilized to analyze the results. Overall, there were 15 dentures in each group, making 30 dentures in total, evenly split between the groups (Propolis and Commercial product)

For the comparison of efficacy between Propolis and the commercial product against Candida albicans, the test statistic (Z) was -0.105 and the p-value was 0.916. The null hypothesis was accepted, indicating no significant difference between the efficacy ranks of Propolis and the commercial product. Similarly, for Streptococcus mutans, the test statistic (Z) was -0.758 with a p-value of 0.448, and for Staphylococcus aureus, the test statistic (Z) was -1.160 with a p-value of 0.246. In both cases, the null hypothesis was accepted, indicating no significant difference.

The results also indicates that both propolis and the commercial product have extremely high efficacy in denture decontamination, with almost identical percentage efficacies of approximately 99.99%. This suggests that propolis is as effective as the commercial product in this context.

IV. DISCUSSION

Dentures play an important role in restoring the structure, function and esthetics for people who have lost teeth. However, denture contamination has arisen as a major concern, with potential hazards to both oral and systemic health. There are various causes of denture contamination and their implications on oral health. It is a complex issue resulting from a variety of reasons, including poor oral hygiene, improper denture maintenance and the existence of microbial biofilms.

Denture hygiene maintenance becomes challenging for the elderly as manual dexterity declines with age. This has been reported by Kanli et al in a study. [6] Food particles might get accumulated in and around the dentures. If not cleaned adequately, they may trigger microbial proliferation and halitosis. Incorrect cleaning procedures or the use of abrasive cleaners may lead to rough denture surfaces, so dentures must be cleaned regularly with a soft bristle brush and a non-abrasive denture cleanser.

Oral health is inextricably linked to overall health as well. There is continuing research exploring the potential linkages between oral health disorders and systemic diseases such as cardiovascular disease, diabetes and respiratory infections. Dentures might serve as a reservoir for pulmonary and general opportunistic infections. They may harbour microorganisms that exhibit resistance to antibiotics, this has been reported by Sumi et al in a study. [7]

Natural denture disinfecting solutions are attracting interest because of their efficiency as well as safety. These natural products may serve as an alternative to traditional chemical disinfectants. Natural compounds that are often used include essential oils with antibacterial effects that are beneficial against a variety of oral infections, such as tea tree, thyme and peppermint oils.

Adoption of natural denture care methods not only shows the rising trend towards sustainable and environmentally friendly options, but it also highlights the significance of a gentler yet efficient oral hygiene methods. Promising results have been observed in the research on natural agents for denture decontamination in recent years. Natural substances such as grapefruit seed extract have demonstrated efficacy against a variety of oral cavity-common bacteria, fungi and viruses in a study done by Waghmare et al. [8] These natural substitutes may help limit the likelihood of oral infections and their related consequences.

Propolis, frequently known as “bee glue", is a resinous material that honeybees gather and produce from a variety of plant sources. To make propolis, bees collect resins from plant buds, sap flows and botanical exudates. Bees then mix the collected resin with beeswax, saliva and other secretions. This substance plays a number of vital roles in the beehive. Propolis also possesses potent antibacterial, antifungal and anti-inflammatory qualities that

contribute to hive sanitation by preventing the onset of infections and safeguarding the colony's integrity, this has been confirmed by Dodwad et al. [9]

Propolis has been used in human applications for possible medicinal benefits, such as an alternative remedy for wounds, infections and other illnesses in traditional medicine. Propolis may be used to clean complete dentures, owing to its antimicrobial properties. Flavonoids and phenolic acids, two substances found in propolis, have antimicrobial effects against both bacteria and fungi, as reported by Wagh et al. [10] Propolis may help prevent the growth of bacteria on denture surfaces, thus lowering the risk of systemic infections and other oral health issues related to dentures. De Souza et al have found that Propolis accomplishes this by intervening with the production of biofilms and limiting microbial adherence. [11]

Important information regarding usefulness of Propolis for regular denture hygiene practices may be obtained by contrasting its efficacy with that of commercial products.

Candida albicans is the predominant fungal species linked to denture stomatitis, a common condition characterized by inflammation of the oral mucosa underlying dentures. It was a relevant choice for this study because it is the most common microorganism found on complete dentures as reported by Abbeele et al. [12]

Streptococcus mutans and Staphylococcus aureus are also frequently found on denture surfaces, especially in those with poor oral hygiene. These findings have been confirmed in a study conducted by Vijita Nair et al. [13]

Numerous studies have demonstrated Propolis' effectiveness against oral infections such as Candida albicans, Streptococcus mutans and Staphylococcus aureus, emphasizing its potential use as a denture decontaminant.

Overnight immersion of dentures in the respective decontaminants was done in this study. This was done because most of the commercial products recommend overnight immersion for optimum efficacy. Joke Duyck et al have reported in their study that overnight immersion of dentures in decontaminating solutions significantly reduced the microbial count. [14]

In the present study, the dilution ratio of Propolis with distilled water was 1:25. Propolis has shown excellent efficacy in this concentration as reported by Loreta et al in their study. [15]

Rattiporn et al have assessed the efficacy of 5 denture cleansers, chlorhexidine, sodium hypochlorite, thymol and geraniol in their study. It was reported that all these decontaminants had an antibacterial efficacy of 99.9%. [16]

In the present study, a comparison of the overall efficacy of propolis and the commercial product against the investigated microbes was done. This resulted in a percentage efficacy of about 99.985% for propolis and a percentage efficacy of approximately 99.999% for the commercial product. These findings reveal that both the products are highly efficient in decontaminating dentures.

Both products exhibited remarkable effectiveness, yet propolis has several advantages over the commercial product. Propolis, being a natural substance, offers inherent advantages over commercial products. Firstly, there has been no studies reporting any discernible alteration of denture base materials, indicating its compatibility and non-invasive nature. Moreover, the absence of reported microbial resistance to propolis emphasizes its efficacy in eradicating microorganisms, a concern often associated with commercial products. Propolis' natural origin inherently reduces the likelihood of triggering allergic reactions or toxicity, enhancing its safety profile compared to commercial alternatives. This aspect is crucial, as patient comfort and acceptance is the most important factor in treatment adherence and satisfaction.

In contrast, commercial products may pose risks such as altering the physical properties of denture materials, potentially compromising their longevity and functionality.

Additionally, the higher incidence of allergic reactions and toxicity associated with synthetic formulations raises concerns regarding their long-term safety and suitability for widespread use. These findings were confirmed in a study done by Prabal Sharma et al. [17]

The results of the study demonstrated that some samples had no Candida albicans present at all, even prior to the dentures being decontaminated. The patients' excellent denture and oral hygiene habits might be one reason for the situation. Previous studies have reported that Candida albicans is unlikely to be present in newer dentures. A study done by Sajid et al has confirmed this finding. [18]

There were no statistically significant differences in the two products' efficacies against the tested microorganisms.

Streptococcus mutans was present in almost all the samples that were analysed in this study. The biofilm formed is very difficult to eradicate using commonly used decontaminants or antibiotics as reported by Tania et al in a study. [19] On the contrary, Propolis was able to effectively eradicate and inhibit biofilm formation on dentures as reported by Carolina et al in a study. [20]

As reported by Lewis et al in their study, Staphylococcus aureus was not found in 73% of the

studied samples. [21] Similarly, in the current study Staphylococcus aureus was absent in most of the samples. However, out of the samples that had Staphylococcus aureus growth, both the products were able to eradicate it effectively post decontamination.

The present study fills a significant gap in current literature by highlighting the advantages of employing natural products for denture decontamination. Additionally, there have been no studies exploring Propolis as a denture decontaminating agent in India so far, however, there has been a single in-vitro study on this topic conducted in Canada, which showed favorable results.[11]

V. CONCLUSION

Propolis has demonstrated an overall efficacy of 99.9% which was equal to that of commercial product.

Propolis has proved to be equally efficient in the reduction of microbial counts of C. albicans, S. mutans and S. aureus.

There was no statistically significant difference between the efficacy of Propolis and commercial product against these microorganisms. This indicates promising prospects for propolis in denture hygiene maintenance.

While the initial findings of this study on propolis are encouraging, additional research is required to assess its shelf life, its potential impact on the mechanical properties of denture bases and the possibility of staining dentures with long term usage.

Overall, this study contributes to the growing evidence supporting the use of propolis in dental care and provides ideas for future research endeavours aimed at denture decontamination strategies and improving oral health outcomes.

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Table 1- T- test for Group Statistics

	Group	N	Mean	Standard Deviation	Standard Error Mean
Candida before	Propolis	15	40206.67	50535.96	13048.33
	Commercial	15	40160.00	50575.02	13058.41
Candida after	Propolis	15	66.80	258.16	66.66
	Commercial	15	94.93	254.25	65.65
Difference	Propolis	15	40139.87	50451.87	13026.62
	Commercial	15	40065.07	50472.14	13031.85
Streptococcus before	Propolis	15	46886.80	51427.79	13278.60
	Commercial	15	34760.40	47866.45	12359.06
Streptococcus after	Propolis	15	681.07	2578.24	665.70
	Commercial	15	67.33	258.03	66.62
Difference	Propolis	15	46205.73	50736.35	13100.07
	Commercial	15	34693.07	47918.01	12372.38
Staphylococcus before	Propolis	15	0.27	0.70	0.18
	Commercial	15	13.93	35.00	9.04
Staphylococcus after	Propolis	15	0.00	0.00	0.00
	Commercial	15	0.00	0.00	0.00
Difference	Propolis	15	0.27	0.70	0.18
	Commercial	15	13.93	35.00	9.04

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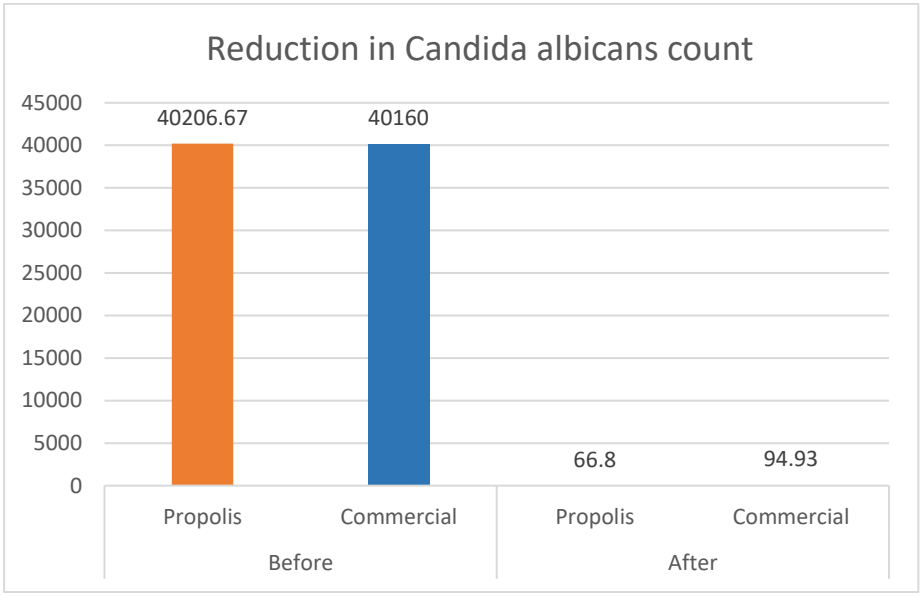

Fig 1- Reduction in Candida albicans count

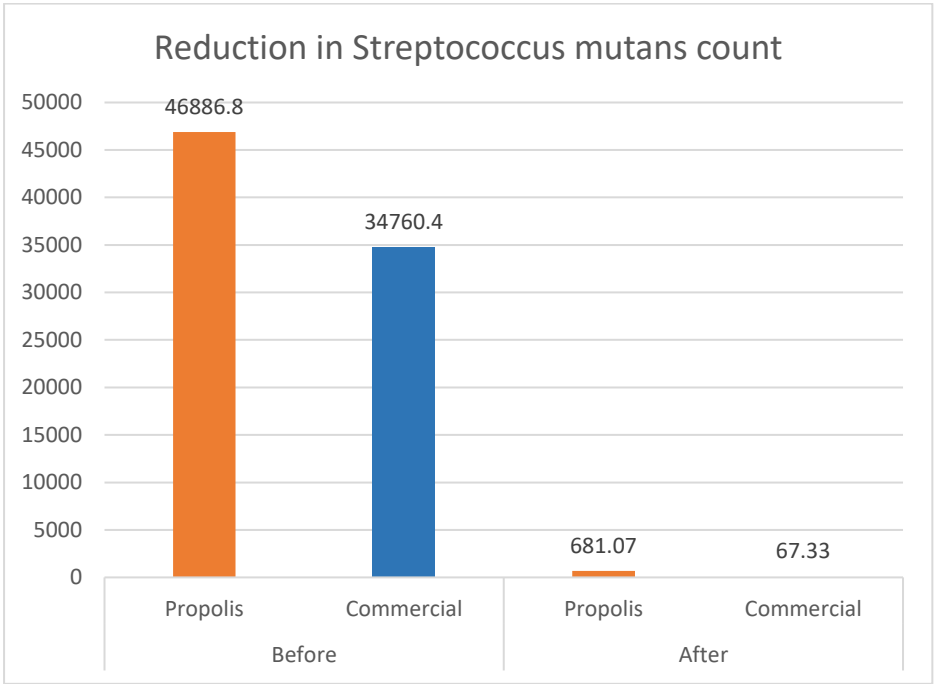

Fig 2- Reduction in Streptococcus mutans count

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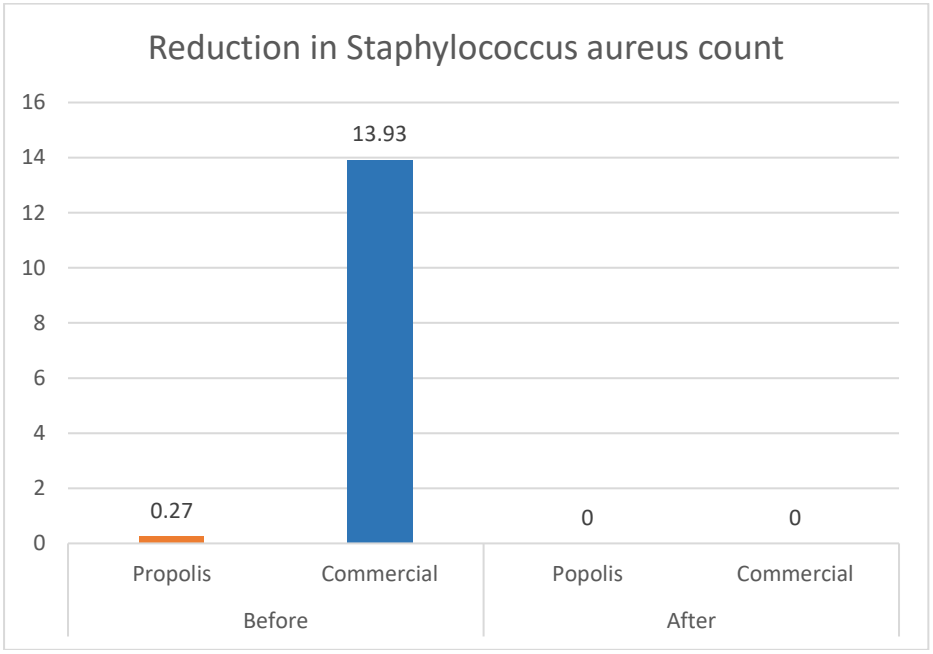
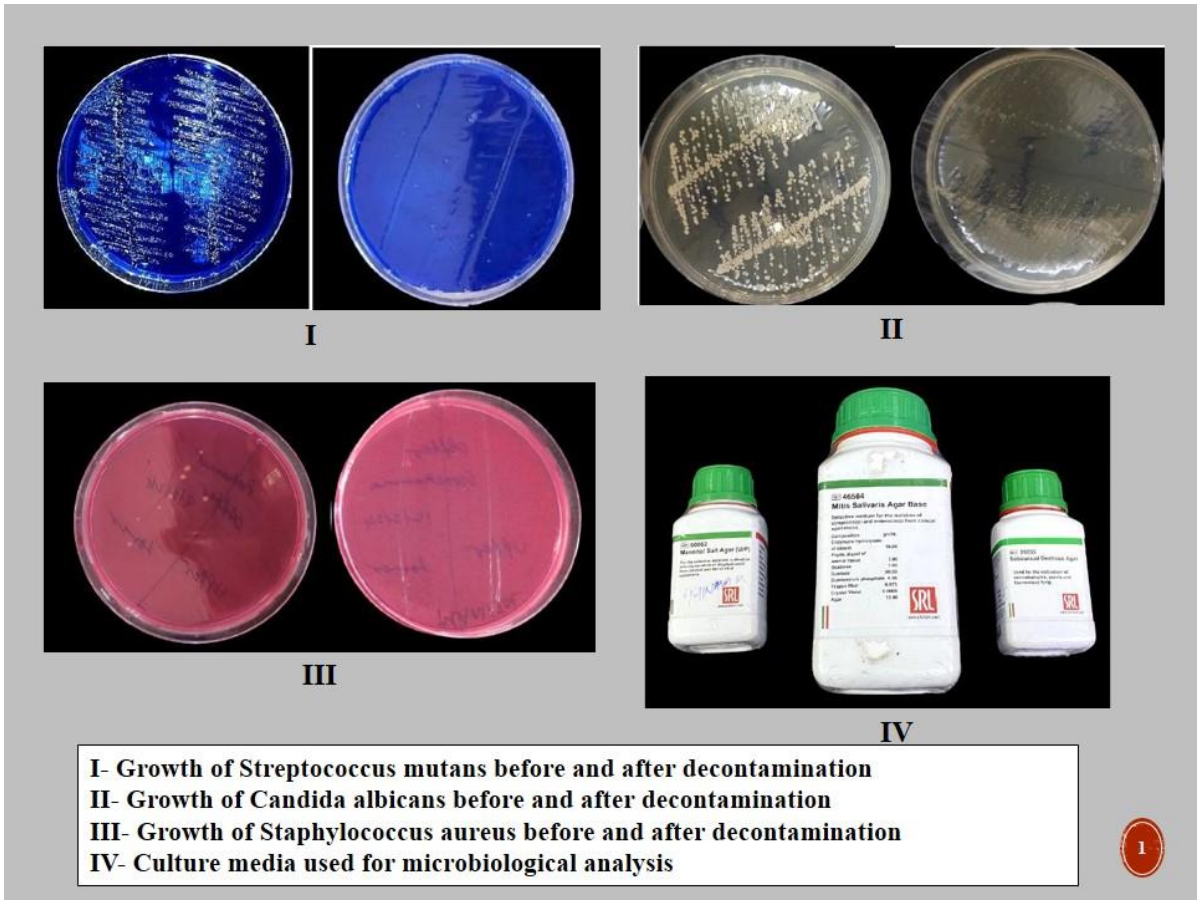


Fig 1- Reduction in Staphylococcus aureus count



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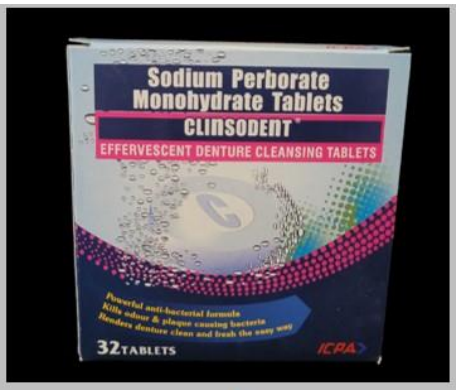

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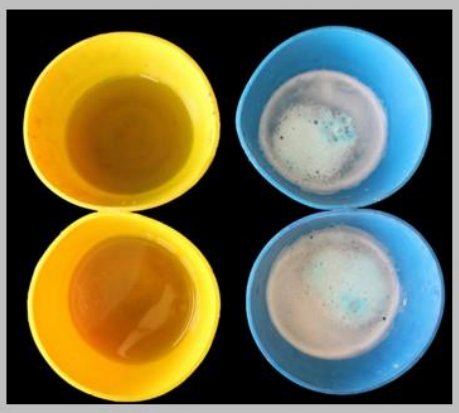

VII

V- Propolis extract
VI- Clinsodent denture cleaning tablets
VII- Decontaminant solutions

2