
**"COMPARATIVE EVALUATION OF SURFACE ROUGHNESS OF
HERBAL DENTURE CLEANSING TABLET CONTAINING ESSENTIAL
OILS OF TULSI & LEMONGRASS, 0.12% CHLORHEXIDINE
DIGULCONATE & COMMERCIALY AVAILABLE DENTURE
CLEANSING TABLET ON HEAT CURE POLYMETHYL
METHACRYLATE DENTURE BASE RESIN: AN INVITRO STUDY."**

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Dissertation

Submitted to

**KLE ACADEMY OF HIGHER EDUCATION & RESEARCH,
Belagavi, Karnataka.**

In partial fulfillment of the requirements for the degree of

MASTER OF DENTAL SURGERY

In

**PROSTHODONTICS AND CROWN & BRIDGE
(BRANCH - I)**

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**DEDICATED TO
MY PARENTS AND
MY GUIDE**

ACKNOWLEDGEMENT

“Feeling gratitude and not expressing it is like wrapping a present and not giving it”.

A sense of triumph is very much justified at this stage of completion of my dissertation, even more so is a sense of gratitude to all my peers, mentors and well-wishers.

My salutations to ALMIGHTY GURU – A tangent between zero and infinity – for his divine grace bestowed when needed.

*In Loving Memory of my Beloved Father Late **Mr. DALJIT SINGH MANAKTALA***

I gladly utilize this opportunity to express my deep sense of gratitude and indebtedness to all my TEACHERS.

“The task of the excellent teacher is to stimulate "apparently ordinary" people to unusual effort. The tough problem is not in identifying winners: it is in making winners out of ordinary people.”

*I feel honored to be a student of my respected teacher and guide **Dr. RAGHUNATH PATIL_{MDS}**, Professor, Department of Prosthodontics and Crown and Bridge, KLE Vishwanath Katti Institute of Dental Sciences, Belagavi, without whose everlasting inspiration, incessant encouragement, constructive criticism, and with valuable suggestions for improvement, the completion of this study would not have been possible. His unlimited patience, meticulous supervision at every step and everlasting zeal for perfection has not only enabled me to complete the dissertation, but has also helped me tremendously during the postgraduate programme.*

I would like to thank Mr. Vijay; KLS Gogte Institute of Technology, Belagavi for assisting me with surface roughness evaluation.

*I am grateful to **Dr. ANAND KUMAR G. PATIL_{M.D.S.}**, and **Dr. RAMESH NAYAKAR_{M.D.S.}** who have been the pillar of support and were always available to me and have been a source of endless guidance all through.*

*I would like to thank **MAHANTESH B. _{M.D.S.}** **Dr. SOUNALAYA_{M.D.S.}**, **Dr. SANTHOSH_{M.D.S.}**, **Dr.**, for their valuable guidance and keen personal interest in critical analysis of this study material, without which this study would be incomplete.*

*I would also like to thank, **Dr. PRASHANT_{M.D.S.}**, **Dr. ABHIJIT_{M.D.S.}**, **Dr. ADITYA_{M.D.S.}**, **Dr. SAYED_{M.D.S.}**, **Dr. SWAPNIL_{M.D.S.}**, **Dr. MALIKARJUN_{M.D.S.}**, **Dr.VEENA_{M.D.S.}** **Dr. SUVIDHA _{M.D.S.}**, for their support, their innovative ideas and friendly association discussions in the matter of mutual professional interest which were of considerable value & helped me to broaden my source of knowledge.*

*I am grateful to **Dr. (Mrs.) ALKA KALE_{MDS,}** Principal, KLE V.K Institute of Dental Sciences, Belagavi, for extending her help and cooperation towards the completion of this dissertation.*

*A word of thanks to **Dr S.B. JAVALI**, statistician, for providing a scientific meaning to this study by way of its statistical analysis.*

I would like to thank all the technicians and non-teaching staff for their services and help whenever required.

*I am thankful to all my colleagues **Dr. RAHUL**, **Dr. RUTVI**, **Dr. DIVYA**, **Dr. VISHAKHA** and **Dr. AAYUSHI** for their co-operation. A note of thanks to my dear seniors, **Dr RICHA**, **Dr. SAYALI**, **Dr. DIVYESH**, **Dr. HARSHALI**,*

Dr. MEEKHA Dr. OVAIS. A special thanks to my dear juniors Dr. PALLAVI, Dr. POONAM, Dr. RAISA, Dr. SONALI, Dr. MITALI, Dr. HIMA, Dr. SHREYA, Dr. SWAPNIL, Dr. YAMINI and Dr. KARUNA, Dr. NISHA, Dr. AISHWARYA, not to forget Dr. SONALI GHOLAP, who have been there with me every minute.

The words “Thank You” seem shallow at best for my mother Mrs. SEEMA MANAKTALA, my dear sister Miss. NEETI MANAKTALA, Mrs. Ramanjeet Kaur for supporting me at my best and at my worst.

“The hand that rocks the cradle may not rule the world, but it certainly makes it a better place.”

I would like to express my heartfelt gratitude to Dr. TEJASHREE CHOUGALE M.D.S, Dr. BHUMIKA SHAHA; MY SENIORS for their tremendously valuable inputs and guidance without whom this study would not be complete.

Thank you, one and all.

Dr. HARPREET SINGH MANAKTALA

LIST OF ABBREVIATIONS USED IN THE STUDY

Group 1	Disinfection was performed on acrylic blocks using phytopharmaceutical denture cleansing tablet
Group 2	Disinfection was performed on acrylic blocks using fittydent denture cleansing tablet
Group 3	Disinfection was performed on acrylic blocks using chlorhexidine
Group 4	The acrylic blocks were immersed in the control (water)
S.D.	Standard deviation
S.E.	Standard error
ANOVA	Analysis of variance
hrs	Hours

ABSTRACT

STATEMENT OF PROBLEM

Poor denture hygiene is a risk factor for oral infections and systemic dissemination. Denture cleanliness is essential to prevent malodor, poor aesthetics and the accumulation of plaque/calculus with its deleterious effect on the mucosa. Denture base resins become colonized by several microbial species in the oral cavity. There are number of tablets, pastes and powders available for cleaning dentures with a variety of manufacturer's claims for their relative efficacies. Due to increase in the use of denture cleaners can lead to more wear off of the heat cure polymethyl surface leading to surface roughness.

The increase in the surface roughness of the heat cure polymethyl methacrylate can lead to increase in denture biofilm. *Candida species* yeasts are the most common species associated with denture biofilm. *Streptococcus mutans* and other bacterial species are also important denture biofilm components.

PURPOSE

The purpose of this study was to evaluate and compare the surface roughness of herbal denture cleansing tablet containing essential oils of tulsi and lemongrass, commercially available denture cleansing tablet and 0.12% chlorhexidine digluconate on heat cure polymethyl methacrylate denture base resins

MATERIALS AND METHODS

84 blocks were fabricated of heat cure polymethyl methacrylate resin and were divided into 4 groups of 21 samples each, which were disinfected using Phytopharmaceutical denture cleansing tablet containing Tulsi and Lemongrass,

fittydent denture cleansing tablets, chlorhexidine to disinfect the polymethyl methacrylate blocks as per the manufacturer's instruction. The last group was control group in which 21 samples were immersed in water without any disinfectant. Surface roughness evaluation was done before and after disinfection of the heat cure polymethyl methacrylate blocks. The difference in values obtained of surface roughness before and after disinfection were tabulated and were subjected to statistical analysis.

RESULTS

The results showed that highest surface roughness was seen with fittydent commercially available denture cleansing tablet followed by chlorhexidine group and least surface roughness was seen with phytopharmaceutical denture cleansing tablet.

CONCLUSIONS

The use of phytopharmaceutical denture cleansing tablet as a denture cleansing agent had the least effect on surface roughness. Phytopharmaceutical denture cleansing agent containing tulsi and lemongrass can be recommended as a denture cleansing agent for complete dentures over chlorhexidine and commercially available denture cleansing tablets

TABLE OF CONTENTS

Sl. No.	Particulars	Page No.
1.	INTRODUCTION	1-3
2.	NEED FOR THE STUDY	4-5
3.	HYPOTHESIS	6
4.	AIM AND OBJECTIVES	7
5.	REVIEW OF LITERATURE	8-28
6.	MATERIALS AND METHOD	29-39
7.	RESULTS	40-47
8.	DISCUSSION	48-50
9.	SCOPE OF THE STUDY	51
10.	LIMITATIONS	52
11.	CLINICAL IMPLICATIONS	53
12.	CONCLUSION	54
13.	SUMMARY	55
14.	BIBLIOGRAPHY	56-62
15.	ANNEXURES	63-67

LIST OF FIGURES

Figure No.	Particulars	Page No.
1.	Fabrication of acrylic blocks	31
2.	Heat cure acrylic resin (DPI, Mumbai)	34
3.	Preparation of wax blocks for surface roughness evaluation	34
4.	Investment of wax blocks to obtain acrylic blocks	35
5.	Acrylic blocks for surface roughness evaluation	35
6.	Profilometer for surface roughness evaluation	36
7.	Surface roughness evaluation prior to disinfection	36
8.	Disinfection of the acrylic blocks carried out	37-38
9.	Surface roughness evaluation after disinfection	39

LIST OF TABLES

Table No.	Particulars	Page No.
1.	Comparison of four groups (Chlorhexidine, Fittydent tablet, Phytopharmaceutical cleansing tablet and control) with respect to pre and post treatment surface roughness (um) by one way ANOVA	40
2.	Pair wise comparison of four groups (Chlorhexidine, Fittydent tablet, Phytopharmaceutical cleansing tablet and control) with respect to pre and post treatment Surface roughness (um) by Tukeys multiple posthoc procedures	41-42
3.	Comparison of pre and post treatment surface roughness (um) in four groups (Chlorhexidine, Fittydent tablet, Phytopharmaceutical cleansing tablet and control) by dependent t test	45

LIST OF GRAPHS

Figure No.	Particulars	Page No.
1	Comparison of four groups (Chlorhexidine, Fittydent tablet, Phytopharmaceutical cleansing tablet and control) with respect to pre and post treatment surface roughness (um)	43
2	Comparison of four groups (Chlorhexidine, Fittydent tablet, Phytopharmaceutical cleansing tablet and control) with respect to pre and post treatment surface roughness (um)	44
3	Comparison of pre and post treatment surface roughness (um) in four groups (Chlorhexidine, Fittydent tablet, Phytopharmaceutical cleansing tablet and control)	46
4	Comparison of pre and post treatment surface roughness (um) in four groups (Chlorhexidine, Fittydent tablet, Phytopharmaceutical cleansing tablet and control)	47

INTRODUCTION

Oral diseases affect a large number of people irrespective of gender, nationality, race or color. The consequences of tooth loss have been described in several literatures. And one such consequence which has been dealt with for decades is complete edentulism. Although edentulism is not a life threatening, morbid condition which precipitates mortality, it has an important and multifaceted impact on the individual. Few exemplifications being functional impairment, morphological changes, alterations in chewing efficiency and thereby nutritional status, oral perception, quality of life and underlying systemic implications.¹

The maintenance of the complete denture hygiene contributes in the overall health and systemic condition of the individual. Positive correlation has been documented between systemic diseases and detrimental oral conditions.¹

Published reports include the ones mentioning relationship between oral micro flora and bacterial endocarditis, aspiration pneumonia, chronic obstructive pulmonary disease, generalized respiratory tract infection etc.²

The micro surface topography of the denture or the surface characteristics of denture is conducive to plaque, biofilm, debris, stain and calculus accumulation. Failure in denture and oral hygiene maintenance is a major risk factor and paves way to denture stomatitis. Therefore, it is vital to maintain the denture hygiene thus preventing oral malodor, poor esthetics, biofilm accumulation which contribute to the onset and progression of oral disease.

DENTURE HYGIENE MEASURES COULD BROADLY BE CLASSIFIED INTO 3 GROUPS:³

1. Mechanical methods
2. Chemical methods
3. Irradiation method

➤ **MECHANICAL METHODS**

These include mechanical scrubbing using a tooth brush.

➤ **CHEMICAL METHODS:**

Chemical dentures could broadly be grouped into 5 categories based on their chemical composition.

a. Alkaline peroxidase

Is the most commonly used ingredient. These ingredients dissolve to form an aqueous alkaline solution. These compositions also include surfactants like detergent to reduce the surface tension along with agents which release oxygen eg. steradent

b. Alkaline hypochlorite

These generic compositions remove stains, debris, cause dissolution of mucinous films and auxiliary organic matter. It has been speculated that hypochlorite directly acts upon the organic niche and scaffold of the denture plaque- biofilm causing dissolution of polymer structure e.g. Dentural

c. Acids

They comprise of inorganic acids as their active ingredient; solutions include 3% to 5 % hydrochloric acid or combination of phosphoric and hydrochloric acids e.g. Denclean

d. Disinfectants

There have been documented reports of experimental disinfecting denture cleansers which are not commercially marketed. These reagents have previously been considered to treat and prevent oral fungal infections beneath removable dentures and appliances.

e. Enzymes

Another experimental approach has been taken towards the control of denture plaque with the help of enzymes eg. polident.

Microwave radiation has been used a simple and quick method to disinfect the dentures at 650 W for 3 cycles. The denture must be immersed in water during irradiation. However, this method reportedly causes surface deterioration which accelerates biofilm formation.

This paves way for further exploration into efficient and efficacious denture cleaning protocols.

An ideal denture cleanser must be easy to use, be bactericidal and fungicidal in nature, be able to remove organic and inorganic matter from denture surface, should have compatibility with denture base materials and should not alter the physical and mechanical properties of denture base material.

NEED FOR THE STUDY

The use of heat cure acrylic resin for denture fabrication is a common routine procedure; however, it has a micro-porous surface which provides a favorable platform for micro-organisms to organize “denture plaque”.

Mechanical cleaning with brushes and toothpaste, soap, or water is the most common routine for denture plaque control. However, the abrasive action of this method, caused by bristle stiffness, toothpaste particle size, and type of abrasive used, can increase the roughness of denture material, causing bacterial growth and biofilm accumulation.

Surface irregularities present in dentures can act as a microorganism reservoir. The adherence of the *Candida* species to cells and the fitting surface of acrylic resins is known to be the first phase of denture stomatitis.

Routine denture cleaning may increase the surface roughness of the material, increasing biofilm formation.

When this intimate relationship is interrupted; the tissue health improves and the most common method of controlling this interface is good denture hygiene, which can be achieved with the proper use of denture cleansers¹.

Denture cleansers can be broadly divided into mechanical, chemical and both; based on their mode of action².

For being effective, a denture cleanser must be capable of removing plaque from not only the polished surfaces of prosthesis, but more importantly from the unpolished tissue surfaces².

In geriatric patient, because of lack of manual dexterity due to age, chemical methods are more advisable³.

Most of commercial denture cleansing products are not widely promoted, so these are not easily available for general public and are quite expensive, if used regularly.

So, keeping in mind all these limitations, this study was designed to evaluate & compare the surface roughness of herbal denture cleansing tablet containing essential oils of tulsi & lemongrass, commercially available denture cleansing tablet and 0.12% chlorhexidine digluconate on heat cure polymethyl methacrylate resins.

HYPOTHESIS

NULL HYPOTHESIS

- There is no difference between surface roughness of herbal denture cleansing tablet containing essential oils of tulsi and lemongrass, commercially available denture cleansing tablet and 0.12% chlorhexidine digluconate on heat cure polymethyl methacrylate.

ALTERNATE HYPOTHESIS

- There is difference between surface roughness of herbal denture cleansing tablet containing essential oils of tulsi and lemongrass, commercially available denture cleansing tablet and 0.12% chlorhexidine di-gluconate on heat cure polymethyl methacrylate.

AIM AND OBJECTIVES

AIM OF THE STUDY:

To evaluate the surface roughness of herbal denture cleansing tablet containing essential oils of tulsi and lemongrass, commercially available denture cleansing tablet and 0.12% chlorhexidine digluconate on heat cure polymethyl methacrylate.

OBJECTIVES OF THE STUDY

1. To evaluate the surface roughness of herbal denture cleansing tablet containing essential oils of tulsi and lemongrass, commercially available denture cleansing tablet and 0.12% chlorhexidine digluconate on heat cure polymethyl methacrylate denture base resins.
2. To compare the surface roughness of herbal denture cleansing tablet containing essential oils of tulsi and lemongrass, commercially available denture cleansing tablet and 0.12% chlorhexidine digluconate on heat cure polymethyl methacrylate denture base resins.

REVIEW OF LITERATURE

- 1. Jørgensen and Budtz (1979):** Described various denture cleansing products and techniques. Proper hygiene of dentures is a vital method to maintain excellent hygiene in denture users. Hygiene of prosthesis is often poor because most commercial products are insufficient and ineffective for the cleanup of dental prostheses. For dentists and patients, unhygienic prostheses are known to damage both oral and patient's general health. The patient has a obligation to keep acceptable oral hygiene in the daily domestic care routine of the prosthesis. The Prosthodontist and dental health care provider is responsible for motivating and instructing the patient and for providing plaque control techniques and methods Future study should be conducted on the development of solution cleansers that are able to maintain plaque-free dentures affecting the color and surface luster of acrylic resin in the denture.⁴
- 2. Moore TC, Smith DE, Kenny GE (1984):** Observed that denture hygiene is a key factor in denture stomatitis treatment, which should be highlighted in patient instructions, they also conducted a two-section study to compare the effectiveness of 8 denture-cleansing methods for denture plaque removal and/or killing. The efficacy of cleaners on a pure yeast *Candida* culture was evaluated in Part I. The methods for denture cleaning have been clinically evaluated in Part II. The results of this study were the following: Miller's and Kleenite were the most efficient denture sanitizing agents.¹³
- 3. Charles J. Palenik, Chris H. Miller (1984):** Two ultrasonic instruments were compared with alkaline peroxide which was often used to remove the plaque from denture surfaces. During the experiment period, thirty dentures were

separated and microbially soiled. Efferdent, Branson and Clairol were similar in nature in regards to antimicrobial efficacy. The Bartlett Chi-Square test was used to conduct a statistical assessment of the data. The Clairol Sonic Scrub cleaner efficiently removed plaque after 15 minutes of usage.¹⁴

4. **S.S. Dills, A.M. Olshan, S.Goldner, C.Brogdon (1988):** The comparative analysis of the anti-microbial efficacy of an abrasive dental paste and the chemical-soak denture cleanser was conducted by two clinical studies which were done to test the efficacy of commercial denture cleansing products. This study verified the decreased sustainable microorganism's concentration retrieved from the in vitro denture bases. The reagents were an abrasive denture cleanser and a chemical soak product based on alkaline peroxide. In 2 experiments the above materials were used separately and later together with no interventional control, therefore to establish the amount of recoverable plaque bacteria from dentures, together with non-interventional approach were studied. In people with good healthy oral mucosa, plaque was retained for 48 or 72 hours and the patients did not clean their dentures for this duration. The findings of research following comparable double-blind cross-over studies were compatible with the fact that abrasive denture cleansers reduced microorganisms considerably than the ones which were soaked with the denture cleanser itself. Combining the brushing with the soaking did not considerably lower the number of recoverable microorganisms than the soak alone. These findings endorsed the necessity of using a denture cleanser along with manual cleaning by brushing for adequate denture hygiene.¹⁵

5. **Nikawa, Yamamoto, Hamada, Sadamori, Agrawal (1995)**, A straightforward criteria had been intended for measuring *Candida* activity using pH-change stomastat, in order to check for the effectiveness of eleven commercially available denture cleansers for *Candidal* biofilm. After 24- & 30-hours incubation ($r=.992$; $n= 988$, respectively; $P <.01$), the highest number of inoculated *Candidal* yeasts correlated with the pH of stomastat which endorsed the effectiveness of the method. Depending on the parts of the agents, their ability to reduce fungal biofilm activity varied. Overall, peroxide denture cleansers, disinfectants and an enzyme-purifiers were more effective than the other kinds of denture cleansers tested in this research.¹⁶

6. **Keng SB, Lim M (1996)**: The efficacy of a perborate-based denture cleanser had been researched by the authors. A coloring agent was used to reveal the plaque and a modified Quigley-Hein scale was used for further analysis. To determine the distribution of plaque, a photographic method was used. An evaluation of the pre-cleaned and the post-cleaned condition of the denture quantified the effectiveness of a Perborate soak-type cleaner. On tissue surfaces of the dentures, denture cleansers were more noticeable and recognizable than on flange, teeth, and palate regions. Thus, they concluded that the use of the cleaner alone of the soak type was not fully efficient in heavy plaque control.¹⁷

7. **Nikawa, Hamada, Yamashiro, Kumagai (1999)**The technique of assessment for denture cleansers was studied by the authors along with and some methods on assessment technique for plaque control. Over 20 papers were compared and the pros and cons of each technique assessing the efficiency of denture cleansers was established. The findings differed, particularly in In-vitro and In-Vivo tests, depending on the technique used to detect the effectiveness of denture cleansers.

It was also noted that chemical denture cleansers in clinical applications were not as efficient as the ones in In-Vitro test. The uncertainty about efficacy could have come about as a result of lack of standardized methodology and conflicting outcomes.¹⁸

8. Sheen SR, Harisson A (2000): had studied In-vivo, two methods that have successfully been used for the evaluation of the dental plaque: the visual scoring system and the digital imaging system. The validated and developing techniques were both able to identify important variations in the biofilm, both clinically appropriate and statistically significant. In one out of the two treatment groups 35 participants were randomized: (1) prosthesis soaked with a fresh cleanser or (2) dentures soaked in water. It took 14 days to receive therapy. Digital image analysis and visual scoring method were used to evaluate the plaque at day 2 and 14. The fresh cleanser showed a statistically important and significant 51% decrease of average visual plaque at day 2 ($P=.0002$) and 42% ($P=.0014$) at day 14 as compared with water. The result of the new cleanser was examined with ANOVA. Digital imaging showed similar outcomes. this study found that the fresh denture cleanser has demonstrated its efficiency in stopping denture plaque build-up. The digital image assessment produced comparable outcomes as the visual measurement technique.²³

9. Paranhos HF et al. (2000): The dental plaque / biofilm removal ability and the antimicrobial effect of the denture cleanser were analyzed by the authors. The effectiveness of denture cleansing paste for complete dentures was evaluated by determining colonies of the *Streptococcus mutans* group and *Candida albicans* group from 120 full-denture wearers with a healthy palatine mucosa in terms of denture plaque / biofilm removal and antimicrobial action against those

microorganisms. In order to assess the experimental product in terms of significant features, patients received a questionnaire. Patients preferred the paste extensively and it was found to be efficient in the biofilm removal and antimicrobial action. The most commonly isolated species were *Candida albicans*, *Candida tropicalis* and *Candida glabrata* species. The study found that wearers of removable dentures could maintain their prostheses and clean them by using a paste-like cleansing product regularly.²⁴

10. **Kulak-Ozkan, E. Kazzoglu, A. Arikan (2002):** Conducted a study to assess oral hygiene habits, cleanliness and presence of yeasts, and dentures of stomatitis in older patients in 70 wearers of complete denture. Subjects were assessed by, denture hygiene, yeast presence, cleanliness of dentures, frequency of brushing of denture and techniques of dental cleaning. Swabs were collected from the palate to recognize the yeast colonies and were confirmed after Mycological examination. No statistical relation between dental stomatitis and denture brushing frequency and techniques of dental cleaning were discovered. However, the connection between stomatitis, existence of yeasts and cleanliness was found to be statistically important and valid.²

11. **Aydan Kanli, Figen Demirel, Yasemin Sezgin (2005):** The oral hygiene habits, denture cleanliness and presence of yeasts in elderly individuals had been researched by the authors. Forty-two wearers of complete dentures were clinically and mycologically investigated. The subjects were assessed based on *Candida albicans* count, denture cleanliness, denture brushing frequency, denture cleaning techniques and duration of denture usage. In order to identify *Candida albicans*, Swabs were taken from the palate and mycologically investigated. The findings were analyzed using a chi-square test. Results of the study showed an

important correlation between oral candidiasis and denture cleanliness. The correlation between denture cleaning and candidiasis was statistically significant. Also, there was an important relationship between duration of denture usage and the existence of *Candida*. The correlation between dental cleanliness and dental age, cleaning method or cleaning frequency was significant. The Survey found that only 16.7% of elderly dentures were cleaned correctly. Older dentures were generally dirtier than newer ones. There, was a substantial link between bad hygiene and prevalence of candidiasis.²

12. Tania Baena, Victor Moreno, Fernando Franco (2005): conducted a study to investigate the occurrence and incidence of *Candida albicans*, *Staphylococcus aureus* and *Streptococcus mutans*. In order to assess their pH, saliva was gathered from 105 patients (62 female and 43 males). Sterile cotton swab was drawn for the microbiological evaluation of the sample of mucus membrane and denture bearing and tissue surface area. The identification by standard microbiological techniques of isolated micro-organisms was carried out. Results showed that the most common systemic diseases were diabetes and hypertension. 50 patients reported atrophic denture stomatitis and average pH was 5 in saliva. 86% of patients with atrophic denture stomatitis showed isolates of candida. Similar isolates of *aureus* (84% of patients) had been reported. Patients with complete denture prosthesis often exhibited colonies of *S. mutans*.²¹

13. Daniluk T et al (2006): studied the incidence of *Candida albicans* induced denture stomatitis in non-diabetic and diabetic patients. The tests were carried out in patients treated by two University Hospital clinical departments. 95 patients were tested. The samples of biofilms from dentures had been drawn from patients with complete dentures with mycological tests on the tongue dorsa and palatal

mucosal surfaces, while those without complete dentures were taken from tongue-and palatal swabs. Standard techniques were used to inoculate and incubate the fungi. The findings had shown *C albicans* In denture wearer patients with Diabetes Mellitus arthritis (p=.0207) and without asthma (p=.0376), *C. albicans* was statistically significantly more frequently occurring compared to those patient groups without dentures. Similar analysis had not shown statistically important variations in the incidence of *C albicans* in 25 patients with abdominal cancer and 15-without cancer. The research found that mycological results from this research did not suggest that *Candida albicans* or other genus of *Candida* have a significant impact upon oral colonization.²²

- 14. Paranhos Hde F et al (2007):** Assessed the distribution of biofilms over inner and exterior surfaces of upper complete dentures. This research revealed that smoking and continually used dentures encouraged oral candidal colonization. The study was also intended to assess the effect of maintenance and hygiene guidelines, instructions before and after domestic use of a disclosing reagent in the form of a solution. The sample consisted of 29 complete denture wearers whose prosthesis was assessed. A 1% neutral red solution was used to disclose surface biofilm and quantify the interior i.e., the tissue and intaglio polished surfaces. Information on oral hygiene was provided to the patients. A quantity of disclosing solution was provided for home uses during the second phase. Tissue surface, polished and flange areas' surfaces showed a similar amount of biofilm focused in the dorsal region, palatine rugae and distobuccal flange area. There's also a further decrease in the biofilm after use of the solution by subjects at home. The conclusion was that regardless of the type of method, bio-film deposits were similar over evaluated surfaces but those on the uneven surfaces

were larger and showed overall reduction in biofilm accumulation. Oral health instructions reduced the biofilm effectively, especially in relation to the home use of a disclosing agent.²⁴

- 15. H. F. O. Paranhos et al. (2007:)** quantified biofilm, using six potential cleansing procedures, on the inner tissue surface of upper complete dentures. The test was conducted for a time-limited trial of thirty-six edentulous subjects, and dentures were cleaned according to six methods: (i) water rinsing; (ii) soaking with a solution of alkaline peroxide (bonyplus); (iii) dentifrice brushing and Johnsons soft toothbrush; (iv) a combination of soaking to the brushing of teeth according to methods 2 and 3. During 21 days, each technique was used randomly. 1 percent neutral red solution was disclosed and quantified with digital photographs taken from the inner layer of denture bio film. The 6 techniques showed considerable distinctions between the percentage of coverage in biofilms (repeat ANOVA measures, $P < 0.0001$). The largest values were shown in Method 1, two were intermediate and other smallest outcomes. The 6 strategy was the most effective. Biofilm tended to accumulate mainly over certain denture base areas, but this pattern did not alter irrespective of the method used. Brushing alone was more efficient than the chemical technique used. It could thus be concluded that a mixture of methods produced the highest impact results.²⁵
- 16. Dilek Nalbant, Ayse Kalkanci (2008):** Studied the impact of Klorhex and Fittydent, which were utilized as cleansing agents against adhesion of *Candida* to the denture and palatine mucosa surfaces were investigated further, yeasts had been assessed for adherence to polymethyl methacrylate after these reagents are applied in vitro. group of 15 patients each washed their dentures with Klorhex or Fittydent. Cleansing their dentures with water was the Control group. Results

revealed that 62.2% of samples had *Candida species* ' colonies on their palatine mucosa which, after using these cleaning agents, had been lowered to 51.1%. The colonization rate on their teeth for the use of these cleaning agents was reduced by 82.2% to 68.8%. The average adherence value for the *Candida* strains separated from the acrylic bands was 75 cell per strip before the Klorhex and Fittydent application, 37.5 cell per strip application and 15 cell / strip after application. above mentioned findings showed that the colonization rate of *Candida species* was prevented by Klorhex and Fittydent.²⁶

- 17. Paranhos HF, Cla'udia H., Raphael F., Patricia C (2009):** Carried out the evaluation of the effects on acrylic resin samples by three denture hygiene techniques with various microbial biofilms. A microbial inoculum was contaminated with a collection of 37 colonies (CFU)/ml (sterile stainless steel). Following inoculation, samples were treated using: (1) the chemical: 5 minutes ' immersion in the solution for alkaline peroxide (Bonyplus tablets), (2) the mechanical: 20 seconds ' denture bubbling for removable protheses (Dentu Creme). On the Petri plate with a suitable medium of culture, reagents were applied for duration of 10 minutes. The samples were subsequently detached and the plate was incubated at 37 °C for 48 hours. There was no significant distinction in reducing CFU in chemical, mechanical and mixture techniques. *S. Mutans*, and *P. aeruginosa* (ATCC & field strain). The techniques of mechanical and combined were analogous and more efficient than the chemical method *E. Faecalis*, *C. Faecalis*, *C albicans* (ATCC), and *C. Glabrata*. The combined technique was superior to the chemical method for *E. Coli*. The three techniques showed distinct efficacy, depending on the type of microbial biofilms created

from PMMA resin samples and the mechanical process showed intermediate results.²⁷

18. Heitor Panzeri, Elza Helena, (2009): In a clinical study examined the physical characteristics of two experimental denture cleansers, their impact on dental biofilm removal and antimicrobial characteristics by two combinations were made of the experimental denture cleansers. First of all, 1% chloramine T (D1) was added, and secondly, 0.01% fluoro-surfactant (D2) was added. Density, rheology, consistency, pH, and other characteristics and abrasivity measurements were carried out. 60 denture wearers were allocated randomly to 3 groups and instructed to brush their dentures with a certain way with tooth brush: (1) Water (control); (2) D1 or (3) D2. For 21 days, every technique was used. A 1% neutral red solution was used as disclosing agent and quantified by digital photographs taken from the tissue surface. In order to quantify *Candida sp* microbiological evaluation was performed. One way ANOVA and Kruskal –Wallis Tukey HSD were used and data was assessed ($\alpha = 0.05$). Results showed that the biofilm coverage reduced in comparison to the control group of both denture cleansers. D1 was the most effective way of reducing *Streptococcus mutans*, while D2 demonstrated an intermediary result (ANOVA, $p < 0.040$). *Candida albicans* or non-candida species (Kruskal-Wallis, $p = 0.163$ and 0.746 , respectively) did not have any influence on therapy modality.²⁸

19. Mario Augusto Brondani, Firoozeh Samim and Hong Feng (2010): Study found that brushing the entire denture with experimental denture cleanser tested could be efficient for removing denture biofilm, with Cochrane Database of Systematic Reviews, Pubmed Central, Google Scholar, Ovid Medline (R), reviewed Microwave Therapy for Denture Cleaning. Every 167 manuscripts were

found and published and 28 accepted and discussed in English, with the purpose of identifying the advantages and disadvantages of using conventional microwave ovens to clean and disinfect complete dentures. For denture cleansing, no standardization was available. The optimal way to monitor fungal infection and denture stomatitis still seemed to be manual cleansing.⁶

20. Jose A et al (2010): Had studied the ability of 4 denture cleansers to decontaminate and sterilize biofilm covered areas efficiently. A total of 16 *Candida albicans* strains isolated from the ATCC 90028 denture stomatitis. Strains were grown in a 96-well and evaluated further. Scanning electron microscopy (SEM), which were used to examine treated biofilms, were then quantified for metabolic activity and biofilms. Results showed Dentural was the most effective cleaner for the dentures, which reduced biofilm in 20 minutes by over 90%. After 10 minutes of immersion, Steradent Active Plus was considerably more efficient than overnight ($p < 0,001$). All cleansers decreased metabolic activity by more than Eighty percent after overnight soaking, however, after only 15 minutes of immersion, Boots Smile demonstrated a significantly decreased metabolic activity ($p < 0.001$). This study found that denture cleansers had an efficient anti-Candidal effect. Candida biofilm activity was noted in terms of both disinfection and removal; however, the retaining ability of remaining biofilms was noted, which might have led to regeneration of biofilm on dentures. Therefore, alternate mechanical disruptive methods were needed to improve plaque and biofilm removal.²⁹

21. De Andrade IM et al (2011): Studied the antimicrobial effects of effervescent tablets and ultrasound on *Candida* species and *Streptococci mutans* from the biofilm of prosthesis. 77 complete denture wearers were allotted randomly to 4

groups: (a) water; (b) water-soluble tablets; (c) Ultrasonicating device; (d) ultrasonic and effervescent tablets. Three times a day, all groups brushed their dentures with a specific method. In baseline and after 21 days Denture biofilm has been collected. The specimens were collected by rubbing the dentures with brush and saline; by placating the detached microbial cells. There was no substantial distinction between the techniques. Compared to other techniques lower levels of biofilm accumulation were discovered for techniques B and D. The complete aerobic figures were smaller than A in Method B, whereas intermediate findings in C and D were present ($p= 0.011$). The effervescent tablets reduced *S mutans* substantially.³

- 22. David Felton et al (2011):** Evidence-based instructions for the care and maintenance of removable complete denture prostheses were drawn up by the authors. In 2009, a task force was established by the American College of Prosthodontists (ACP) to create evidence-based guidelines for treatment and maintenance of the complete dentures. The taskforce included the Academy of General Dentistry ACP members, the Council of Scientific Affairs of the United States Dental Association (ADA), and the National Association of Dental Laboratories and GlaxoSmithKline Consumer Healthcare representatives. This review method included an evaluation of more than 300 abstracts and choice of more than 100 items fulfilling the criteria for inclusion. The task force assessed literature synopses and formulated 15 recommendations for denture care and maintenance based on evidence. These guidelines have been evaluated by the clinical specialists of participating agencies and released in The Journal of the American Dental Association, February 2011.⁵

23. Chethan, S.Azhagarasan (2011): Contrasted and Measured the effectiveness of denture cleansing agents on complete dentures in healthy individuals using the microbial quantification technique of four chemically distinct kinds of commercially accessible immersion denture cleansers. Ten healthy individuals between 60 and 70 years of age had been chosen. The chemical cleansers were split into four groups: Group I–0.02% solution of the sodium hypochlorite; Group II–phosphate of Trisodium; Group III–perborate of Sodium; and Group IV–gluconate of Chlorhexidine. The dorsal half of the tissue surface of the denture was swabbed and cultivated on blood agar by the use of the sterile cotton swabs at four different locations. Calculated, tabulated and analyzed statistically for the net % reduction in the colony forming units prior to and after treatment of each of the above mentioned test agents at cultures from above 4 sites. The results showed that a statistically significant difference in means was seen in all groups. For Groups I, II, III & IV, the percentage decrease in the number of streptococcus species was 28%, 16%, 10% and 9%, respectively. The research has found that cleansing agents, the Sodium hypochlorite (0.02%), Trisodium phosphate, Sodium perborate and Chlorhexidine gluconate, were found to be efficient in the following order (0.2%). The usage of denture cleansers reduced the quantity of plaque that is subsequently reduced significantly. The significant reduction in the number of micro-organisms found in this research indicated that the use of chemical cleaners is an appropriate technique for the removal and treatment of candidiasis in the oral cavity.⁸

24. Byadarahally Raju and Shashanka Rajappa (2011): Studied different available techniques for *Candida* isolation in the oral cavity. The diagnosis and management of oral candidiasis was influenced by such methods. Candida is

becoming more and more important, in part because HIV is developing and immunosuppressive chemotherapy is being more widely used. The importance of non-candida species in oral candidiasis was more widely recognized along with *Candida albicans*. They identified the infecting pathogenic strains of *Candida*, which varied considerably in the capacity of both infective and antifungal susceptibility, because the isolates of *Candida* species varied considerably. Thus, this review offered an overview of the reliable techniques of isolating *Candida* and identifying oral cavity isolates among them.³⁰

- 25. Mithilesh M, Ashok Pakhan, Ram U. and Shyam L(2012):** Studied biofilm formed on denture resins. Comparing and evaluating the impacts of *Candida* removal from thermal polymerized acrylic resins of three most frequently available variants of commercial dental cleansers. The metal mould was designed to receive wax plates with uniform dimensions used to produce acrylic resin heat cured treatment plates. A 15 mm and 1.5 mm thick, square-shape window was fitted in a metal mould to simulate the denture base's thickness. This mould was used to prepare all samples for this research. On these acrylic resin panels. Biofilm sample removal was tested using a photo-colorimeter. The most significant activity in *Candida* removal relative to D1, D3 and control solution has been shown in the denture cleaner D2. Prothesis cleaners D2, when contrasted with dental cleaners D1, D3, and control solution, demonstrated enhanced yeast lytic capacity. More time precipitated a certain impact on the denture cleanser's yeast lytic capacity. The impact of cleaning agents on the colonized yeast biofilm removal was assessed for clinical implications, in particular with the fungal biofilm from acrylic resins. Compared to D1 and D3 they all fell under the same chemical group of alkaline peroxide, cleaners D2

showed superior performance. The increased efficacy may have been caused by existence of sodium lauryl sulphate in the D2 formula.³³

26. Renu Yadav, Vikender Yadav, Shushant Garg, Sanjeev Mittal, Rahul Garg

(2013): Studied the efficacy of different denture cleansing techniques, i.e., mechanical, chemical, and combination techniques for removing plaque and biofilm from the tissue surface of denture. A total of 50 maxilla dentures were randomly washed with, toothpaste, vinegar, denture cleansing tablet and then brushed with toothbrush. During the experiment, a two-tone plaque revealing agent was used to reveal accumulated plaque on the tissue surface for 48 hours, prior and after the denture was cleaned and plaque was scored according to the plaque index. For all treatment methods it was statistically significant that the pre-and post-cleansing plaque score had changed ($P < 0,001$). The plaque removal efficiency with the combination technique which used mechanical and chemical methods has been shown to be significantly higher. As a denture cleanser, vinegar has been shown to be less effective. The findings of this research suggest combining brushing and alkaline peroxides to guarantee effective purification of dentures.³⁴

27. Newton Sesma, Alessandra Lima Rocha (2014):

The hypothesis that disinfection with a microwave killed micro-organisms but did not purge dead bacteria from denture surface has already been investigated by the authors. The effective technique of dental prosthesis disinfection was used as microwave irradiation. Although current techniques remove denture microorganisms, a recurrence in denture stomatitis is still seen among denture wearers. The biofilm isolated in the dentures of 10 patients with denture stomatitis was examined. Additionally, the effects of microwave irradiation combined with brushing on the

denture and the combination of denture cleanser with microwave irradiation and brushing intended towards the removal of denture plaque accumulating microorganisms were investigated. Microbiological data was analyzed statistically by nonparametric analysis (Friedman/Wilcoxon, $\alpha=0.05$). MW+B and MW+DC+B were effective in reducing the presence of microorganisms (99.2 percent and 99.5 percent respectively), but without significant difference amongst them. ($p=0.553$). However, it was seen that the absolute and complete removal of microbes from denture surface was only possible when all recommended regimens were combined (MW+DC+B). Microwave irradiation along with soaking in denture cleanser and brushing efficiently disinfected the dentures and eradicated the denture biofilm sufficiently.³⁵

- 28. Silvia Carneiro, Indira Moraes (2015):** Conducted a study to investigate if daily soaking in denture cleansing reagent reduced microbial counts on Removable partial prosthesis framework (RPD) biofilm. 25 RPD wearing volunteers were selected and were instructed to complement the cleaning of their dentures by immersing them in an enzyme-based peroxide-based denture cleanser (Polident® 3 minute) one time a day for 3 min for a duration of 15 days. The biofilm was sampled from RPD surfaces with a swab immediately before and after the experimental period. The samples were put in sterile saline solution, ultra-sonicated at 7 W and then inoculated on specific culture media to quantify total microorganisms, total *Streptococci* and *Candida spp.* counts. Data from both collections were compared by paired t-test ($\alpha=0.05$). It was observed that a significant reduction was seen in total microorganisms' counts in RPD biofilm after denture cleanser use ($p=0.007$). This reduction was also observed for total *Streptococci* ($p=0.0428$), but no difference was observed on *Candida spp.* counts.

It was concluded that regular daily use of denture cleanser enhanced denture hygiene by lessening the total microorganisms and total *Streptococci* from RPD surface but did not have an effect on *Candida* population³⁶

- 29. Prasanna Kadakol, Nadiger K.R. (2015)** Compared and evaluated the efficacy of four commercially available denture cleaners on recently fabricated dentures in healthy patients. Sod.hypochlorite (0.02 percent), Fittydent tablets, Clinsodent powder and Chlorohexidine gluconate. (0.2 percent) on recently fabricated maxillary complete dentures of healthy patients. Total of 40 samples, were divided in to four groups of (T1 to T4) 10 patients each. After initial plaque scoring, the dentures were put in a beaker containing denture cleaner and left for 8 hours. Then the dentures were re-stained with Alpha-plaque disclosing solution and the resultant plaque score recorded by Modified Quigley Hein scale. The total plaque scores of before and after denture cleansers were noted and the difference of scores were calculated and tabulated. The results were statistically analyzed to compare efficiency of each denture cleansers. Reduction in plaque scores after using denture cleansers were analyzed by Wilcoxon's paired test. Reduction in between the groups were compared by one way ANOVA followed by Duncan's multiple range tests. For all the group's results were statistically significant, and the percentage reduction scores of Groups T1, T2, T3 and T4 was found to be 50 percent, 40 percent, 36 percent, and 26 percent respectively. Cleansing agents were found to be effective in the following given order 0.02 percent sodium hypochlorite followed by Fittydent tablets, Clinsodent powder and 0.2 percent Chlorhexidine gluconate.³⁷

30. Sílvia Carneiro, Antonio Pedro, da Silva, Jaime Cury, Altair Cury (2016)

Conducted a study to study the effect of daily exposure to a denture cleanser on a multiple species' biofilm. For this, Multiple species biofilm (five Bacteria and *Candida albicans*) were developed for 64.5 hours on PMMA resin specimens and randomly assigned into control and experimental groups. In the experimental group, complete dentures were soaked in denture cleanser for 3 min per day for 7 consecutive days. In control group, the biofilms were allowed to develop with no interventional treatment. The numbers of microorganisms and polysaccharide levels were evaluated and biological films from both groups were calculated after 1, 4 and 7 days. Scanning electron microscopy (SEM) was conducted and analysis was carried out of confocal microscopy (CLSM). Results showed that in the experimental group the total number of micro-organisms and bacteria populations were lower than in the control group over all evaluated periods. After 7 days, polysaccharide levels in the experimental group were considerably greater. This study found that daily biofilm exposure to a denture cleanser reduced the total amount of microorganisms.³⁸

31. Bakthavatchalam Pallavan, Venkatapathy Ramesh (2017): Conducted the study on occurrence and severity of candidal colonization in patients with diabetes and normal persons by cytologic method. DM is a commonly seen endocrine disorder which lowers host immunity. These microorganisms are opportunistic in healthy people but even in the lack of any clinical oral candidiasis, they develop serious colonization in diabetic patients. This kind of subclinical colonization may render it more susceptible to further blood dissemination by deeper mucosal colonization. In order to establish oral candida colonization using oral exfoliative cytological techniques, 30 cases of diabetes

patients and 30 cases of normal, healthy people were examined. The Chi-square test was used to conduct statistical assessment. The results showed that the rise of *Candida* colonization in diabetic patients was statistically important as compared to ordinary patients.³⁹

32. Karin Hermans Neppelenbroek (2018): In the conclusion of their research, published an article in which highlighted the significance of the daily removal of biofilms for oral and systemic disease prevention. The research found that the oral exfoliating cytological method is a simple and efficient chair side method to assess oral candida colonization. The adoption of routine, mechanical, oral hygiene procedures in combination with immersion in denture cleaners is crucial for oral health and denture biofilms are to be carefully removed every day. This was the key to minimizing the danger of opportunistic infections, contributing to excellent oral and systemic health and maintaining odor-free dentures in an esthetically pleasing way. The clinician should advise patients on the importance of the removal of the denture biofilm for oral and general health maintenance and instruct the wearer of the denture to use denture cleaners correctly⁴⁰

33. J Duyck et al (2019): In a cross-over randomized trial test in Thirteen older adult subjects, 4 conditions were considered with two distinct processes of mechanical cleansing and 2 overnight storage conditions. (i) brushing and immersion in water without a denture cleaning tablet, (ii) brushing and soaking in water with a cleansing tablet, (iii) ultrasonic cleaning and immersion in water without a cleansing tablet, and (iv) ultrasonic cleaning and soaking in water with a cleansing tablet. A 5 successive day, preceded by 2-day wash-out period, was conducted. Samples of biofilm were drawn at the end of every test session. A polymerase reaction (PCR) method was used to establish the identity of the

species and estimate the total and individual level of selected oral microorganism (n= 20) and *Candida albicans*. The biofilm coverage of denture was scored using an analog plaque score. For comparing test conditions, paired t-tests and Wilcoxon tests were used. There was no statistically significant difference in the complete bacterial concentration between the two mechanical cleaning techniques.⁴¹

- 34. Alyson S., Roshan Varghese, Marlynn Bosma, Nicola Kitson, and David J (2019):** conducted a study to gauge the student dental professionals ' attitudes and suggestions to denture cleaning and cleaning schemes. Questionnaire was used to evaluate the variety of suggestions for denture cleaning. In developed countries, denture cleansing tablets were most frequently suggested, while in developing countries toothpaste was the most common aid. The study found that DHCP recommendations and practices for the denture wearer are varied without agreement on best techniques for denture cleaning. This indicated that there is not one clear, systematic evidence to support recommendations on denture hygiene maintenance.⁴²
- 35. Andreas k., Sandra S., Robert L., Alyson S., David J (2020):** Conducted a study to identify compatibility of dentures with typical cleaning agents and their anti-microbial effects. The treatments assessed were based upon research of dental professionals and patient practices, including toothpaste, mouthwash, isopropyl alcohol (IPA), bleach, soap and vinegar. Scanning electron microscopy and profilometry after laboratory regimes have been assessed to simulate the material integrity of dentures, including polymethacrylate and metals. The two years of typical use had been simulated. Treatments for a variety of oral microorganisms with typical treatment regimens were assessed in a microbial kill

time trial. Results demonstrated that Cleanser tablets worked against all organisms at 5 minutes time interval. Effectiveness was observed and studied for use of vinegar, soap, salt and sodium bicarbonate. The study found that a nice combination of antimicrobial effectiveness and sensible material compatibility have been obtained from denture cleanser tablets.⁴³

MATERIALS AND METHODOLOGY

SOURCE OF DATA/ LABORATORY DETAILS:

This study was conducted in-

- KLE Academy of Higher Education and Research, KLE V. K. Institute of Dental Sciences, Belagavi
- Department of Prosthodontics and Crown & Bridge – (For fabrication of polymethyl methacrylate blocks)
- Department of Pharmaceutics, KLE College of Pharmacy, Belagavi – (For fabrication of herbal denture cleansing tablet)
- KLS Gogte Institute of Technology, Belagavi – (For evaluation of surface roughness)

PERMISSIONS TAKEN:

1. Ethical committee approval
2. KLE Academy of Higher Education (KLE University's)
 - The Department of Prosthodontics and Crown and Bridge, KAHER's KLE V. K. Institute of Dental Sciences, Belagavi KLE college of Pharmacy, Belagavi.
 - The Department of Microbiology, Jawaharlal Nehru Medical College, Belagavi.

SAMPLE SIZE ESTIMATION:

- Sample size was obtained using following formula:

$$n = \frac{2S^2(Z_{1-\alpha/2} + Z_{1-\beta})^2}{d^2}$$

= 21 samples in each group

Where, S = standard deviation = 0.05

$Z_{1-\alpha/2}$ = α -error at 5% = 1.960

$Z_{1-\beta}$ = power of test at 95% power = 1.682

d = mean difference = 0.058

- The study was divided into 4 groups of 21 samples in each

INCLUSION CRITERIA:

- Polymethyl Methacrylate Heat cure acrylic specimens with identical size and shape in the dimensions 4 cm x 1 cm x 0.7 cm (ADA specification no. 12).
- Specimens free of any voids.
- Specimens with Ra (surface roughness) value less than 0.30 μm was included in the study.

EXCLUSION CRITERIA:

- Specimens with surface defects and deformities.
- Specimens with inaccurate dimensions.
- Specimens with Ra value (surface roughness) more than 0.30 μm was excluded.

STUDY DESIGN:

An In-Vitro Comparative Study.

MATERIALS & ARMAMENTARIUM USED IN THE STUDY:

Material	Description
0.12% Chlorhexidine gluconate	Listerine
Addition silicone putty consistency impression material	-
Modelling wax	-
Heat cure polymethyl methacrylate denture base resin	DPI Company
Beaker	-
Distilled water	-
Phytopharmaceutical denture cleansing tablet containing Tulsi and Lemongrass	-
Commercially available denture cleansing tablet	Fitty Dent

METHODOLOGY:

Fabrication of heat cure polymethyl methacrylate resin blocks:

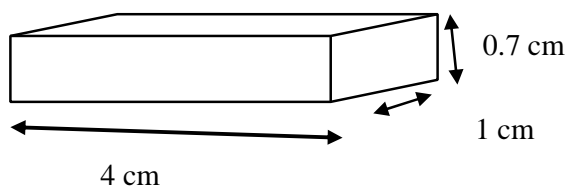


Fig 1: Fabrication of acrylic blocks

Polymethyl methacrylate heat cure acrylic resin blocks in the dimensions of 4 cm x 1 cm x 0.7 cm was fabricated as per ADA specification No. 12.

Modelling wax block in the dimension of 4 cm x 1 cm x 0.7 cm was made.

To standardize the modelling wax block, putty index was taken.

After fabrication of the wax blocks, investing of the wax blocks was done followed by dewaxing & packing of the mould. Acrylisation of the acrylic blocks was done as per the specification provided by the manufacturer. The blocks after fabrication was finished and polished as per manufacturer's recommendation.

After finishing & polishing of heat cure acrylic blocks, surface roughness of the acrylic blocks was evaluated with **PROFILOMETER** with accuracy of ± 0.01 μm .

Specimens was kept perpendicular to the profilometer. Reading was measured by passing the stylus over the length of specimens. Surface roughness of each block was calculated for 3 readings consecutively. Average of each of them, gave the value of surface roughness

Specimens with Ra value (surface roughness) more than $0.30 \mu\text{m}$ was excluded.

FABRICATION OF HERBAL DENTURE CLEANSING TABLET

Herbal denture cleansing tablet (Phytopharmaceutical denture cleansing tablet) as per specifications applied in Indian patent application no: 201941043681 A

It contains essential oils of tulsi and lemongrass.

After evaluation of surface roughness of heat cure polymethyl methacrylate blocks, it was cleansed using:

Group 1: Phytopharmaceutical denture cleansing tablet: Dissolving the herbal denture cleansing tablet in a beaker containing 200 ml of water & inserting the specimen for 30 mins (as per manufacturer's recommendation).

Group 2: Fittydent commercially available denture cleansing tablet: Dissolving the commercially available denture cleansing tablet in 200 ml of water & inserting the specimen for 30 mins (as per manufacturer's recommendation).

Group 3: 0.12% of chlorhexidine digluconate: Immersing the specimen in 200 ml of 0.12% chlorhexidine digluconate for 15 mins (as per manufacturer's recommendation).

Group 4: Water: Inserting the specimen in 200 ml of water (Control)

ASSESSMENT OF SURFACE ROUGHNESS:

After successful cleansing of heat cure polymethyl methacrylate blocks using different cleansing methods, surface roughness was evaluated using a Profilometer with an accuracy of $\pm 0.01 \mu\text{m}$. Specimens was kept perpendicular to the profilometer. Reading was measured by passing the stylus over the length of specimens. Surface roughness of each block was calculated for 3 readings consecutively. Average of each of them, gave the value of surface roughness.

The difference in the values of surface roughness before and after cleansing formed the results for the study. Results were subjected to statistical analysis.



Fig. 2 Heat cure acrylic resin (DPI, Mumbai)

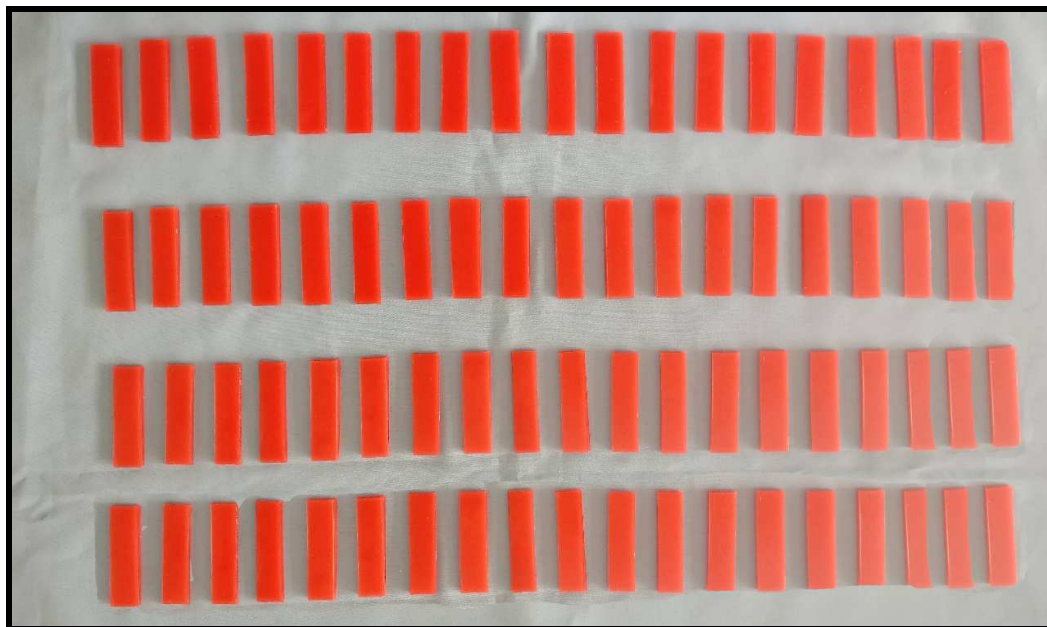


Fig. 3 Preparation of wax blocks for surface roughness evaluation



Fig. 4 Investment of wax blocks to obtain acrylic blocks

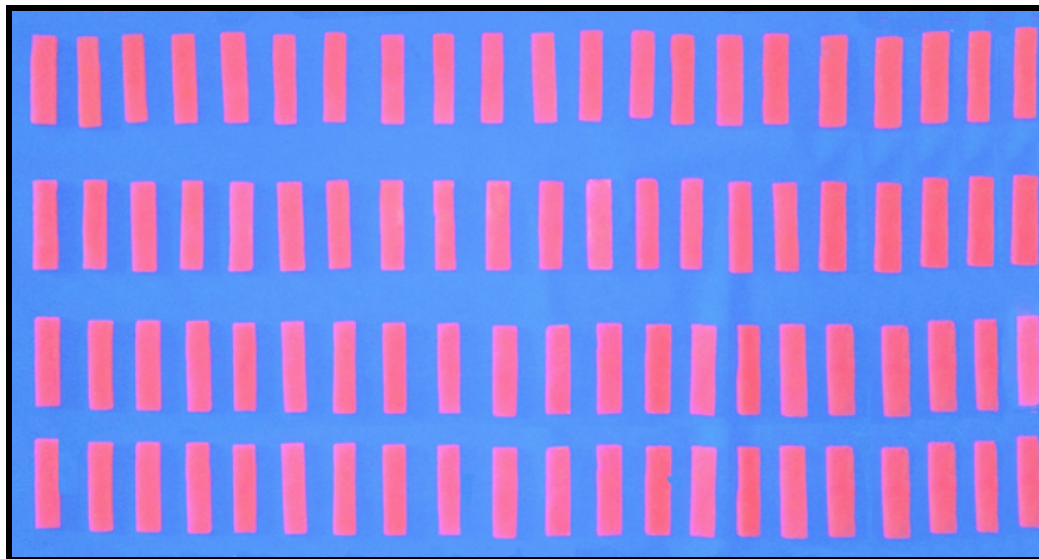


Fig. 5 Acrylic blocks for surface roughness evaluation

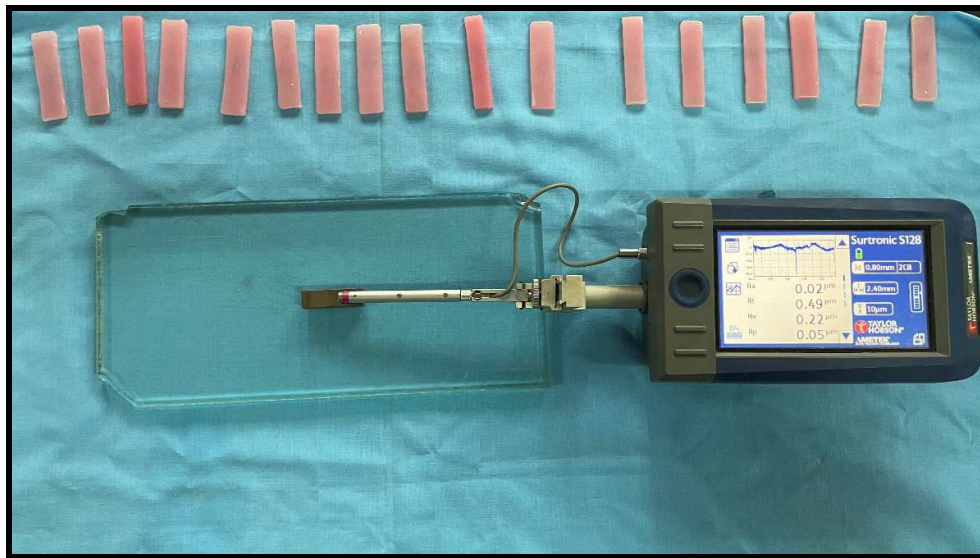


Fig. 6 Profilometer for surface roughness evaluation

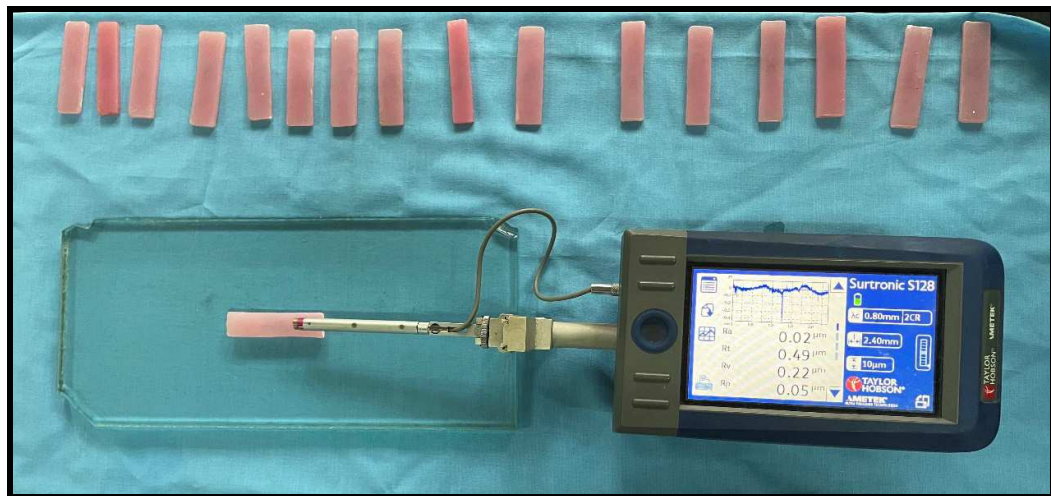
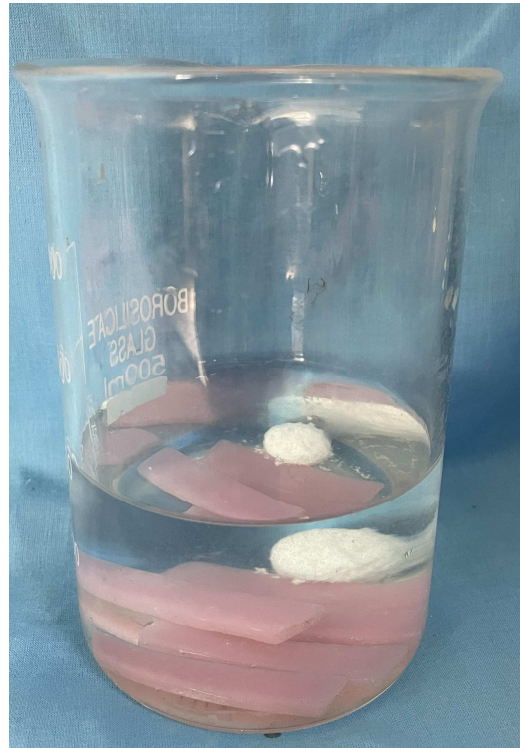


Fig. 7 Surface roughness evaluation prior to disinfection.

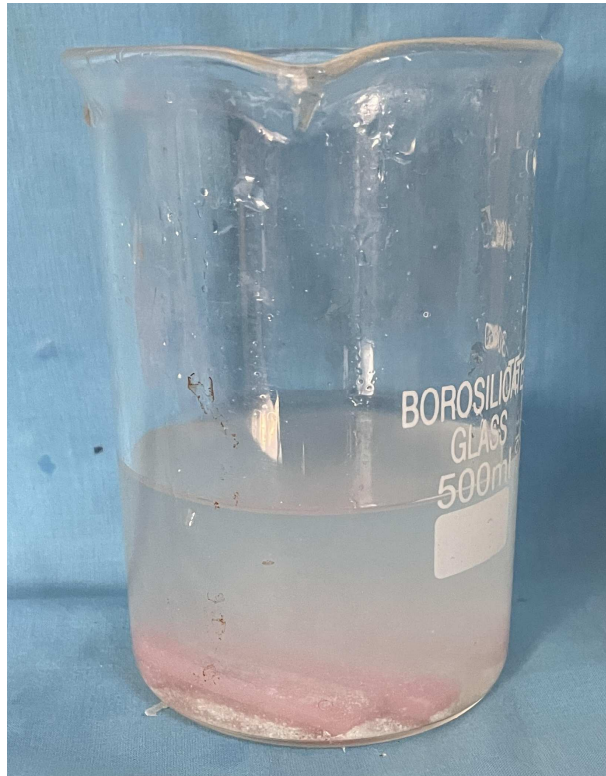
Fig. 8 Disinfection of the acrylic blocks carried out.



A: Disinfection of acrylic blocks using phytopharmaceutical denture cleansing tablet



B: Group 2: Disinfection using Fittydent denture cleansing tablet



C: Group 3: Disinfection using 0.2% chlorhexidine digluconate



D. Control group

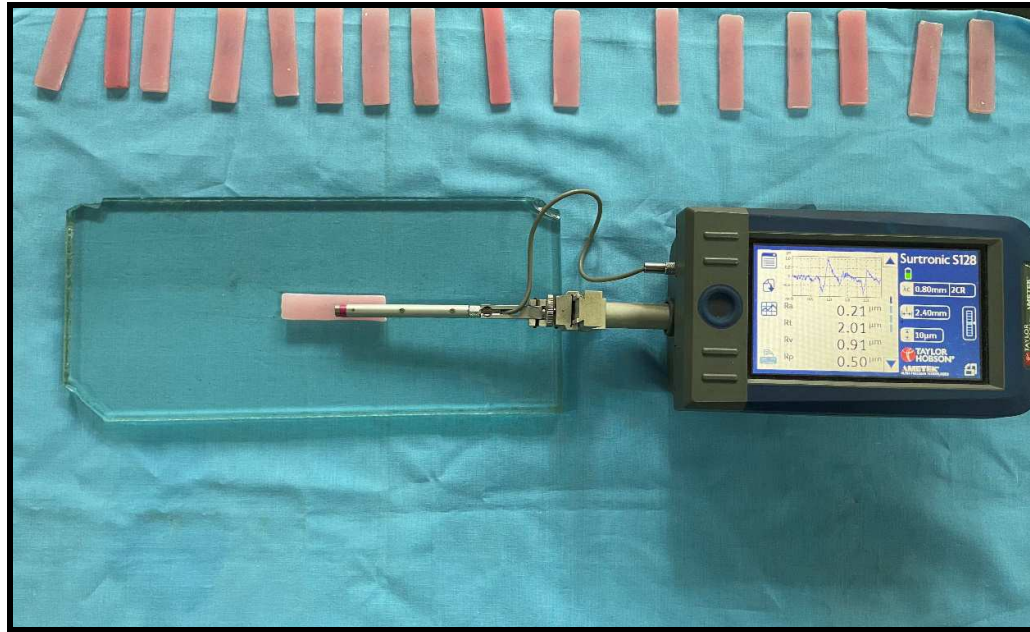


Fig. 9 Surface roughness evaluation after disinfection.

RESULTS

The values of surface roughness after treating the acrylic blocks with 4 different group, were subjected to statistical analysis to draw a conclusion from experimental data.

Comparison of four groups (Chlorhexidine, Fittydent tablet, Phytopharmaceutical cleansing tablet and control) with respect to pre and post treatment surface roughness (um) was analyzed by one way ANOVA and the results were statistically significant within the groups.

Table 1: Comparison of four groups (Chlorhexidine, Fittydent tablet, Phytopharmaceutical cleansing tablet and control) with respect to pre and post treatment surface roughness (um) by one way ANOVA.

Times	Sources of variation	Sum of squares	DF	Mean square	F-value	p-value
Pre treatment	Between groups	0.0070	3	0.0020	1.3410	0.2670
	Within groups	0.1330	80	0.0020		
	Total	0.1400	83			
Post treatment	Between groups	0.4260	3	0.1420	62.1950	0.0001*
	Within groups	0.1830	80	0.0020		
	Total	0.6080	83			
Difference	Between groups	0.3800	3	0.1270	90.9910	0.0001*
	Within groups	0.1110	80	0.0010		
	Total	0.4910	83			

Pair wise comparison of four groups (Chlorhexidine, Fittydent tablet, Phytopharmaceutical cleansing tablet and control) with respect to pre and post treatment Surface roughness (um) was done by Tukeys multiple posthoc procedures and the results were statistically significant among the groups. In the post treatment group statistically significant results were found when 2 groups were compared. Fittydent tablets lead to more surface roughness followed by chlorhexidine group. Least surface roughness was seen with Phytopharmaceutical denture cleansing tablet.

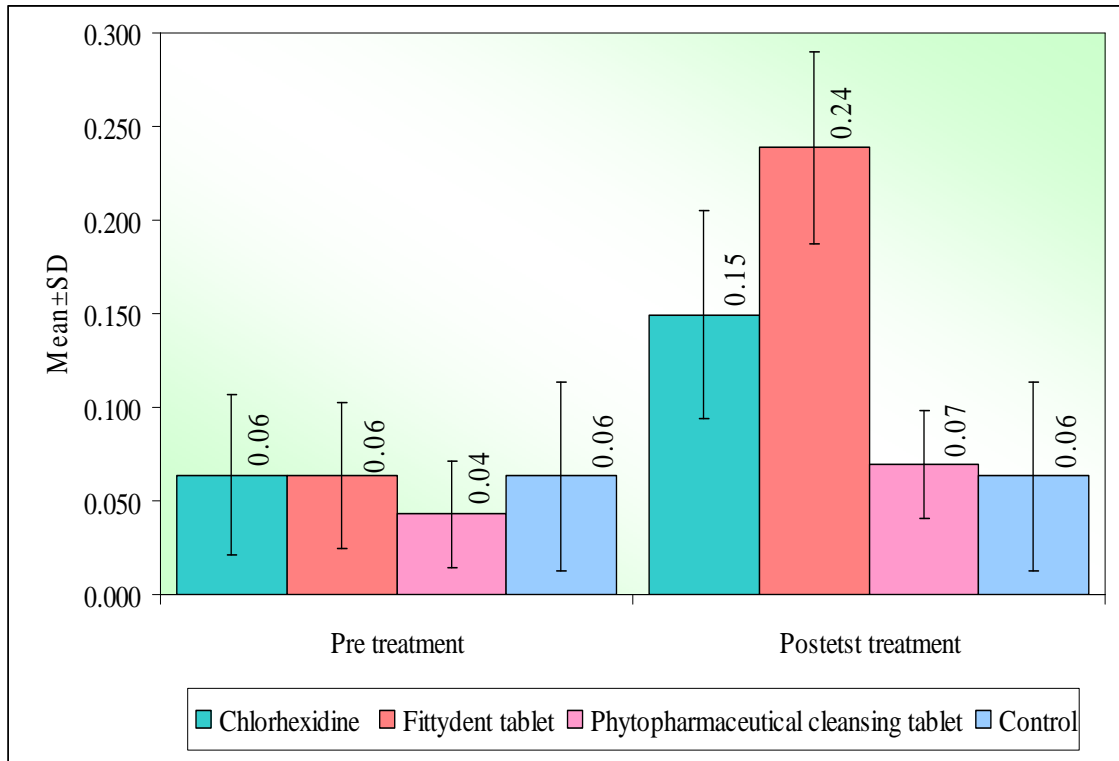
Table 2: Pair wise comparison of four groups (Chlorhexidine, Fittydent tablet, Phytopharmaceutical cleansing tablet and control) with respect to pre and post treatment Surface roughness (um) by Tukeys multiple posthoc procedures

Times	Pair wise comparison	Mean Difference	SE	P-value
Pre treatment	Chlorhexidine vs Fittydent tablet	0.0005	0.0126	1.0000
	Chlorhexidine vs Phytopharmaceutical cleansing tablet	0.0210	0.0126	0.3500
	Chlorhexidine vs control	0.0005	0.0126	1.0000
	Fittydent tablet vs Phytopharmaceutical cleansing tablet	0.0205	0.0126	0.3710
	Fittydent tablet vs control	0.0000	0.0126	1.0000
	Phytopharmaceutical cleansing tablet vs control	-0.0205	0.0126	0.3710
Post treatment	Chlorhexidine vs Fittydent tablet	-0.0891	0.0147	0.0001*
	Chlorhexidine vs Phytopharmaceutical cleansing tablet	0.0800	0.0147	0.0001*

	Chlorhexidine vs control	0.0862	0.0147	0.0001*
	Fittydent tablet vs Phytopharmaceutical cleansing tablet	0.1691	0.0147	0.0001*
	Fittydent tablet vs control	0.1752	0.0147	0.0001*
	Phytopharmaceutical cleansing tablet vs control	0.0062	0.0147	0.9750
Difference	Chlorhexidine vs Fittydent tablet	-0.0895	0.0115	0.0001*
	Chlorhexidine vs Phytopharmaceutical cleansing tablet	0.0591	0.0115	0.0001*
	Chlorhexidine vs control	0.0857	0.0115	0.0001*
	Fittydent tablet vs Phytopharmaceutical cleansing tablet	0.1486	0.0115	0.0001*
	Fittydent tablet vs control	0.1752	0.0115	0.0001*
	Phytopharmaceutical cleansing tablet vs control	0.0267	0.0115	0.1030

*p<0.05

Graph 1: Comparison of four groups (Chlorhexidine, Fittydent tablet, Phytopharmaceutical cleansing tablet and control) with respect to pre and post treatment surface roughness (um)



Graph 2: Comparison of four groups (Chlorhexidine, Fittydent tablet, Phytopharmaceutical cleansing tablet and control) with respect to pre and post treatment surface roughness (um)

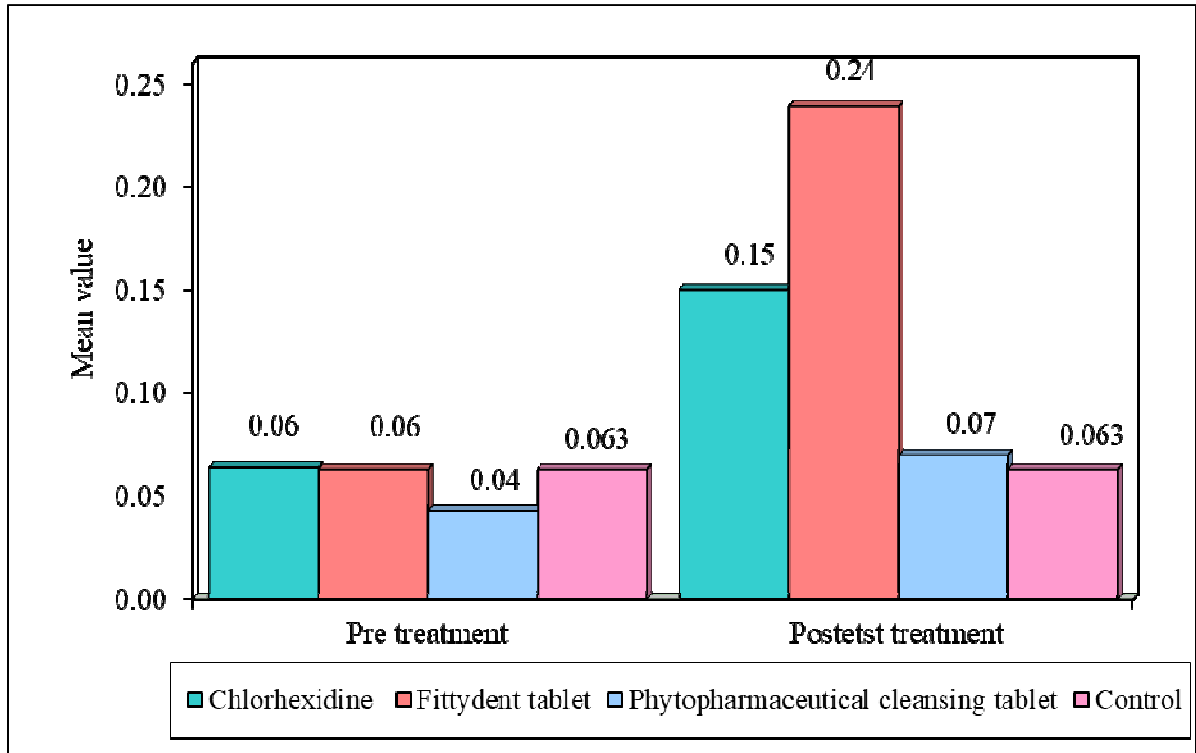
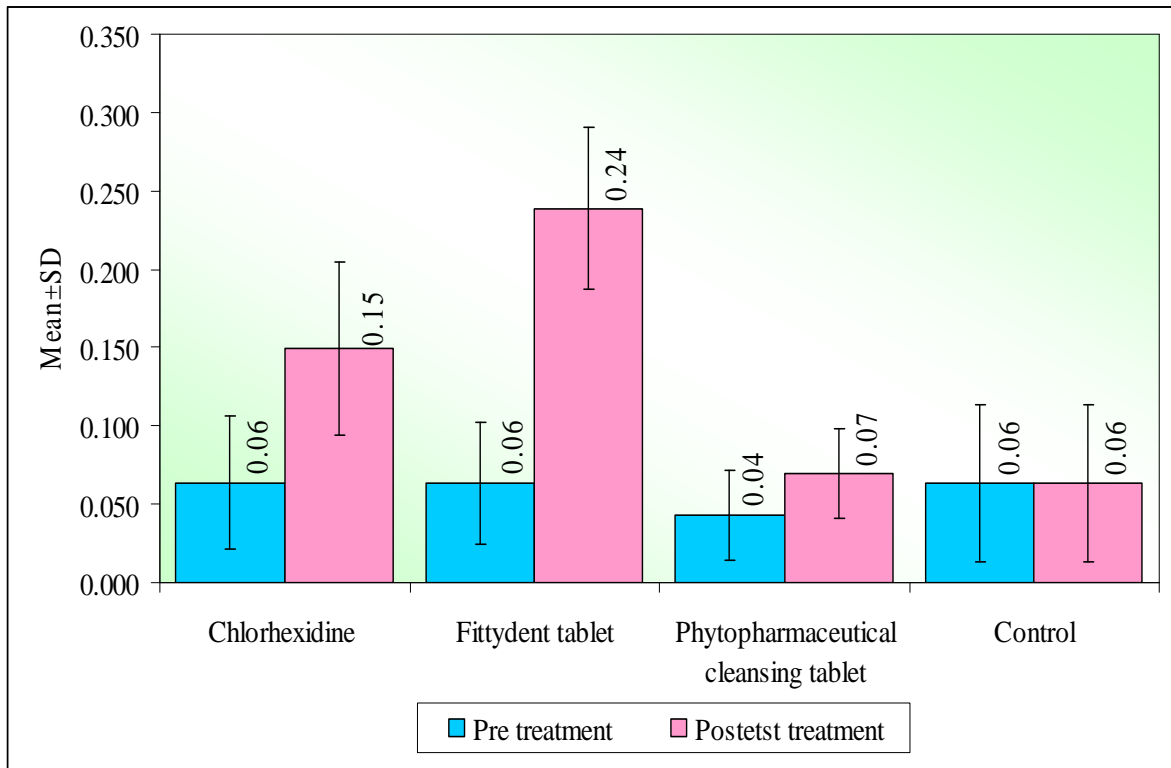


Table 3: Comparison of pre and post treatment surface roughness (um) in four groups (Chlorhexidine, Fittydent tablet, Phytopharmaceutical cleansing tablet and control) by dependent t test

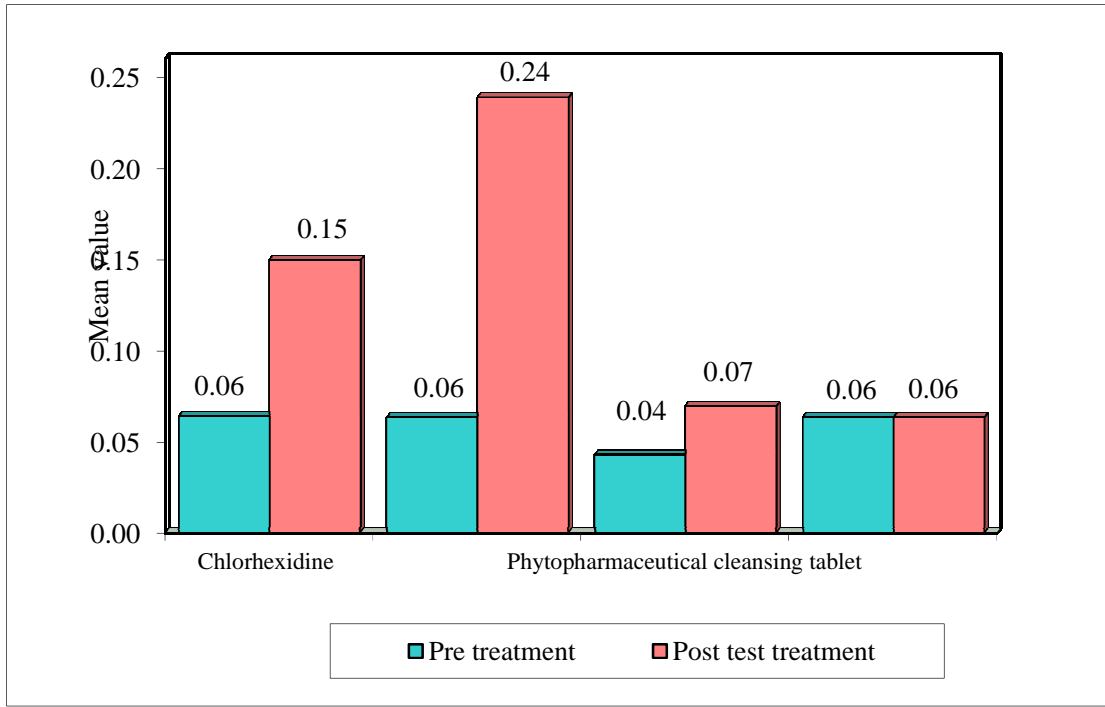
Groups	Times	Mean	SD	Mean Diff.	SD Diff.	% of change	t-value	p-value
Chlorhexidine	Pre treatment	0.06	0.04					
	Post treatment	0.15	0.06	-0.09	0.05	-134.33	-8.1101	0.0001*
Fittydent tablet	Pre treatment	0.06	0.04					
	Post treatment	0.24	0.05	-0.18	0.06	-276.69	-14.2490	0.0001*
Phytopharmaceutical cleansing tablet	Pre treatment	0.04	0.03					
	Post treatment	0.07	0.03	-0.03	0.01	-62.22	-18.5638	0.0001*
Control	Pre treatment	0.06	0.05					
	Post treatment	0.06	0.05	-	-	-	0.0000	1.0000

*p<0.05

Graph 3: Comparison of pre and post treatment surface roughness (um) in four groups (Chlorhexidine, Fittydent tablet, Phytopharmaceutical cleansing tablet and control)



Graph 4: Comparison of pre and post treatment surface roughness (um) in four groups (Chlorhexidine, Fittydent tablet, Phytopharmaceutical cleansing tablet and control)



DISCUSSION

Proper hygiene of the removable complete denture prosthesis is essential in preserving the health of the patient as well as the healthy condition of oral mucosa. Dentures may contain bacterial and mycological micro-organisms as *Streptococcus*, *Candida spp.* Brushing with abrasive paste was the first and the second most commonly used technique for denture cleaning was chemical soak.^{44,48}

To maintain proper hygiene of their dentures there are various methods available and patients are instructed on the different methods used to clean the dentures; But these can increase the surface roughness associated of the dentures.^{47,51}

In the current research, taking this into account in the comparison was done between different denture cleaning methods and surface roughness evaluation between 3 different groups such as Phytopharmaceutical denture cleansing tablet, commercially available denture cleansing tablet (Fittydent) and chlorhexidine digluconate. The Phytopharmaceutical Denture Tablet was Formulated and Prepared for the purpose of this study and Tulsi and Lemongrass were incorporated as the plant extracts for their established antifungal and antimicrobial properties in previous studies. Also these plants are Indigenous and locally abundantly available and thereby acceptable for clinical use and have an added economic advantage. Fittydent was selected for the study for comparison and control purpose as it is the most common, economical and easily available denture cleansing brand in Indian markets.

The study was divided into 4 groups which had 21 samples of acrylic in each group which was disinfected using Phytopharmaceutical denture cleansing tablets, FittyDent Denture Cleansing tablets, chlorhexidine and control group in which no

disinfection treatment was done. The disinfection was done as per the manufacturer's instruction. The evaluation and comparison was carried out by measuring the amount of surface roughness using the Profilometer.

Results of the present study showed that by using of all the method of Disinfection i.e Fittydent denture cleansing tablet, Chlorhexidine, and Phytopharmaceutical tablets had some amount of surface roughness, however statistical analysis revealed that the surface roughness of the Phytopharmaceutical Denture cleansing tablet was least among the other disinfection groups This observation could be due to the mechanism of action of the denture cleansers.

Phytopharmaceutical denture cleansing tablets contain alkaline peroxidases; the commercially available denture cleansing tablet used in this study contains sodium perborate monohydrate. These peroxidases produce effervescence in alkaline solutions of hydrogen peroxide along with active oxygen in water.^{36,42,49}

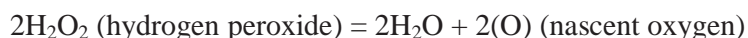
Similarly chlorhexidine contains 1,1- hexanethykebe bis [5-(p-chlorophenyl) biguanide] di-D-gluconate), which has high abrasive action on the surface of PMMA.^{47,50}

Chemical reaction can be summarised as:-



Mechanism of action:

Alkali (chemical reagents) = decreased surface tension



Nascent reactive oxygen produced has a mechanical impact on cleansing. A denture cleaning agent or tablet typically includes abrasive substances to support mechanical denture cleansing; The result is a mechanical action by removing debris and the effects of oxygen are antimicrobial and blemish removal. These abrasives abrade the denture surface and can cause rugging and increase micro-organisms' adhesion.⁵²⁻⁵⁴

The Phytopharmaceutical tablet contains bioactive ingredients from *Oscimum sanctum* and *Cymbopogon citratus* which contribute to the antimicrobial action of the tablet. the basic composition of the tablet is inclusive of Sodium hydrogen Carbonate, Citric acid, Tartaric Acid in a suitable ratio which contributes to the effervescing action of this tablet. These ingredients are chemically less harsh and are hence expected to damage the denture surface in a lesser proportion than the reagents like hydrogen peroxide and sodium perborate monohydrate.

This prompts further probing and research into the various therapeutic and pharmacologic applications of lemongrass in one such attempt the bioactive ingredients were incorporated into denture cleansing tablet. The synergistic action of Phyto ingredients of *Oscimum sanctum and Cymbopogon citratus* gave a statistically significant, clinically relevant and over all acceptable action against biofilm formation and candida colonisation.as was confirmed after application of various statistical tests as mentioned earlier.

Also, during this study, no apparent deformation or color changes were observed on the dentures following usage of the tablets. hence the usage of the Phytopharmaceutical denture cleansing tablet is a lucrative and reliable alternative for maintenance of denture hygiene.

LIMITATIONS OF THE STUDY

There is scope for further research involving a wider area of interest as follows:

1. In the present given study the microbial growth assessment was not carried out, only the surface roughness caused by the phytopharmaceutical denture cleansing tablet, commercially available denture cleansing tablet and chlorhexidine was evaluated
2. The identification of varying microbial strains and the effect of denture cleansing tablet usage on specific strain or particular species of microorganisms was not evaluated.
3. Adherence of microorganisms to denture base materials depends on the micro surface topography of the denture. The ability of microorganisms to form biofilm and there by colonize depends on the physical characteristics and material surface properties. Hence various types of denture base materials need to be considered for the study which was not done in this study.

SCOPE OF THE STUDY

In accordance with the results of the present study, comparatively less surface roughness was observed by the phytopharmaceutical denture cleansing tablet as compared with chlorhexidine and commercially available denture cleansing tablets.

This study could also act as a basis to further research the microbial growth on the roughened area caused by both Phytopharmaceutical denture cleansing tablet, commercially available denture tablet as well chlorhexidine.

Further studies could also include the effect of long term phytopharmaceutical denture cleansing tablet, commercially available denture cleansing tablet as well as chlorhexidine on Different Properties of Denture base material.

CLINICAL IMPLICATIONS

- In the present study the use of Phytopharmaceutical denture cleansing tablet showed least surface roughness when compared to commercially available denture cleansing tablet and chlorhexidine. Hence phytopharmaceutical tablet containing Tulsi and Lemongrass could be recommended as a denture cleansing aid to denture patients to maintain oral hygiene
- The use of phytopharmaceutical denture tablet containing Tulsi and Lemongrass is preferred over commercially available denture cleansing tablet as it was found to be more effective than commercially available denture cleansing tablet, it also overcomes the anticipated ill effects of long term usage of chemical denture cleansers as it does not contain abrasives or any other form of harsh chemical.
- It is important to give proper oral hygiene instructions to patients and educate them, about the importance of maintaining proper oral and denture hygiene along with regular recall and follow up of complete denture patients

CONCLUSION

In view of the results of the study the following conclusions could be drawn:

1. The use of phytopharmaceutical denture cleansing tablet as a denture cleansing agent had the least effect on surface roughness
2. Phytopharmaceutical denture cleansing agent containing tulsi and lemongrass can be recommended as a denture cleansing agent for complete dentures over chlorhexidine and commercially available denture cleansing tablets.

SUMMARY

The present study was conducted with the aim of evaluating and comparing the effect of two denture cleansers and chlorhexidine namely Phytopharmaceutical denture cleansing tablet containing Tulsi and Lemongrass and Commercially available denture cleansing tablet on surface roughness of heat cure polymethyl methacrylate denture base resins.

84 polymethyl methacrylate heat cure acrylic blocks were made as per the inclusion criteria. 4 Groups were made with 21 samples in each group. one group was subjected to the treatment with the phytopharmaceutical denture cleansing tablet, the second group was subjected to treatment with commercially available denture cleansing tablet which acted as positive control, the third group was subjected to treatment with chlorhexidine, and the fourth group was subjected to treatment with water which acted as negative control. The study was done with the null hypothesis that there is no difference between the surface roughness of phytopharmaceutical denture cleansing tablet, commercially available denture cleansing tablet and chlorhexidine.

The data obtained was subjected to statistical analysis using SPSS software version 20. In order to collectively compare the means of two study groups repeated measures ANOVA with a Greenhouse - Geisser correction, Post hoc tests using the Bonferroni correction, Mann Whitney U test for comparison between baseline and after treatment Within the limitations of the study, it was concluded that the acrylic blocks subjected to treatment with Phytopharmaceutical denture cleansing tablet showed least surface roughness when compared to commercially available denture cleansing tablet and chlorhexidine.

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ANNEXURE – I - ETHICAL CLEARANCE LETTER



Research and Ethics Committee
KLE V K INSTITUTE OF DENTAL SCIENCES
KLE University



Accredited 'A' Grade by NAAC

Placed in Category 'A' by MHRD (GoI)

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CERTIFICATE

This is to Certify that the synopsis titled

Comparative evaluation of surface roughness of herbal denture cleansing tablet containing essential oils of Tulsi and lemon grass, 0.12% Chlorhexidine digluconate & commercially available denture cleansing tablet on heat cure polymethyl methacrylate denture base resin - An invitro study. Submitted by

Dr. Dr. Harpreet Singh Manaktala P. G. Student /

Staff, Guided by Dr. Raghunath Patil from Department of Prosthodontics & Crown and Bridge has been critically evaluated by committee members and granted ethical clearance to conduct the above mentioned study

Date : 15/11/20

Member Secretary

Research and Ethical Committee
KLEVK Institute of Dental Sciences
Belagavi

Chairman

Research and Ethical Committee
KLEVK Institute of Dental Sciences
Belagavi

Research and Ethical Committee
KLE V K Institute of Dental Sciences
Belgaum

ANNEXURE - II**Chlorhexidine group**

Before Treatment	After Treatment
0.07 um	0.11 um
0.05 um	0.14 um
0.07 um	0.30 um
0.17 um	0.20 um
0.03 um	0.03 um
0.19 um	0.24 um
0.07 um	0.14 um
0.08 um	0.18 um
0.03 um	0.11 um
0.04 um	0.15 um
0.03 um	0.11 um
0.07 um	0.14 um
0.07 um	0.15 um
0.05 um	0.14 um
0.04 um	0.11 um
0.03 um	0.20 um
0.07 um	0.11 um
0.05 um	0.15 um
0.03 um	0.14 um
0.07 um	0.18 um
0.03 um	0.11 um

Fittydent tablet group

Before Treatment	After Treatment
0.04 um	0.12 um
0.05 um	0.22 um
0.12 um	0.21 um
0.04 um	0.19 um
0.16 um	0.28 um
0.15 um	0.25 um
0.08 um	0.21 um
0.08 um	0.35 um
0.06 um	0.16 um
0.04 um	0.19 um
0.02 um	0.22 um
0.08 um	0.24 um
0.03 um	0.26 um
0.07 um	0.28 um
0.03 um	0.24 um
0.03 um	0.29 um
0.07 um	0.30 um
0.05 um	0.25 um
0.03 um	0.24 um
0.07 um	0.29 um
0.03 um	0.22 um

Phytopharmaceutical cleansing tablet

Before Treatment	After Treatment
0.03 um	0.05 um
0.02 um	0.05 um
0.12 um	0.15 um
0.02 um	0.04 um
0.03 um	0.06 um
0.03 um	0.05 um
0.08 um	0.11 um
0.07 um	0.09 um
0.07 um	0.10 um
0.02 um	0.06 um
0.02 um	0.04 um
0.08 um	0.10 um
0.06 um	0.09 um
0.02 um	0.06 um
0.02 um	0.05 um
0.02 um	0.05 um
0.03 um	0.05 um
0.04 um	0.06 um
0.03 um	0.05 um
0.07 um	0.10 um
0.02 um	0.05 um

Control Group

Before Treatment	After Treatment
0.02 um	0.02 um
0.02 um	0.02 um
0.02 um	0.02 um
0.02 um	0.02 um
0.03 um	0.03 um
0.03 um	0.03 um
0.03 um	0.03 um
0.06 um	0.06 um
0.06 um	0.06 um
0.06 um	0.06 um
0.05 um	0.05 um
0.04 um	0.04 um
0.08 um	0.08 um
0.04 um	0.04 um
0.06 um	0.06 um
0.12 um	0.12 um
0.24 um	0.24 um
0.10 um	0.10 um
0.07 um	0.07 um
0.07 um	0.07 um
0.11 um	0.11 um