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Efficacy Analysis of Denture Cleansing Chemicals on Microbial Flora – An *in vitro* Study

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Abstract It is of paramount importance to maintain denture hygiene in denture wearers. It is important to know the effectiveness of denture cleaning chemicals in the elimination of known pathogenic microorganisms and efficacy of each chemical in reduction of known microbial flora**.** The test group commenced by placing 20 samples of sterilized acrylic resin blocks into the (BHI) broth inoculated with *Staphylococcus aureus* standard strain. After incubation, 5 samples of acrylic blocks were transferred from broth to 5 different test tubes containing solution 'A'. Similarly 5 samples to solution 'B' and 5 to solutions 'C'. All the test samples present in solution 'A', 'B' and 'C' after 7 hours were individually used to make the primary inoculums onto the agar plate. Remaining 5 samples present in the BHI broth which would serve as the control were directly used to make the primary inoculums onto the BHI agar plate. *Staphylococcus aureus* colonies were counted from BHI agar plates once the desired incubation period was achieved. In case of *Candida albicans*, 20 samples of sterilized acrylic blocks were immersed into the BHI Broth inoculated with *Candida albicans* of standard strain. After incubation, similarly 5 samples of each were immersed in test tubes containing solution 'A', 'B' and solution 'C'. After completion all the test samples were individually used to make the primary inoculums onto the Sabouraud dextrose agar plate. Remaining 5 samples which will serve as control were directly used to make the primary inoculums into the Sabouraud dextrose agar plate. The fungal colonies were counted both in treated acrylic blocks and untreated acrylic blocks using colony counter unit once the desired incubation period was achieved. All the reading obtained from both the groups were statistically analyzed and it was concluded that 'A' denture cleansing agent brought about a reduction in the *Staphylococcus aureus* as well as *Candida albicans* to the highest degree as compared to `B' and `C'. However there was no significant difference in the reduction brought about by 'B' and 'C'. Nonetheless, `B' proved to be better than 'C' in bringing about a reduction in Candida colonies which also proved to be statistically significant. It is important for every dentist to inspire and educate their patient and more essentially to offer various means and methods of plaque control.

Keywords: *Acrylic Resins, Denture Cleansers, Hygiene*

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1. Introduction

Dental plaque is considered as the cause of two major critical oral diseases such as dental caries and periodontal disease among dentate patients. However, with complete denture wearers, there has been less concern about plaque control and denture maintenance [1]. The plaque formation on surfaces of the denture is a common problem often leading to halitosis and mucosal inflammation with its associated complications like denture stomatitis, chronic candidiasis and inflammatory papillary hyperplasia [2,3].

Whilst the diversity of organisms present in the oral cavity is well established, *Staphylococcus aureus* plays a considerable role in the environment of the normal oral flora with its importance being documented as a medical pathogen for many years. Various strains of *staphylococcus aureus* are known to cause some infection or the other in the circumoral region. These includes angular cheilities, endodontic infection, osteomylitis of the jaw, parotitis and oral mucositis in elderly [4]. Another common inhabitant of the oral cavity is the *Candida albicans*, a fungus that can move through the blood vessels and affect throat, intestines, and heart valves. *Candida albicans* becomes an infectious agent when there is some change in the body atmosphere that allows it to grow out of control. Denture stomatitis, angular cheilities and median rhomboid glossitis are referred to as *Candida* associated infections, Oral hygiene and topical antifungal agents are usually adequate to cure such infection [5].

Various methods have been reported in the literature regarding denture cleansing and maintenance. These have been broadly classified as mechanical and chemical. The former group includes soaps used in association with brushes and ultrasonic cleaners [6]. Effective plaque removal requires a degree of physical energy that is often lacking among geriatric, physically handicapped, mentally retarded and non-motivated patients. In such situations use

of chemical denture cleansers can be more advantageous [7].

Good oral hygiene practice and adequate care for removable dentures is thereby important for maintaining the health of the denture bearing area and associated structures in denture wearers. It has been hoped that proficient chemical denture cleansers could become a significant alternative to mechanical cleaning especially among geriatric or handicapped denture wearers. Yet, although many commercial chemicals are available to the general community, their effectiveness has not been proved convincingly.

The purpose of this study is to evaluate the effectiveness of three commercially available denture cleansing chemicals in reducing cultured microorganisms known to cause frequent infections among denture wearers and to compare their relative ability to sanitize the dentures.

2. Materials and Methods

The growing importance of denture cleansers has been due to the implication of dental plaque as the etiological factor for denture stomatitis. Denture cleanliness is often poor mainly because of improper denture maintenance practice and relative inefficiency of most commercially available denture cleansing products. In order to fulfill the purpose, this *in vitro* study was carried out to evaluate the effectiveness of three commercially available denture cleansing products in reducing cultured microorganisms known to cause frequent infections among denture wearers.

This study was conducted at the Department of Prosthodontic and Crown & Bridge, Manipal College of Dental Sciences, Manipal.

2.1. Materials Used to Prepare Acrylic Block

- Base plate wax
- Dental stone
- Pink heat cure acrylic resin
- Cold mould seal

2.2. Materials Used for the Microbiological Study

- BHI broth (Brain heart infusion broth)
- BHIA (Brain heart infusion agar)
- SDA (Sabourauds dextrose agar)
- Distilled water

2.3. Microorganisms Used for Study

- *Staphylococcus aureus* (ATCC 25923)
- *Candida albicans* (standard strain)

2.4. Denture Cleansing Chemicals Used for Study

- Denture cleansing tablets A.
- Denture cleansing tablets B.
- Denture cleansers powder C.

2.5. Preparation of Heat Cured Acrylic Resin Block

40 samples of acrylic block with dimension 6x6x4mm were prepared strictly following the long curing cycle protocol. These samples were finished, polished and sterilized. Each sample was sterilized using an autoclave and stored in sterilized bottle containing distilled water.

2.6. Preparation of Denture Cleansing Solution

The 3 commercially available denture cleansing chemicals which were used in this study were denture cleansing tablets and denture cleansing powder. Its chemical composition is mention in table.

Commercially available denture cleansing chemicals	Chemical compositions
Tablet 'A'	Sodium perborate, sodium bicarbonate , potassium monopersulphate , trisodium phosphate , sulfonic anid,PEG-240,TAED,PVP, sodium methyl ,oleoyl taurate,cellulose lactose
Tablet 'B'	sodium carbonate, sodium perborate, potassium monosulphate , sodium lauryl sulphate
Powder 'C'	Potassium persulphate ,sodium perborate , sodium carbonate, sodium sulphate , trisodium phosphate ,sulphamic acid, tetra potassium pyro phosphate ,EDTA,sodium lauryl sulphate , papermint powder

The cleansing chemicals were handed over to the person who prepared the solutions. The solution was coded as solution 'A', 'B' and 'C' so that the operator and the microbiologist would not know which cleansing chemicals the bottle contained. The contents of the different groups of bottles were recorded in a register by the person who had bottled the solutions, and this person otherwise didn't participate in the study to avoid potential bias.

2 grams of denture cleansing tablets `A' were weighed in a digital electric balance and suspended in 200ml of distilled water in a sterilized bottle and the bottle was labeled as solution 'A'. Similarly done for 2grams of tablets `B' and powder `C'.

2.7. Microbiological Procedures

2.7.1. Asepsis

Throughout the study, an effort was made to work aseptically; a standard barrier technique was used with sterile latex gloves, facemask and head cap. All laboratory procedures including plating of agar plates were carried out in a laminar air flow cabinet hood.

2.7.2. Microbiological Evaluation of Antibacterial Activity against *Staphylococcus aureus*

The test group commenced by placing 20 samples of sterilized acrylic resin blocks into the Brain Heart Infusion

(BHI) broth inoculated with *Staphylococcus aureus* standard strain. Sterilized pointed forceps was used to introduce acrylic blocks into the bottle containing inoculated (BHI) broth and incubated at 37^0 C aerobically for 18 to 24 hours. After incubation, 5 samples of acrylic blocks were transferred from BHI broth to 5 different test tubes containing solution 'A' aseptically. 5 samples of acrylic blocks to 5 different test tubes containing solution 'B'. Likewise 5 samples to 5 different test tubes containing solution 'C'. All the test tubes containing cleansing solution with test samples were kept in the test tube rack inside the laminar air flow cabinet hood for 7 hours.

All the test samples present in solution 'A', 'B' and 'C', after 7 hours immersion in the cleansing solution were individually used to make the primary inoculums onto the agar plate. Then an inoculating wire was used to streak rest of the plate and incubated at 37^0 C for 18 to 24 hours aseptically for the growth of the bacteria.

Remaining 5 samples present in the BHI broth which would serve as the control were directly used to make the primary inoculums onto the BHI agar plate.

Staphylococcus aureus colonies were counted from BHI agar plates once the desired incubation period was achieved, samples treated with cleansing solution 'A', 'B', and 'C' as well as untreated samples for (control) samples using colony counter unit.

2.7.3. Microbiological Evaluation of the Denture Cleansers for Antifungal Activity against *Candida albicans*

In case of *Candida albicans*, 20 samples of sterilized acrylic blocks were immersed into the Brain Heart Infusion Broth inoculated with *Candida albicans* of standard strain which were incubated at 37^0 C for 24 to 48 hours in an incubator. After incubation, 5 samples of acrylic blocks were transferred to 5 different test tubes containing solution 'A'. Similarly 5 samples each were immersed in test tubes containing solution 'B' and solution 'C'. After completion of 7 hours immersion, all the test samples were retrieved from the test tube and individually used to make the primary inoculums onto the Sabouraud dextrose agar plate. An inoculating wire was used to streak rest of the plate and incubated at 37°C for 24 to 48 hours in the incubators to see the growth of the fungus.

Remaining 5 samples present in the BHI broth which will serve as control were directly used to make the primary inoculums into the Sabouraud dextrose agar plate and streaked with the help inoculating wire to the rest of the plate and incubated at 37°C aerobically for 24 to 48 hours in an incubator.

The fungal colonies were counted both in treated acrylic blocks with cleansing solution and untreated acrylic blocks using colony counter unit once the desired incubation period was achieved.

3. Results

The present study was conducted to evaluate the effectiveness of three commercially available denture cleansing chemicals in reducing cultured microorganisms *Candida albicans* and *Staphylococcus aureus* known to cause frequent infections among denture wearers.

The denture cleansers used in this study were commercially available chemicals with different chemical compositions. All the denture cleansers used in this study were alkaline peroxide based denture cleansing chemicals but each cleanser to be studied contained different amount of sodium perborate in them.

All the readings so obtained from both the groups were tabulated and statistically analyzed (1 to 4)

All the analysis was done using SPSS version 14. Mean cfu/ml at baseline and post treatment with various cleansing solution for *Staphylococcus aureus* and *Candida albicans* groups were compared using Wilcoxon signed ranks test. Multiple comparisons within the *Staphylococcus aureus* and *Candida albicans* groups at post treatment were compared using ANOVA followed by Post hoc Tukey's HSD test. The cut-off level for statistical significance was taken at $p = <0.05$.

Table 1. Representing cfu/ml of *Staphylococcus aureus* on BHIA

S. aureus before treatment (cfu/ml)		Bacterial colony after treatment (cfu/ml)		
		`A' (cfu/ml)	`B' (cfu/ml)	`C'(cfu/ml)
Sample 1	$1x10^5$	$6x10^3$	$12x10^3$	$7x10^3$
Sample 2	$1x10^5$	$3x10^3$	$7x10^3$	$5x10^3$
Sample 3	$1x10^5$	$6x10^3$	$6x10^3$	$8x10^3$
Sample 4	$1x10^5$	$4x10^3$	$7x10^3$	$9x10^3$
Sample 5	$1x10^5$	$3x10^3$	$7x10^3$	$9x10^3$

Table 2. Representing cfu/ml of *Candida Albicans* on SDA

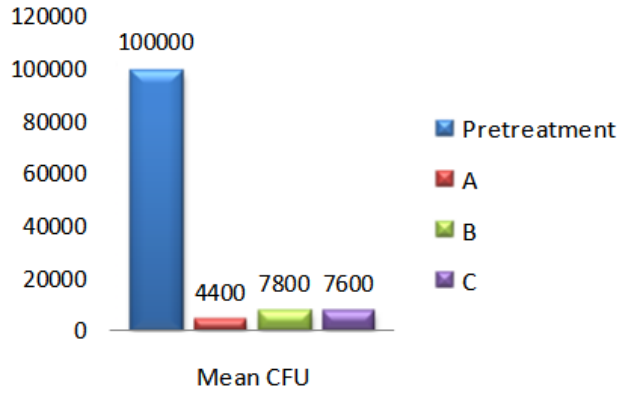
C. albicans before treatment (cfu/ml)		Fungal colony after treatment (cfu/ml)		
		`A' (cfu/ml)	`B' (cfu/ml)	`C'(cfu/ml)
Sample 1	$1x10^5$	$4x10^3$	$5x10^3$	$1x10^4$
Sample 2	$1x10^5$	$5x10^3$	$5x10^3$	$1x10^4$
Sample 3	$1x10^5$	$4x10^3$	$7x10^3$	$9x10^3$
Sample 4	$1x10^5$	$4x10^3$	$7x10^3$	$1x10^4$
Sample 5	$1x10^5$	$5x10^3$	$7x10^3$	$9x10^3$

Table 3. Wilcoxon Signed Ranks Test: Non Parametric Test

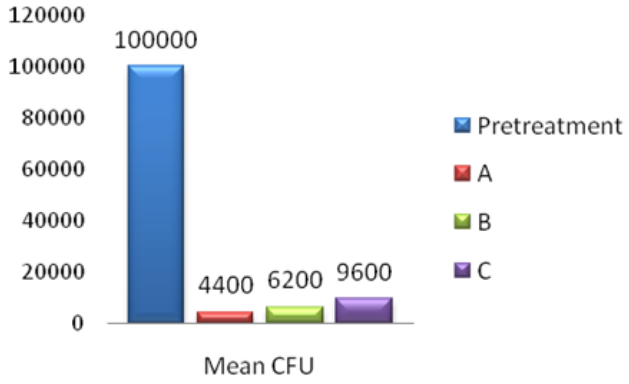
Group	Baseline	Disinfectant	N	Mean	SD	p-value
Staphylococcus aureus	10^5 cfu/ml	`A'	5	4400	1516.58	0.041
		`B'	5	7800	2387.47	0.039
		`C'	5	7600	1673.32	0.042
Candida albicans	10^5 cfu/ml	`A'	5	4400	547.72	0.038
		`B'	5	6200	1095.45	0.038
		`C'	5	9600	547.72	0.038

Table 4. ANOVA Followed by Post Hoc Tukey Test

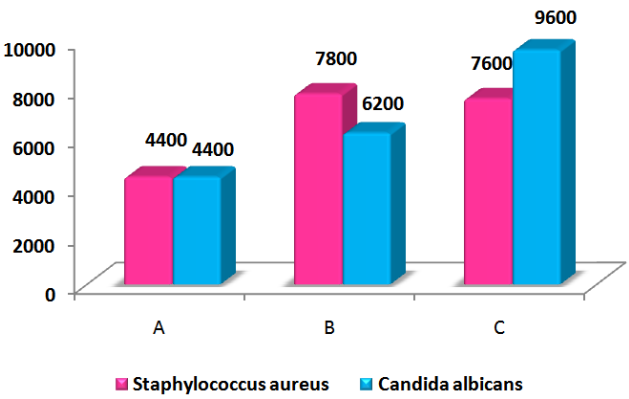
Group	Baseline	N	Mean	SD	p-value	Post hoc tests
Staphylococcus aureus	`A'	5	4400	1516.58	0.026	2>1, 3>1
	`B'	5	7800	2387.47		
	`C'	5	7600	1673.32		
Candida albicans	`A'	5	4400	547.72	<0.001	3>2>1
	`B'	5	6200	1095.45		
	`A'	5	9600	547.72		



Graph 1. Mean cfu/ml of Staphylococcus aureus before and after treatment



Graph 2. Mean cfu/ml of Candida albicans before and after treatment



Graph 3. Mean cfu/ml of Staphylococcus aureus and Candida albicans

4. Discussions

Edentulism interferes with mastication, speech and esthetics. Providing dentures can overcome the problem of edentulousness and improve the quality of life, but can be harmful when the denture hygiene is not maintained and properly cared for.

The phenomenon of initial adherence representing the first step in the colonization process is plaque formation that results from the formation of a thin biofilm, followed by a multilayer, which culminates into denture plaque [8]. Plaque formation on the surfaces of denture is a common problem among denture wearers often leading to halitosis and gingival inflammation [9] with its related complications like denture stomatitis, inflammatory papillary hyperplasia and chronic candidiasis [3]. A variety of soft tissue changes are allied with it. Lack of denture cleanliness is the most commonly cited etiologic factor for these entities [1,3,8,10] There is copious documented data showing the connection between good oral health and denture sanitations. A significant relationship between poor denture cleanliness and denture stomatitis was first described by Jorgenson EB and Betramin 1970. [10] According to Jorgenson EB [10] this infection can be best prevented by through oral and denture hygiene.

Correct regular cleansing of the tissue surface of denture is essential for the maintenance of healthy oral mucosa in the elderly. Frequently used methods of denture cleansing seem to be the usage of soap and brush, which decrease the denture cleansing ability with increasing age. Geriatric patients have reduced manual dexterity and are handicapped hence cannot achieve denture cleansing effectively. The practice of immersion type of cleansing chemicals helps them to keep the dentures clean and devoid of any deposits.

Although a large number of denture cleansers are available for the removal of organic and inorganic deposits from the denture, they are not supported by considerable investigational evidence. In the past the purpose of denture cleansers has been to remove deposit and stain from the dentures. With the present knowledge, poor denture hygiene induced complications therefore more emphasis is placed on denture cleansing agent to sanitize dentures. A high antifungal and antibacterial activity is considered a desirable property of a denture cleansing agent for the prevention of denture hygiene related complications.

Conventional alkaline soak types of denture cleansing chemicals are by far more widely used by most of the patients than others to clean denture.

Hence keeping with the trend, three alkaline peroxide type of denture cleansers have been evaluated in this study which was commonly available in the local pharmacy. It was observed that mere immersions of dentures in the cleaners were incapable of removing deposits and stains on the denture surface to an appreciable degree. 30 mins immersion and light mechanical brushing of the dentures was not adequate in removal of the deposits and stains, confirming the past recommendations of Anthony and

Gibbons (1958) [11] and Neil (1968) [12] that 20 mins immersion of denture in denture cleansers is inadequate and that overnight immersion is essential for the cleansers to act its optimum.

Hence, this study was carried out following the overnight immersion protocol conducted by Nikawa H. et al [13], Tarbet WJ. Etal [1] who stated that the efficacy of denture cleansers should be done under conditions alike to a normal overnight cleansing regimen (6–8 hours) in addition to recommendations given by the manufacturer.

The present study was conducted on 40 samples of acrylic blocks of which 20 blocks were inoculated in BHI broth contaminated with *staphylococcus aureus*.. All the test samples present in solution 'A', 'B' and 'C' after 7 hours immersion in the cleansing solution were individually inoculated in BHI agar plate. Remaining 5 samples present in the BHI broth which would serve as the control were directly used to make the primary inoculums onto the BHI agar plate. *Staphylococcus aureus* colonies were counted from BHI agar plates once the desired incubation period were achieved, samples treated with cleansing solution 'A', 'B' and 'C' as well as untreated samples using colony counter unit.

Similar procedure was carried out for *Candida albicans* with the remaining 20 samples of acrylic resin blocks. All the test samples present in solution 'A', 'B' and 'C' after 7 hours immersion in the cleansing solution were individually inoculated in Sabouraud dextrose agar plates. The fungal colonies were counted both in treated as well as untreated acrylic blocks using colony counter unit once the desired incubation period was achieved.

In the current study it was found that all the three denture cleansing chemicals `A', `B' and `C' have high antifungal activity against *Candida albicans* and antibacterial activity against *Staphylococcus aureus*. Thus satisfying an important criterion which qualifies antifungal and antibacterial activity as important requisites of denture cleansers.

The use of alkaline denture cleansers in this study to sanitize dentures further gains importance as it is in accordance with the study conducted by Dills, Olshan, Goldners and Brogdon (1988) [7] who compared the antimicrobial activity of an alkaline peroxide cleansers which displaced a greater and broader antimicrobial activity against gram positive facultative cocci and gram negative anaerobic cocci as well as reduction in the total recoverable microorganisms after denture cleanser treatment.

The effect of alkaline peroxides as a denture cleanser in reducing the Candidal colonies was supported by Nokamoto, Tomamoto and Hamada (1991) [14] who evaluated the cleansing efficacy of four denture cleansers containing enzymes and alkaline peroxide type cleansers under the similar conditions.

The study was aimed at testing the effectiveness of the cleansers on pure bacterial culture of *Staphylococcus aureus* and pure fungal culture of *Candida albicans*. Even though all the denture cleansers contained alkaline peroxides, variants of the same as 3 different denture cleansers were selected as they contained different amounts of sodium perborate in them.

The culture media used in the present study was Brain Heart Infusion (BHI) broth for the growth of *Staphylococcus aureus* bacteria and *candida albicans*. Colonies of *Staphylococcus aureus* were counted from the BHI agar plates once the desired incubation period was achieved for samples treated with 3 different cleansing solutions. Similar colony counting procedure was carried out for *Candida albicans*.

All the reading obtained from both the groups were statistically analyzed and it was concluded that `A' denture cleansing agent brought about a reduction in the *Staphylococcus aureus* as well as *Candida albicans* to the highest degree as compared to `B' and `C'. However there was no significant difference in the reduction brought about by `B' and `C'. Nonetheless, `B' proved to be better than `C' in bringing about a reduction in Candidal colonies which also proved to be statistically significant.

5. Conclusion

It is of paramount importance to maintain denture hygiene in order to maintain a healthy oral mucosa in denture wearers. The accumulation of the microbial flora on the dentures can lead to denture related associated problems. In order to maintain the cleanliness of the dentures, chemical denture cleansers are available in marketplace.

Denture cleansers which have flooded the commercial market have always made strong claims about their abilities. Keeping in view of these claims, the current study was undertaken to assess the efficacies of three commercially available immersion types of denture cleansers chemicals in the elimination of known pathogenic microorganisms under the same condition.

The results obtained from the present study was encouraging enough to show a great reduction in *Staphylococcus aureus* bacteria and *Candida albicans* fungi after treating with `A', `B' and `C' denture cleansing chemicals when treated for a period of 7 hours.

Among the three test agents used in the current study, `A' denture cleansing chemical had a highest reduction in colony forming units in both *Staphylococcus aureus* and *Candida albicans.*

Further research needs to be carried out to evaluate various other denture cleansers on optimal disinfection of dentures. Besides, the oral cavity comprises of a myriad of microorganisms which necessitates an in vivo study to correlate the effect of denture cleansers in lysis of a wide gamut of microorganism. Deleterious effects on long term use of these denture cleansers on color stability are also warranted.

Nonetheless, the study reiterates the importance of patient education in maintaining denture hygiene for over all patient well being.

To conclude, now that the dental fraternity is awakening to the problem of denture hygiene with the realization that microbial plaque on dentures may be dangerous to both the oral mucosa and the patient's general health. Patient should be responsible in maintaining their oral hygiene through a daily home care regime. It is the responsibilities of every dentist to encourage and educate their patient and more essentially to advice the means and methods of plaque control.

Declaration of Conflicting Interests

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References

[1] Tarbet WJ, Axelrod S, Minkoff S .Denture Cleansing: A comparison of two methods. J Prosthet Dent 1984 Mar; 51(3):322-324.

[2] Ghalichebaf M, Graser GN and Helmut A. The efficacy of denture cleansing agents. J Prosthet Dent 1982Nov; 48(5):515-520.

[3] Abelson DC. Denture plaque and denture cleansers. J Prosthet Dent 1981 Apr; 45(4):376-379.

[4] Species in the oral cavity. J Medical Microbiology 2001; 50:940-946. Smith AJ, Jackson MS and Bagg J. The ecology of *Staphylococcus*

[5] Akpan A, Morgan R. Oral Candiasis Postgrad Med J 2002; 78:455-459

[6] Hoyle DE, Cavano J and Taylor DF. Evaluation of the effectiveness of methods for cleaning dentures. J Prosthet Dent 1977 Dec; 38(6): 659.

[7] Dills SS, Goldner OS and Brogdon C. Comparison of the antimicrobial capability of an abrasive paste and chemical soak denture cleansers.J Prosthet Dent 1988 Oct; 60(4):46-470.

[8] Nicholson RJ, Stark MM and Scott HE. Calculus and stain removal from acrylic resin dentures. J Prosthet Dent 1968 Oct; 20(4):326-328.

[9] Augsburger RH and Elahi JM. Evaluation of seven proprietary denture cleansers. J Prosthet Dent 1982 Apr; 47(4):356-358.

[10] .Jagger DC and Harrison A. Denture cleansing – the best approach. British Dent J 1995 Jun; 178:41-417.

[11] Anthony DH, Gibbons P. The Nature and Behavior of Denture Cleansers. J Prosthet Dent 1958 Sep-Oct; 8(5):796-810.

[12] Neil DJ. A study of materials and methods employed in cleaning dentures. British Dent J 1968 Feb; 124(3):10-115.

[13] Nikawa H, Hamada T, Yamashiro H and Kumagai H. A review of In Vitro and In Vivo methods to evaluate the efficacy of denture cleansers. Int J Prosthodont 1999; 12(2):153-159.

[14] Nakamoto K, Tamamoto M and Hamada T. Evaluation of denture cleansers with and without enzymes against Candida albicans. J Prosthet Dent 1991; 66:792-795.