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Comparative Evaluation of the Effect of Two Plant Extract and Denture Cleanser on the Staining and Anti-Fungal Efficacy of Denture Base Resin: An *In Vitro* Study

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Abstract

Introduction: Edentulism is the most common oral problem encountered in the human population and the commonest remedy to it is dentures. Natural products and essential oils are promising therapeutic tools for oral infection. The increasing awareness towards the varied uses of natural products has made them a popular alternative to synthetic materials. Therefore, a study is planned to evaluate and compare the antifungal efficacy of triphala and *Aloe vera* when combined with denture cleansers on heat activated polymethyl methacrylate resin.

Materials and Method: In the present study 30 samples of polymethyl methacrylate resin of 20 mm × 10 mm × 2.5 mm were fabricated. All the samples will be grouped into three groups of ten samples each and will be immersed in three test solutions for 8 h daily for 30 days. The samples will be tested by spectrophotometer. Another test will be that all samples will be first inoculated with *candida albicans* mature biofilm, after which they will be dipped in the three solutions to observe the decrease in colony forming units per millimeter.

Results: There was a statistically significant reduction in CFU/ml of both triphala and *Aloe vera* solution. However, no statistically significant difference was found in color stability among the two groups.

Conclusion: Within the limitations of this study, it was found that both the denture cleansers showed a significant difference decrease in CFU/ml for anti-fungal efficacy on denture base resins when compared to control group. However, both the denture cleansers did not show a significant difference on the color stability of denture base resins.

Keywords: *Candida albicans*, Denture cleansers, *Aloe vera*, triphala, Staining, Denture base resins

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Introduction

Oral hygiene is an important aspect in maintaining the well-being of an individual since ages. There is a lack of awareness regarding the maintenance of oral hygiene and management in elderly individuals. Denture-related stomatitis or *Candida*-associated denture-induced stomatitis is common condition seen in geriatric patients, where mild inflammation and redness of the oral mucosa occurs beneath a denture [1]. The prevalence of Candida has been observed to be around 60% to 100% [1]. It binds to dentures and if left to accumulate over a short period of time can cause mucosal inflammation and halitosis [2]. Denture cleansers are a popular method used by denture wearers for cleaning. There are wide varieties of denture cleansers used to remove soft food and hard deposits of calculus and stains on denture base and teeth [3]. Deposits that form on the acrylic resin denture bases and on the teeth are assumed to be caused by the same mechanisms and substances that cause deposits on natural teeth, of which salivary calculus and tobacco tars are most common and most difficult to remove [4]. Natural products and essential oils are promising therapeutic tools for oral infection [5]. *Aloe vera* and triphala is the oldest medicinal plant ever known. Both have significant antimicrobial property [1]. The increasing awareness towards the varied uses of natural products has made them a popular alternative to synthetic materials. Therefore, a study was planned to evaluate and compare the antifungal efficacy of triphala and *Aloe vera* when combined with denture cleansers on heat activated polymethyl methacrylate resin.

Materials and Method

For the study, 30 samples of heat cure acrylic resin of 20 mm × 10 mm × 2.5 mm dimensions were fabricated and were divided into three groups-

1. **GROUP 1:** denture cleanser + triphala solution in tap water
2. **GROUP 2:** denture cleanser + *Aloe vera* solution in tap water
3. **GROUP 3:** denture cleanser in tap water (control)

For the fabrication of 30 samples, pre-fabricated metal die of 21 mm × 11 mm × 2.6 mm was used and putty index was formed. Then modeling wax (DPI) was taken, melted and was poured into the putty index that was formed. The denture base resin patterns were then fabricated according to manufacturer's technique.

Initial color evaluation

The initial color evaluation (CIE L*a*b* value) for each group was done by color spectrophotometer (SI. No-1004545, Model CM 2600D).

Preparation of test solutions

For evaluation of color stability of polymethyl methacrylate acrylic resin and efficacy of denture cleansers to remove *Candida albicans* biofilm, two test solutions were prepared. Plant extract test solution were prepared in the ratio of (1:10) by dissolving one tablet of fittydent denture cleanser in 100 ml tap water (to simulate environmental conditions) of 10 ml plant extract solution.

Final color evaluation

The solution was prepared and placed in 30 small containers. Into each container one die of polymethyl methacrylate was dipped overnight (8 h duration) for a period of 30 days in separate containers. After the treatment, each specimen was removed; cleaned and dried the color change (AE) of each specimen was calculated as follows:

$\Delta E\ [(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2\ 1^12$

Revival of culture

Candida albicans pure strain powder was obtained. It was placed in a 40 mL container of BD Bactec Mycosis broth and cultured in a Bactec culture machine at 35°C for 48 h. With the pour plate lawn culture method, 2 ml of the revived *Candida albicans* suspension in the broth was placed on the Sabouraud dextrose Agar plate. Subculture was carried out in an incubator at 37°C for 48 h.

Method for Preparing the Standardized Candida Suspension for Biofilm Formation

With the use of a Densitometer, the Candida density was standardized to 2×10^5 CFU/ml. Three milliliters of normal saline were placed in two polypropylene tubes. Colonies were picked from the culture plates and transferred to the tubes using loop. The densitometer was zeroed in order to standardize the density. The colony count was increased until the densitometer read 2×10^5 CFU/ml. Sabouraud dextrose agar was put into three culture plates. When the agar was semisolid, 10 polymethyl methacrylate acrylic resin samples were inserted horizontally in each plate and left to freeze. In each culture plate, 1.5 mm of the standardized suspension was poured over the samples. All of the plates were incubated for 72 h at 37°C.

Positive control

After 72 h, one sample was taken out, sonicated for 30 sec, and the sonicated solution was serially diluted with pH buffer saline before being placed on a new agar plate with 2 ml of the suspension. At 37°C, this was incubated for 24 h. The plate showed *Candida albicans* colonies after 24 h, indicating that the yeast incubation process was successful.

Treatment of samples

After 72 h, all of the samples were removed from the plate and washed in PBS for 2 sec to remove the loosely adherent *Candida*. It was then immersed in the test solutions for eight hours. Each specimen was gently washed in 2 mL of Phosphate Buffer Saline solution for 2 sec in a sterile sonicator tube. Sonication at 8 W for 30 sec was used to remove adherent bacteria from the sample. The sonicated solution was diluted in PBS and plated on a freshly prepared sabouraud dextrose agar plate with 1 ml of the suspension. The plates were incubated for 72 h at 37°C in the incubator.

Final culture count

After 72 h, 30 polypropylene tubes were numbered and filled with 3 mL distilled water. A loop of colony was harvested from the plate and suspended in the appropriate tubes. The densitometer was taken, and was zeroed. For the treated samples, all of the tubes were analyzed for CFU/ml.

Results

Table 1.1, 1.2 and Graph 1 shows that after treatment, except for Group III which showed an increase in colony count all other two groups showed a reduction in colony count. This reduction was maximum in Group I and minimum in Group III.

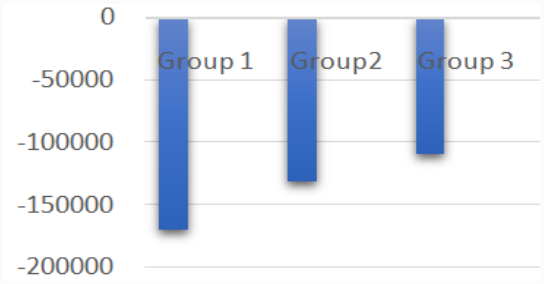
Table 2 shows a statistically significant intergroup difference with respect to change in mean colony forming unit in different groups. It was observed that change in the three groups were in negative direction. Among the three groups. Group I showed change values at

Table 1.1: Comparison of colony count in different groups after treatment.

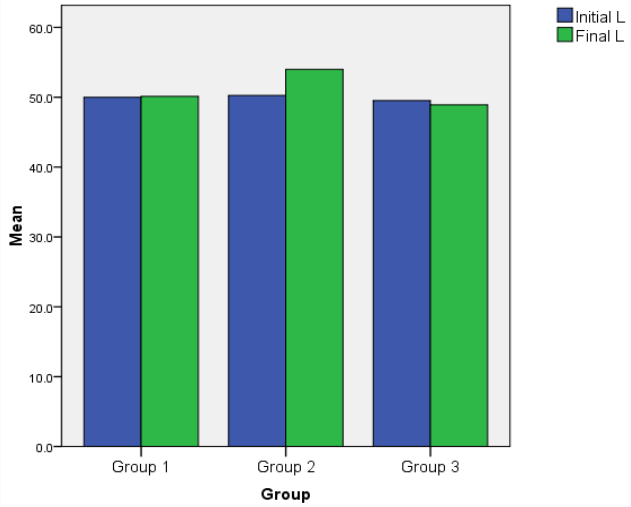
S. No.	Group I	Group II	Group III
1	29090.91	67272.73	92727.27
2	25454.55	74545.45	85454.54
3	30909.09	61818.18	96363.64
4	36363.64	63636.36	94545.45
5	27272.73	72727.27	87272.73
6	25254.55	67272.73	98181.82
7	32727.27	63636.36	89090.91
8	30909.09	70909.09	85454.54
9	34545.45	74545.45	87272.73
10	27272.73	69090.91	92727.27
Mean	29980	68545	90909

Table 1.2: Comparison of mean colony count change from baseline in different groups.

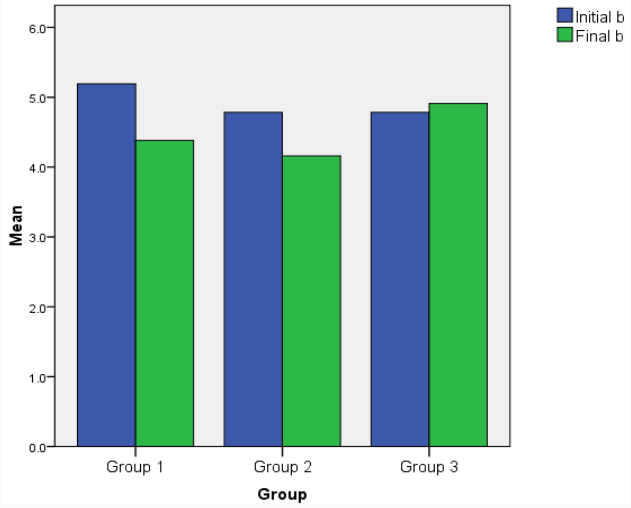
	Group I	Group II	Group III
Mean	-170020	-131455	-109091
SD	3788	4620	4616
Min	-174745	-138182	-114545
Max	-163636	-125455	-101818
Mean	-85	-65.7	-54.5
SD	1.9	2.3	2.3



Graph 1: Comparison of mean colony count change in different groups after treatment.



Graph 2: Distribution of means of color on position L at two-time interval.

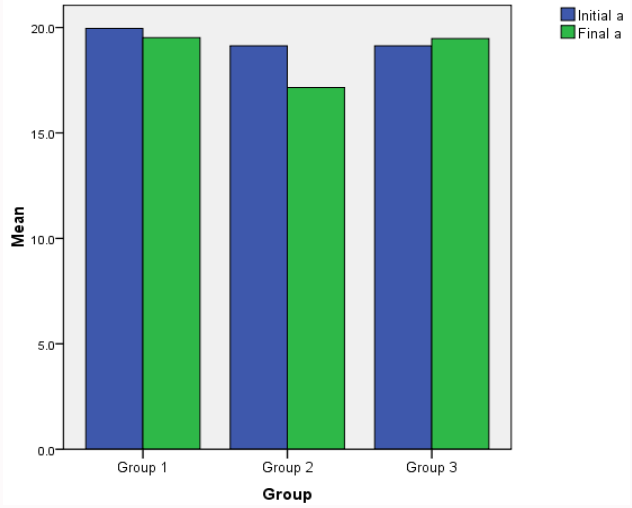


Graph 3: Distribution of means of color on position b at two-time interval.

the most negative value while Group III showed change values at the least negative order.

Inter group comparison (Table 3) revealed a statistically significant difference for all the comparisons.

Table 4 and Graphs 2-4 shows distribution of mean and S.d. of color at positions L, b and a, at two-time interval of three groups.



Graph 4: Distribution of means of color on position a, at two-time interval.

Table 2: Analysis of variance for mean change in colony count in different groups.

	Sum of Squares	Df	Mean Square	F	Significance
Inter Groups	3.0 X 10^{11}	3	1.0 X10^{11}	390.728	<0.001
Intra Group	9.3 X 10^{9}	36	2.5 X10^{9}		
Total	3.1 X 10^{11}				

Table 3: Inter group comparison of change in colony count.

	Comparison	Absolute change		% Change		P-value
		Mean	SE	Mean	SD	
1	I *vs.* II	228020	7199	114.01	3.6	<0.001
2	I *vs.* III	167091	7199	83.55	3.6	<0.001
3	II *vs.* III	-60929	7199	-30.46	3.6	<0.001

Table 5 on comparison (Inter group comparison) of means of color at positions L, b and a, at two-time interval among three groups by one way ANOVA, there was a significant difference in means of color at position L on Final time, p=0.025, p<0.05. The reaming means of color were not significant p>0.05.

Table 6 shows the multiple comparison of means of color at positions L, b and a, at two-time interval among groups by Tukey's HSD test. The mean difference of color Final L between group 2 and group 3 (5.0882) was significant, p<0.05. So the means of color Final L in group 2 (53.997) was significantly higher than group (348.909).

Table 7 showed the distribution of mean and S.d. of Color change ΔE of three groups. From Table 8, on comparison of mean of Color change ΔE among three groups by one way ANOVA, there was no significant difference in the means of Color change ΔE among three groups, p>0.05.

Table 9 showed the intra group comparison of means of color between two-time intervals on different positions of three groups paired t- test. The mean difference (0.8106) of color between Initial b and Final b of group 1 was significant <0.05.

Discussion

Oral microbial flora is comprised of numerous microorganisms like *Streptococcus species*, *Staphylococcus species*, *Escherichia coli*, *Pseudomonas species* and *Candida* species [6,7]. The most commonly

Table 4: Change in lightness ΔL (brightness) in different groups.

	Group	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean	Upper Bound	Minimum	Maximum
						Lower Bound			
Initial L	I	10	49.997	1.414	0.4471	48.986	51.009	47.9	52
	II	10	50.249	1.6838	0.5325	49.045	51.454	47.9	53.1
	III	10	49.523	1.1049	0.3494	48.733	50.314	47.9	51.1
Initial b	I	10	5.192	1.1114	0.3515	4.397	5.987	2.7	6.1
	II	10	4.784	0.8731	0.2761	4.16	5.409	2.7	6.1
	III	10	4.784	0.8731	0.2761	4.16	5.409	2.7	6.1
Initial a	I	10	19.954	1.6198	0.5122	18.795	21.112	17.1	21.8
	II	10	19.128	1.8455	0.5836	17.808	20.449	16.2	20.6
	III	10	19.128	1.8455	0.5836	17.808	20.449	16.2	20.6
Final L	I	10	50.119	2.5039	0.7918	48.328	51.911	47.9	55.1
	II	10	53.997	6.581	2.0811	49.29	58.705	47.9	67.7
	III	10	48.909	0.5848	0.1849	48.491	49.328	47.9	49.6
Final b	I	10	4.382	0.9905	0.3132	3.673	5.09	2.7	5.4
	II	10	4.161	1.5743	0.4979	3.035	5.287	1.6	6.1
	III	10	4.91	1.2188	0.3854	4.038	5.782	2.7	6.4
Final a	I	10	19.518	2.0563	0.6502	18.047	20.989	15	21.4
	II	10	17.154	3.9716	1.2559	14.312	19.995	10.8	21.2
	III	10	19.476	1.3296	0.4204	18.525	20.427	17.1	20.7

Table 5: Comparison (Inter group comparison) of means of color at positions L, b and a at two time interval among three groups by one way ANOVA.

ANOVA						
	Groups	Sum of Squares	Df	Mean Square	F	P value
Initial L	Inter Group	2.718	2	1.359	0.673	0.518
	Intra Group	54.499	27	2.018		
	Total	57.218	29			
Initial b	Inter Group	1.109	2	0.554	0.603	0.555
	Intra Group	24.837	27	0.92		
	Total	25.946	29			
Initial a	Inter Group	4.537	2	2.269	0.721	0.495
	Intra Group	84.921	27	3.145		
	Total	89.459	29			
Final L	Inter Group	141.311	2	70.655	4.246	.025*
	Intra Group	449.287	27	16.64		
	Total	590.598	29			
Final b	Inter Group	2.963	2	1.482	0.899	0.419
	Intra Group	44.506	27	1.648		
	Total	47.47	29			
Final a	Inter Group	36.621	2	18.31	2.523	0.099
	Intra Group	195.924	27	7.256		
	Total	232.545	29			

*p=0.025, p<0.05

found in denture wearers is the *Candida* species [2,8-12]. The present study was undertaken to see the effect in combination of denture cleanser along with plant extract solution for polymethyl methacrylate resin.

Several studies [2,13-16] have evaluated the effect of denture cleansers on initial Candida (24 h to 48 h of biofilm) on denture base materials, however not much attention has been paid on the effect of these cleaning agents on *Candida* associated mature biofilm. The fungus grows in number, invades tissues, and causes illness over time, most likely as a result of the creation of more harmful cells aided by the phenotypic switching mechanism. Such qualities enable *Candida albicans* fungal cells to adapt quickly to changes in the host, such as evading immune system elements, acquiring antifungal resistance,

Table 6: Multiple Comparison of means of color at positions L, b and a, at two-time interval among groups by Tukey's HSD test.

Dependent Variable	(I) Group *vs.* (J) Group	Mean Difference (I-J)	Std. Error	P value	95% Confidence Interval	
					Min	Max
Initial L	1 *vs.* 2	-0.2519	0.6354	0.917^{NS}	-1.827	1.323
	1 *vs.* 3	0.4742	0.6354	0.738^{NS}	-1.101	2.05
	2 *vs.* 3	0.7261	0.6354	0.497^{NS}	-0.849	2.301
Initial b	1 *vs.* 2	0.4078	0.4289	0.614^{NS}	-0.656	1.471
	1 *vs.* 3	0.4078	0.4289	0.614^{NS}	-0.656	1.471
	2 *vs.* 3	0	0.4289	1.000^{NS}	-1.063	1.063
Initial a	1 *vs.* 2	0.825	0.7931	0.559^{NS}	-1.141	2.791
	1 *vs.* 3	0.825	0.7931	0.559^{NS}	-1.141	2.791
	2 *vs.* 3	0	0.7931	1.000^{NS}	-1.966	1.966
Final L	1 *vs.* 2	-3.878	1.8243	0.103^{NS}	-8.401	0.645
	1 *vs.* 3	1.2102	1.8243	0.786^{NS}	-3.313	5.733
	2 *vs.* 3	5.0882	1.8243	0.025*	0.565	9.611
Final b	1 *vs.* 2	0.2204	0.5742	0.922^{NS}	-1.203	1.644
	1 *vs.* 3	-0.5286	0.5742	0.632^{NS}	-1.952	0.895
	2 *vs.* 3	-0.749	0.5742	0.405^{NS}	-2.173	0.675
Final a	1 *vs.* 2	2.3645	1.2047	0.141^{NS}	-0.622	5.351
	1 *vs.* 3	0.0421	1.2047	0.999^{NS}	-2.945	3.029
	2 *vs.* 3	-2.3224	1.2047	0.150^{NS}	-5.309	0.665

*p<0.05

Table 7: Distribution of mean and S.d. of Color change ΔE of three groups.

Group	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
1	10	2.0394	1.60703	0.50819	0.8898	3.189	0	4.45
2	10	5.5759	6.44404	2.03778	0.9661	10.1857	0	15.23
3	10	2.6263	1.97781	0.62544	1.2115	4.0412	0.53	4.7

Table 8: Comparison of mean of Color change ΔE among three groups by one way ANOVA.

ANOVA					
ΔE					
	Sum of Squares	**df**	**Mean Square**	**F**	**P value**
Between Groups	71.838	2	35.919	2.244	0.125^{NS}
Within Groups	432.179	27	16.007		
Total	504.017	29			

Table 9: Intra group comparison of means of color between two time intervals on different positions of three groups paired t- test.

Group		Paired Differences					t	df	P value
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
1	Initial L - Final L	-0.122	1.9228	0.6081	-1.4975	1.2535	-0.201	9	0.845^{NS}
	Initial b - Final b	0.8106	1.0735	0.3395	0.0427	1.5785	2.388	9	0.041*
	Initial a - Final a	0.4355	1.1819	0.3738	-0.41	1.281	1.165	9	0.274^{NS}
2	Initial L - Final L	-3.7481	5.9601	1.8847	-8.0117	0.5155	-1.989	9	0.078^{NS}
	Initial b - Final b	0.6232	1.5862	0.5016	-0.5115	1.7579	1.242	9	0.245^{NS}
	Initial a - Final a	1.975	4.2021	1.3288	-1.031	4.981	1.486	9	0.171^{NS}
3	Initial L - Final L	0.614	1.4893	0.4709	-0.4514	1.6794	1.304	9	0.225^{NS}
	Initial b - Final b	-0.1258	1.3634	0.4311	-1.1011	0.8495	-0.292	9	0.777^{NS}
	Initial a - Final a	-0.3474	2.6322	0.8324	-2.2304	1.5356	-0.417	9	0.686^{NS}

and maximizing colonization and invasion of the host epithelial surface. Hence, in the present study, a time period of 72 h [17] has been given for mature biofilm formation so that the effectiveness of the denture cleansers can be evaluated.

Shetty et al. [18], and Shireen et al. [19], evaluated the effect of *Aloe vera* on anti-fungal efficacy and found to be effective. The results were consistent with the Iseri et al. [10], and however he compared the efficacy against mouth rinses. The effect was similar with Ferreira et al. [12] when compared with multispecies biofilm.

The results of the present study are in consistent with several authors [20,21] in which the authors did not detect any color changes in the use of denture cleansers. Whereas, the results are not consistent with several authors [13,22-24] in which the authors detected a significant color change. The difference in results might be due to the fact that the samples were immersed with denture cleansers for ninety days, whereas in this study the time period selected was thirty days.

Only chemical cleansing can be considered in the present study, chemical cleansing could be a good choice for the elderly too, who require adjunctive measures to clean their dentures. Several studies [25,26] showed combination methods was more effective than chemical cleansing alone.

Sodium perborate is a peroxide type denture cleanser. When dissolved in water, it forms a solution of hydrogen peroxide. This type of cleanser combines alkaline detergents to reduce surface tension and chemicals that release oxygen from the solution. The oxygen bubbles exert a mechanical cleansing effect [27]. The denture cleansers that have been selected in the present study is evaluated for its effectiveness against *Candida albicans* mature biofilm by dipping the polymethyl methacrylate acrylic resin samples with the *Candida* biofilm in it for 8 h [8,28]. The literature has stated various time intervals for evaluating the same [29].

In this study out of the two plants extract solutions triphala solution showed increase in anti-fungal efficacy than *Aloe vera* solution when compared to the control group whereas there was no significant difference in the color stability however out of the two solutions *Aloe vera* showed better stability than triphala solution when compared to the control group.

The present study provides some clinical implications which are of benefit to the denture wearers as well as for the clinician. For use by patients with severe denture stomatitis, a denture cleanser with highest ability of biofilm removal should be recommended like combination of denture cleanser and triphala solution and if color stability is more important for the patient than a combination of denture cleanser and *Aloe vera* solution is recommended.

The limitation of this study was that mixed microbial biofilms were not assessed. In the oral cavity, microorganisms exist in polymicrobial communities and different species interact in a complex manner to modulate biofilm nature. Also, this study did not simulate the oral environment conditions in which the *Candida* biofilms develop on denture. Time of dipping the samples into denture cleansers was another factor which would have been varied to longer.

Durations to see the long term effect. Also, other cleansing aids like brushing or ultrasonic cleansing were not used to assess the efficacy of denture cleansers.

Hence, further studies should look at the *in vivo* as well as *in vitro* response of mixed communities with longer time intervals and using other denture cleaning aids also.

Conclusion

The following conclusions can be drawn:

1. Both the plant extract solutions showed significant decrease in CFU/ml for *Candida albicans* biofilm on denture base resins when compared to control group.

2. Among the two plant extract denture cleansers used, the most effective in reduction of CFU/ml for *Candida albicans* was triphala solution followed by *Aloe vera* solution.

3. The plant extract were not significantly effective in removing stains from heat cure denture acrylic resins on short term basis.

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