

Anti candidal efficacy of commercially available triphala, neem, denture cleanser and natural aloe vera leaf on heat polymerized acrylic resin

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Abstract

Aim: The aim of the study was to evaluate the anticandidal efficacy of Triphala, aloe vera, Neem, and denture cleanser on heat polymerized acrylic resin.

Settings and Designs: *In vitro* - experimental study.

Materials and Methods: In this study, forty denture wearer patients were selected and were divided into four groups consisting of ten patients, namely, Group I, Group II, Group III, and Group IV, in which dentures were cleansed with denture cleanser (tablets), Triphala (churna), aloe vera (leaf), and Neem (tablets), respectively, and stored in copper containers. Swabs were collected from the dentures before and after the use of Denture cleanser, Triphala, aloe vera, and Neem. Thereafter, the swabs were cultured on Sabouraud dextrose agar and the total Candida counts (CFU/4 cm²) were determined.

Statistical Analysis Used: One way ANOVA and Tukeys HSD post hoc test were used.

Results: The pre- to post-reduction in mean Candida count was found highest in Denture cleanser followed by Neem, Triphala, and aloe vera. Further, both Denture cleaner and Neem showed statistically significant reduction ($P < 0.001$) in the mean Candida count when compared to Triphala and aloe vera. However, reduction in the mean Candida count of both Denture cleanser and Neem was found statistically similar ($P > 0.05$).

Conclusion: The anticandidal efficacy of denture cleanser was found to be the highest. The cost-effective Neem can be used as anticandidal modality in place of denture cleanser.

Keywords: Anticandidal efficacy, Candida count, denture stomatitis, Denture wearer, heat polymerised acrylic resin, oral cavity

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Submitted: 03-Dec-2020, **Revised:** 22-Feb-2021, **Accepted:** 11-Mar-2021, **Published:** 28-Apr-2021

INTRODUCTION

Oral cavity is a site where a diverse, complex, and abundant microbial community exists. This diverse flora is seen in

edentulous patients who wear complete dentures. Dentures enhance the accumulation of food debris and formation of denture plaques, both of which can initiate a condition

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How to cite this article: Ojah P, Luniyal C, Nair C, Astekar M, Pal A, Chopra M. Anti candidal efficacy of commercially available triphala, neem, denture cleanser and natural aloe vera leaf on heat polymerized acrylic resin. *J Indian Prosthodont Soc* 2021;21:167-72.

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Quick Response Code:	Website: www.j-ips.org
	DOI: 10.4103/jips.jips_599_20

called as denture stomatitis.^[1] Candida-associated denture stomatitis is found underneath the palatal region of the denture. Commercially, there are chemical products which can serve the purpose of controlling this problem of geriatric patient, but there is an obligation to discover few more natural products that can be easily and economically available without any perilous effect. An ideal denture cleanser should be biocompatible, fungicidal, bactericidal, and nontoxic to the intrinsic structure of denture. It should effectively remove deposits and be easy to use. Natural products can also be used effectively as an alternative to synthetic chemical substances.

Ayurveda is considered as one of the world's oldest medical system. The term "Ayurveda" is a combination of word ayur signifies "Life" and word veda denotes the "system of knowledge." It originated more than 5000 years ago from Atharva Veda. According to the WHO, 80% population in the developing countries are dependent on traditional medicine for their primary health-care needs. Among the traditional medicines, the implication of plant extracts or herbs are most pervasive.

Triphala (*Phyllanthus emblica*, *Terminalia chebula*, and *Terminalia bellerica* fruits powders in equal proportion). Tannin found in triphala has a potent antifungal activity. Aloe vera extract has been traditionally used in India as therapeutic and antimicrobial aids in various ailments and are potent antifungal products effective against Candida.^[2-7] Neem (scientific name *Azadirachta indica*) is also known for its antimicrobial property. The increasing demand in Ayurveda lead to the study of these natural products to test and compare the anticandidal efficacy of triphala, aloe vera, neem, and denture cleansing tablets on heat-cured acrylic resin dentures. Copper is an essential element in trace amounts for humans. Novel coronavirus (SARS-CoV2) which is responsible for the current pandemic is very sensitive to copper surface. Copper affects the virus morphology, causes disintegration of envelope, and dispersal of surface spikes.^[8] Recent Study by US National Institute of Health showed that SARS-CoV-2 virus survives not more than 4 h on copper surfaces as compared to 48 h for stainless steel and 72 h for plastic.^[9] Copper may act like a preventive and therapeutic regimen against the COVID-19 pandemic and reducing environmental contamination.

For maintaining the hygiene of dentures various chemical, mechanical, or a combination of chemical and mechanical methods are recommended. Several elderly patients find the mechanical method difficult due to impaired motor function or physical limitation. Hence, routine use of denture cleansers is recommended. Studies have reported that prolonged use of denture cleansers can

cause undesirable effect on color, surface roughness, and hardness of denture base resins.^[10,11]

This study was undertaken to study the effect of various alternate natural products on Candida. The objective of the present study was to compare the anticandidal efficiency of triphala, neem, aloe vera, and denture cleansers on complete dentures.

MATERIALS AND METHODS

The study consisted of forty denture wearer patients that were selected randomly who had reported to the Department of Prosthodontics and Crown and Bridge. Ethical clearance was obtained from the University Ethical Committee - IRB number (IEC/142). The patients were informed about the study procedure in their mother tongue. The written informed consent of the patients was taken before they participated in the study. Selection of the patients was based on the following inclusion and exclusion criteria.

Inclusion criteria

Patients wearing a complete denture regularly for more than 6 months and not using denture cleansers were included in the study.

Exclusion criteria

Participants undergoing antifungal treatment, patients with systemic conditions or diseases, and those with denture stomatitis were excluded from the study.

They were divided into four groups; each group having ten participants each. Four groups were given either of the cleaning measures:

In denture cleanser group (Clinsodent, ICPA), patient were asked to immerse the denture into effervescent solution for 30 min. For triphala churna (Dabur India) group, 10 g of powder into 100 ml of water was taken as standardized solution and dentures were then dipped into the solution and kept overnight. Two tablets of neem (Himalaya) were dissolved into 100 ml of water which acted as a solvent and then was instructed to keep the denture immersed into the solution for overnight as in the triphala churna group. In the aloe vera group, patients were given freshly cut leaves and were advised to clean the surfaces of the dentures using those cut leaf pieces of aloe vera.^[12] The patients were asked to follow this procedure every alternate day for 15 days and report back to the department on the 16th day following the denture cleansing protocol. The patient was instructed to keep the dentures in copper vessel due to the current COVID-19 pandemic. Use of denture cleansing brushes was discouraged during the study period as it would compromise the clinical result.

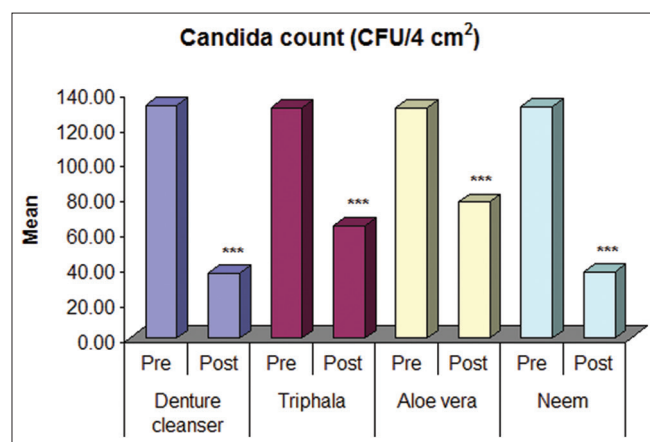
The swabs were taken from palatal surface of upper denture according to a 2 cm × 2 cm template before the start of the treatment protocol. Swabs were then taken on the 16th day after the treatment protocol. The swabs were placed in a transport medium and transported to the laboratory. The swabs were placed in a test tube containing 1 ml sterile distilled water, vortexed for 1 min. Then, using a 4-mm loop, the sample was spread in Sabouraud dextrose agar medium, incubated at 37°C for 48 h. The candidal colonies appeared as cream, pasty, smooth colonies. Colony count was determined using digital colony counter.^[12]

Statistical analysis

Candida count data were summarized as mean ± standard deviation pre- and post-candida count were compared by paired *t*-test. The change in the pre to post difference in Candida count of four groups (Denture cleanser, Triphala, aloe vera, and Neem) were compared by one-way analysis of variance (ANOVA). The significance mean difference between the groups was done by Tukey's honestly significant difference *post hoc* test after ascertaining normality by Shapiro–Wilks test. A two-tailed ($\alpha = 2$) $P < 0.05$ was considered statistically significant. Analysis was performed on SPSS software (Statistical Package for Social Sciences by IBM, New York, USA, Windows Version 17.0).

RESULTS

The pre- and post-Candida count of four groups is summarized in Table 1 and also depicted in Graphs 1 and 2.



Graph 1: Comparison of pre and post mean candida count of four groups. *** $P < 0.001$ - as compared to pre

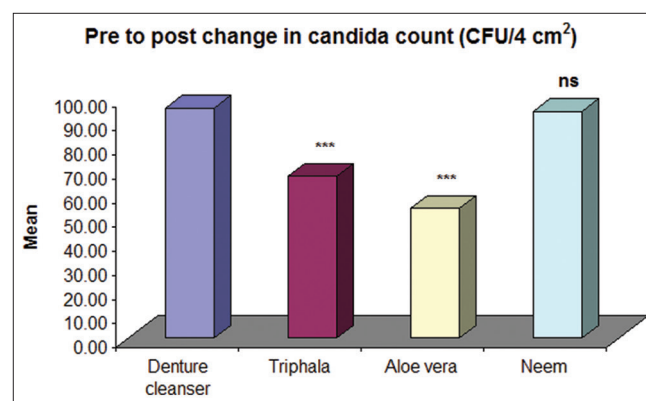
In all groups, mean Candida count decreased comparatively at posttreatment as compared to pretreatment. The decrease in mean Candida count was highest in denture cleanser followed by Neem, Triphala, and aloe vera (Aloe vera < Triphala < Neem < Denture cleanser).

For each group, comparing the difference in pre- and post-mean Candida count, paired *t*-test showed a significant ($P < 0.001$) decrease in Candida count at post as compared to pre in all groups.

To find out anticandidal efficacy of four treatments, the net mean decrease in Candida count (i.e., pre to post mean change) of four groups is further summarized in Table 2. Comparing the pre to post mean decrease in Candida count of four groups, ANOVA showed significantly different decrease in Candida count among the groups ($F = 393.70$, $P < 0.001$). Further, comparing the difference in mean decrease in Candida count between the groups, Tukey test showed significantly ($P < 0.001$) different and higher decrease in Candida count of both Denture cleanser and Neem as compared to both Triphala and aloe vera [Table 3]. Further, the decrease in mean Candida count of Triphala was also found significantly ($P < 0.001$) different and higher as compared to aloe vera. However, it did not differ ($P > 0.05$) between denture cleanser and neem, i.e., found to be statistically the same.

DISCUSSION

The term “Candida” was originated from Latin word



Graph 2: Comparisons of pre- to post-mean change in candida count of four groups. ns $P > 0.05$ or *** $P < 0.001$ - as compared to Denture cleanser

Table 1: Pre-and post-candida count (CFU/4 cm²) summary of four groups

Group	Pre (n=10)	Post (n=10)	Change (pre-post)	Paired <i>t</i>	<i>P</i>
Denture cleanser	132.70±2.95	37.20±4.18	95.50±4.28	70.64	<0.001
Triphala	131.40±2.72	64.20±3.85	67.20±1.75	121.30	<0.001
Aloe vera	131.40±3.03	77.60±2.17	53.80±3.82	44.49	<0.001
Neem	132.10±3.70	37.90±2.92	94.20±2.66	112.10	<0.001

Pre-and post-candida count were summarized in mean±SD and compared by paired *t*-test. SD: Standard deviation

candid, means white. The most common mainspring of fungal infection is the spores of *Candida* which belongs to the genus of yeast. Two distinct forms of *Candida* are present in the oral cavity, i.e., blastopores, blastoconidia in an organized biofilm, and are also known as floating planktonic cells. Structured microbial community or accumulation of biofilm occurs when there is poor oral hygiene and such doings include inadequate denture cleansing and failure to remove the denture from the mouth while sleeping.^[13-15] Approximately 10¹¹ microorganisms per gram of dry weight, surrounded by a self-produced extracellular matrix are attached to the surface.^[13,16] The pervasiveness of *Candida* increases in denture wearer patients to 60%–100% and the organism becomes expedient in nature as the denture causes decrease in the flow of oxygen and saliva to the underlying tissue thus favors the growth of *Candida* and produces an anaerobic environment. In addition, *Candida* has propinquity for the acrylic dentures. The hydrophobicity has generally been acknowledged to be one of the factors contributing to the adhesion, which is a crucial step in biofilm formation. Copper ions can inhibit RNA polymerase activity by more than 60%. It interferes with proteins which is necessary for the functions of the virus. It also generates reactive oxygen species that can kill the virus. SARS-CoV-2 virus survives not more than 4 h on copper surfaces. Hence, during the study, patients were asked to immerse the dentures in copper containers when removed from the mouth. In dentistry, use of copper has been recommended for contact surfaces in dental office. This reduces environmental contamination and thwarting the viral spread in nonoptimal use. Copper can also eradicate pathogenic organisms such as Corona virus, influenza virus, and fungi after a short period of exposure.^[17]

Table 2: Pre to post mean change in candida count (CFU/4 cm²) of four groups

Group	Change (pre-post)	F	P
Denture cleanser	95.50±4.28	393.70	<0.001
Triphala	67.20±1.75		
Aloe vera	53.80±3.82		
Neem	94.20±2.66		

Pre to post mean change in candida count of four groups were summarized in mean±SD and compared by ANOVA (F value). SD: Standard deviation, ANOVA: Analysis of variance

A. indica (Neem) has potential therapeutic values, and it is efficacious against periodontal microorganism and oral bacterial flora causing dental caries and dental plaque. Anti-candidal activity is shown by ethanolic and aqueous extract of neem against *Candida albicans*.^[18] A clinical study revealed that the leaf aqueous extract from *A. indica* (Neem) affects adhesion, cell surface hydrophobicity, and biofilm formation and its colonization by *C. albicans*. Thus, neem leaves have a potential antiadhesive effect.^[19]

When effervescent denture cleansing tablets is dissolved in water, sodium perborate perishes to form an alkaline peroxide solution that releases oxygen and enables a mechanical cleaning by oxygen formation in addition to the chemical removal of the biofilm.

Triphala meaning “three” [tri] “fruits” [phala], is a traditional ayurvedic herbal formulation. It has synergy of three “fruits” *Embolica officinalis* (Amla), *Terminalia bellerica* (Bahera), and *Terminalia chebula* (Harar). Gallic acid components present in triphala attributes to the anticandidal activity and also aqueous, ethyl acetic, and ethanolic fractions of *Terminalia Chebula* has shown a repressive action on *Candida* species.

Aloe Vera botanical name is *barbadensis miller* exhibits inhibitory action on fungi, bacteria, and viruses. It contains several nutrients and antiseptic agents such as lupeol, salicylic acid, urea nitrogen, cinnamonic acid, phenols, sulfur that have inhibitory action on fungi, bacteria, and viruses. In the present study, denture cleansing tablets, triphala churna, aloe vera, and neem showed a reduction in total *Candida* count. The total reduction in candidal count was statistically insignificant between denture cleanser tablets and neem. Individually, these products showed a potent reduction in the total *Candida* count. There were statistically significant results for all other intergroup comparison ($P < 0.05$). Neem was found to be as effective as denture cleansing tablet against *Candida*, followed by triphala and aloe vera. Hamid *et al.*^[20] reported that addition of neem powder to the denture base polymer during fabrication significantly decreased the *C. albicans* count. Studies have shown that the use of plant extract (Thyme)

Table 3: Comparison of difference in pre to post mean change in candida count (CFU/4 cm²) between groups by Tukey test

Comparisons	Mean difference	q	P	95% CI of difference
Denture cleanser versus triphala	28.30	27.28	<0.001	24.35-32.25
Denture cleanser versus aloe vera	41.70	40.20	<0.001	37.75-45.65
Denture cleanser versus neem	1.30	1.25	>0.05	2.66-5.26
Triphala versus aloe vera	13.40	12.92	<0.001	9.45to 17.35
Triphala versus neem	27.00	26.03	<0.001	23.05-30.95
Aloe vera versus neem	40.40	38.95	<0.001	36.45-44.35

q: Tukey test value, CI: Confidence interval

as denture cleanser on heat cure acrylic resin has increased structural strength as compared to denture-based resin.^[21]

Shetty *et al.*^[12] conducted a study which shows comparison of the anticandidal efficacy of the denture cleansing tablets, triphala, cashew leaf, aloe vera, and water (control) on heat polymerized acrylic complete denture. It was concluded that there was statistically significant reduction in *Candida* counts using denture cleansing tablet and triphala churna which is in agreement with the present study.

The regular use of commercially available denture cleansers has some disadvantage and cause degradation of acrylic dentures and ingestion of these products affect the burns in the esophagus, severe edema of epiglottis, pharynx, and widespread gastrointestinal ulceration.

Hence, in the current study, the natural products were used which were nontoxic, inexpensive, easy to use, and readily available. Prakash *et al.*^[22] reported higher level of *Candida* species on the denture surface as compared to the palatal mucosa. Denture wearers though may be free from diseases could be asymptomatic carriers of multispecies *Candida*. Hence, regular and adequate cleaning of dentures is important for improved oral health. This study however has its limitations as the sample size used was small based on certain criteria that might limit the extensive results. It is also vacillating whether patients strictly adhered to the set concordant instructions that were given to the patients. More studies are needed to evaluate the efficacy of these natural products as denture cleansers and people with less oral hygiene will be benefitted by the use of these natural products. Further studies are also needed to evaluate the role of dentures stored in copper containers would provide an additive effect along with the natural products as an antifungal agent.

CONCLUSION

The present study showed that, among the denture cleanser, denture cleansing tablets had the highest reduction in the candidal colonies. Among the natural products, neem had a better antifungal property on the heat-cured acrylic denture base followed by triphala churna and aloe vera. The availability, cost-effectiveness, and colossal advantages make this herb one of the best alternative to the present denture cleansing tablet agents that are used. It is, therefore, highly essential that medicinal plants extract whose properties have not been yet identified should form a top agenda in developing countries where citizens are sometimes incapable to purvey expensive devout medicine.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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