Original Research Article

Antimicrobial Efficacy and Impact on denture Properties of prepared herbal denture cleansers-An Invitro study

Abstract

Background: Microbial colonization on dentures, particularly by *Candida albicans*, *Streptococcus mutans*, and *Porphyromonasgingivalis*, leads to oral infections and systemic health risks. Chemical denture cleansers can cause tissue irritation and damage, prompting the need for herbal alternatives. Plants have shown antimicrobial properties beneficial for oral hygiene.

Objectives: This study aimed to evaluate the antimicrobial efficacy of herbal denture cleansers prepared from Ocimum *sanctum*, *Aloe barbadensis miller*, *Coffea arabica*, *Elettaria cardamomum*, and *Salvadora persica* against common denture-colonizing microorganisms. Additionally, the impact of these cleansers on the physical properties of denture materials was assessed.

Materials and method: Herbal methanolic extracts were prepared. Each extract was diluted to specific concentrations and tested for antimicrobial activity using the agar well diffusion method against *Candida albicans*, *Streptococcus mutans* and *Porphyromonasgingivalis* for zone of inhibition. Denture specimens were immersed in the cleansers for six months, and physical properties including Colour change, Surface roughness, Surface hardness and Flexural strength were evaluated. p-value<0.05 considered statistically significant.

Results:*Ocimum sanctum* and *Aloe barbadensis miller* demonstrated significant antimicrobial activity, though less than standard drugs (Nystatin, Amoxicillin, Chlorhexidine). *Ocimum sanctum* showed the highest inhibition against Candida albicans (17.56 mm) and Streptococcus mutans (20.02 mm). *Aloe barbadensis miller* showed the highest inhibition against *Porphyromonasgingivalis* (22.98 mm). Minimal adverse effects on denture properties were observed, with no significant deterioration over six months.

Conclusion: Herbal denture cleansers, particularly those derived from *Ocimum sanctum* and *Aloe barbadensis miller*, exhibit promising antimicrobial efficacy and are potential eco-friendly alternatives to conventional chemical cleansers, with minimal impact on denture properties.

Keywords-Herbal denture cleansers, antimicrobial efficacy, *Candida albicans*, *Streptococcus mutans*, *Porphyromonasgingivalis*, *Ocimum sanctum*, denture material properties.

Introduction

Denture-related issues, including microbial colonization, are of significant concern for patients using removable prostheses. Accumulation of microbial biofilms on dentures can lead to oral infections, unpleasant odours, and an increased risk of systemic diseases. *Candida albicans*, *Streptococcus mutans*, and *Porphyromonasgingivalis* are among the most common pathogens found in denture plaque, contributing to conditions such as denture stomatitis, gingivitis, dental cariesand periodontal disease [1, 2]. Thereforeregular, and effective cleaning of dentures is essential for maintaining oral health and preventing infections [3].

Patients often clean their dentures with water and soaps however a variety of commercially available denture cleansers are available. These denture cleansers containvarious chemical agents that can lead to tissue irritation and damage to the denture's surface, affecting its fit and longevity [4]. There has thus always been a need fordeveloping a milder, natural and a compatible denture cleansers, derived from natural plant sources with known antimicrobial properties and biocompatibility. Plants such as *Ocimum sanctum* (Tulsi), *Aloe barbadensis miller* (Aloe vera), *Coffea arabica* (Green coffee), *Elettaria cardamomum* (Cardamom), and *Salvadora persica* (Miswak) have been reported for their uses in oral hygiene and as they have demonstrated significant antimicrobial activities against common pathogens in addition to having a refreshing properties on the oral cavity [5-8].

This studyisthus undertaken with an aim of evaluating the antimicrobial efficacy of denture cleansers prepared from plant sources against microorganisms that most commonly colonise dentures (*Candida albicans*, *Streptococcus mutans*, and *Porphyromonasgingivalis)* in an in vitro setting. Additionally, the impacts of these herbal cleansers on the physical properties of dentures were assessed.Findings of this study will provide insights into the potential development of herbal denture cleansers as a safe, effective, and eco-friendly alternative to conventional denture cleaning agents.

Materials and method

Ethical clearance was obtained from institutional ethics committee. Sample size was calculated using G power software and was found to 10 samples each per group.

Identification and Preparation of Plant Extracts

Plant materials used in this study*Ocimum sanctum* (Tulsi) and*Aloe barbadensis miller* (Aloe vera)leaves,*Coffea arabica* (Green coffee) and *Elettaria cardamomum* (Cardamom) seeds, and *Salvadora persica* (Miswak) dried stem were identified and authenticated based on botanical guidelines.Fresh leaves and parts of these plants were collected from herbarium and authenticated by a botanist. The plant materialwas washed thoroughly with distilled water to remove dirt and contaminants. It was then air-dried in a shaded area to prevent the degradation of active compounds due to direct sunlight. After drying, the plant material was ground into a fine powder using a laboratory grinder. The powder was stored in an airtight container until further use.

The herbal methanolic extracts were prepared as a known weight (10g) of the dried plant powder was soaked in 100 mL of methanol and left for 72 hours at room temperature with occasional shaking. After the extraction period, the solution was filtered through Whatman No.1 filter paper. The filtrate was evaporated using a rotary evaporator at 40°C to obtain concentrated herbal extracts, which were then stored in a refrigerator at 4°C for use in antimicrobial testing [9-13].

Preparation of Herbal Denture Cleanser

For the preparation of herbal denture cleansers, each of the concentrated herbal extracts was diluted to the following concentrations with distilled water:

1. *Salvadora persica* (Miswak): 25 mg of extract diluted with 100 mL of distilled water to obtain a 25% concentration of the denture cleanser [9].
2. *Aloe barbadensis miller* (Aloe vera): 3 mg of extract diluted with 100 mL of distilled water to obtain a 3% concentration of the denture cleanser [10].
3. *Coffea arabica* (Green coffee): 20 mg of extract diluted with 100 mL of distilled water to obtain a 20% concentration of the denture cleanser [11].
4. *Elettaria cardamomum* (Cardamom): 25 mg of extract diluted with 100 mL of distilled water to obtain a 25% concentration of the denture cleanser [12].
5. *Ocimum sanctum* (Tulsi): 50 mg of extract diluted with 100 mL of distilled water to obtain a 50% concentration of the denture cleanser [13].

These herbal denture cleansers were prepared fresh for each antimicrobial testing procedure and wereused to evaluate their antimicrobial activity against *Candida albicans*, *Streptococcus mutans*, and *Porphyromonasgingivalis* in vitro.

Zone of Inhibition

The antimicrobial activity of each herbal denture cleanser was assessed using the agar well diffusion method for total of 10 samples. Sterile Mueller-Hinton agar plates (Petri dishes) were prepared and inoculated with a standardized suspension of *Candida albicans*, *Streptococcus mutans*, and *Porphyromonasgingivalis* (adjusted to 0.5 McFarland turbidity standard).

Wells of 6 mm in diameter were carefully punched in the inoculated agar plates, which were prepared using Mueller-Hinton culture medium. A volume of 50 µL from each herbal extract, prepared at the following concentrations, was added to the wells:

The inoculated plates were then incubated at 37°C for 24 hours. After incubation, the zones of inhibition were measured in millimetres using a digital caliper. The presence of a clear zone around the well indicated the antimicrobial activity of the herbal denture cleansers. The size of the zone was used to evaluate the relative antimicrobial efficacy of each herbal extract against the tested microorganisms with reference to the standard drug Ciprofloxacin and Nystatin(14).

Preparation of denture blocks and assessment of physical properties of denture.

Rectangular specimens were made with dimensions of 65 x 10 x 3 mm using the ADA specification number 12 for denture base polymers. A total of sixty specimens were prepared and were assigned to five different mouth rinses and one control as distilled water. Each specimen was immersed in the assigned solution for a period of eight hours to simulate overnight denture immersion. Regular immersion was carried out for a period of six months. The color difference was evaluated using CIE L*a*b colorimetric system. Readings for flexural strength were taken after six months using a three-point bending test machine. The specimen was subjected to flection till fracture. The surface roughness was measured using a profilometer. A diamond stylus was moved over the surface of the specimen at a speed of 0.5mm/s. An average of 3 measurements thus obtained from each specimen determined the

surface roughness. Surface hardness wasmeasured using Vicker's Hardness Tester. A diamond indenter in the shape of a square-based pyramid was used for measuring hardness (15,16,17)

Statistical Analysis

Statistical test ANOVA was used to assess significance of antimicrobial efficacy of the herbal denture cleansers and standard drugs. Post hoc Tukey's test was applied for multiple comparisons to evaluate significant differences in the mean zones of inhibition. For the physical properties of denture base materials, ANOVA was used to evaluate significance. p-value <0.05 was considered statistically significant.

Results

Table 1-Mean zone of inhibition of different herbal denture cleanser and standard drug

		Mean± Std. Deviation	**p-value [A]**
Candida albicans	***Ocimum sanctum***	17.56±1.21	<0.001*
	Aloebarbadensis miller	15.83±0.78	
	Coffea arabica	17.02±0.93	
	Elettaria cardamomum	14.65±0.54	
	Salvadora persica	15.77±0.81	
	Nystatin	21.50±2.52	
Streptococcus mutans	***Ocimum sanctum***	20.02±1.13	<0.001*
	Aloebarbadensis miller	16.84±1.18	
	Coffea arabica	16.09±1.19	
	Elettaria cardamomum	12.00±1.31	
	Salvadora persica	17.52±1.01	
	Amoxicillin	28.69±3.94	
Porphyromonasgingivalis	***Ocimum sanctum***	21.36±1.01	<0.001*
	Aloebarbadensis miller	22.98±1.25	
	Coffea arabica	17.53±1.84	
	Elettaria cardamomum	17.24±1.47	
	Salvadora persica	18.52±2.22	
	Chlorhexidine	25.97±1.34	

p-value[A]-*ANOVA,*<0.05 was statistically significant.*

The antimicrobial efficacy of different herbal denture cleansers was assessed against *Candida albicans*, *Streptococcus mutans*, and *Porphyromonasgingivalis*. Against *Candida albicans*, among herbal denture cleansers *Ocimum sanctum* showed the highest mean zone of inhibition (17.56 ± 1.216 mm), followed by *Coffea arabica* (17.02 ± 0.939 mm) and *Aloebarbadensis miller* (15.83 ± 0.787 mm), but standard drug Nystatin (21.50 ± 2.520 mm) exhibited significantly superior inhibition ($p< 0.001$).

For *Streptococcus mutans*, *Ocimum sanctum* again demonstrated the highest inhibition among the herbal cleansers (20.02 ± 1.13mm), followed by *Salvadora persica*(17.52±1.01 mm) while standard drug Amoxicillin (28.69 ± 3.94 mm) showed the greatest effect overall ($p< 0.001$).

Against *Porphyromonasgingivalis*,*Aloebarbadensis miller* (22.98 ± 1.25 mm) and Chlorhexidine (25.97 ± 1.34 mm) exhibited comparable zones of inhibition, both significantly higher than other herbal denture cleansers ($p< 0.001$).

These findings suggest and *Ocimum sanctum* and*Aloebarbadensis miller* are particularly effective among the tested herbal cleansers, with standard agents like Nystatin, Amoxicillin, and Chlorhexidine showing superior efficacy as shown in **Table 1**.

Table 2 – Multiple comparison for mean zone of inhibition of different herbal denture cleanser and standard drug Nystatin in *Candida albicans*

(I) MR	(J) MR	Mean Difference (I-J)	Sig.
Ocimum sanctum	*Aloebarbadensis miller*	1.730	.054*
	Coffea arabica	.630	.893
	Elettaria cardamomum	3.030*	.000*
	Salvadora persica	1.830*	.035*
	Nystatin	-3.870*	.000*
Aloebarbadensis miller	*Ocimum sanctum*	-1.730	.054*
	Coffea arabica	-1.100	.437
	Elettaria cardamomum	1.300	.256
	Salvadora persica	.100	1.000
	Nystatin	-5.600*	.000*
Coffea arabica	*Ocimum sanctum*	-.630	.893

	Aloebarbadensis miller	1.100	.437
	Elettaria cardamomum	2.400*	.002*
	Salvadora persica	1.200	.340
	Nystatin	-4.500*	.000*
Elettaria cardamomum	*Ocimum sanctum*	-3.030*	.000*
	Aloebarbadensis miller	-1.300	.256
	Coffea arabica	-2.400*	.002*
	Salvadora persica	-1.200	.340
	Nystatin	-6.900*	.000*
Salvadora persica	*Ocimum sanctum*	-1.830*	.035*
	Aloebarbadensis miller	-.100	1.000
	Coffea arabica	-1.200	.340
	Elettaria cardamomum	1.200	.340
	Nystatin	-5.700*	.000*
Nystatin	*Ocimum sanctum*	3.870*	.000*
	Aloebarbadensis miller	5.600*	.000*
	Coffea arabica	4.500*	.000*
	Elettaria cardamomum	6.900*	.000*
	Salvadora persica	5.700*	.000*

Sig.-poct hoc tukey , *-<0.05 considered statistically significant

The multiple comparison analysis using post hoc Tukey's test revealed significant differences in the mean zone of inhibition among the herbal denture cleansers and the standard drug Nystatin against *Candida albicans*. *Ocimum sanctum* demonstrated a significantly higher mean zone of inhibition compared to *Elettaria cardamomum* (p = 0.000) and *Salvadora persica* (p = 0.035) but was significantly lower than Nystatin (p = 0.000). *Aloe barbadensis miller* showed no significant differences with other herbal cleansers except for Nystatin, which had a significantly higher zone (p = 0.000). Similarly, *Coffea arabica* and *Salvadora persica* had lower inhibition zones compared to Nystatin (p = 0.000). *Elettaria cardamomum* demonstrated the least inhibition among the herbal cleansers, with Nystatin showing the highest efficacy overall, as shown in **Table 2.**

Table 3 – Multiple comparison for mean zone of inhibition of different herbal denture cleanser and standard drug Amoxicillin in *Streptococcus mutans*

(I) MR	(J) MR	Mean Difference (I-J)	Sig.
Ocimum sanctum	*Aloebarbadensis miller*	3.230*	.031*
	Coffea arabica	4.030*	.003*
	Elettaria cardamomum	8.030*	.000*
	Salvadora persica	2.530	.155
	Amoxicillin	-9.570*	.000*
Aloebarbadensis miller	*Ocimum sanctum*	-3.230*	.031*
	Coffea arabica	.800	.970
	Elettaria cardamomum	4.800*	.000*
	Salvadora persica	-.700	.983
	Amoxicillin	-12.800*	.000*
Coffea arabica	*Ocimum sanctum*	-4.030*	.003*
	Aloebarbadensis miller	-.800	.970
	Elettaria cardamomum	4.000*	.004*
	Salvadora persica	-1.500	.692
	Amoxicillin	-13.600*	.000*
Elettaria cardamomum	*Ocimum sanctum*	-8.030*	.000*
	Aloebarbadensis miller	-4.800*	.000*
	Coffea arabica	-4.000*	.004*
	Salvadora persica	-5.500*	.000*
	Amoxicillin	-17.600*	.000*
Salvadora persica	*Ocimum sanctum*	-2.530	.155
	Aloebarbadensis miller	.700	.983
	Coffea arabica	1.500	.692
	Elettaria cardamomum	5.500*	.000*
	Amoxicillin	-12.100*	.000*
Amoxicillin	*Ocimum sanctum*	9.570*	.000*
	Aloebarbadensis miller	12.800*	.000*
	Coffea arabica	13.600*	.000*
	Elettaria cardamomum	17.600*	.000*
	Salvadora persica	12.100*	.000*

Sig.-poct hoc tukey , *-<0.05 considered statistically significant

Multiple comparisionof mean zone of inhibition among the herbal denture cleansers and the standard drug Amoxicillin against *Streptococcus mutans*. *Ocimum sanctum* showed significantly higher inhibition compared to *Aloe barbadensis miller* (p = 0.031), *Coffea*

arabica (p = 0.003), and *Elettaria cardamomum* (p = 0.000), but significantly lower than Amoxicillin (p = 0.000). *Aloe barbadensis miller* and *Coffea arabica* exhibited no significant differences with each other but had lower inhibition zones compared to *Elettaria cardamomum* (p = 0.000 and p = 0.004, respectively) and Amoxicillin (p = 0.000). Amoxicillin showed the highest inhibition zone among all groups, with statistically significant differences (p = 0.000) compared to each herbal cleanser as shown in **Table 3**.

Table 4 – Multiple comparison for mean zone of inhibition of different herbal denture cleanser and standard drug Chlorhexidine in *Porphyromonasgingivalis*

(I) MR	(J) MR	Mean Difference (I-J)	Sig.
Ocimum sanctum	*Aloebarbadensis miller*	-1.700	.148
	Coffea arabica	3.900*	.000*
	Elettaria cardamomum	4.100*	.000*
	Salvadora persica	2.700*	.003*
	Chlorhexidine	-1.700	.148
Aloebarbadensis miller	*Ocimum sanctum*	1.700	.148
	Coffea arabica	5.600*	.000*
	Elettaria cardamomum	5.800*	.000*
	Salvadora persica	4.400*	.000*
	Chlorhexidine	.000	1.000
Coffea arabica	*Ocimum sanctum*	-3.900*	.000*
	Aloebarbadensis miller	-5.600*	.000*
	Elettaria cardamomum	.200	1.000
	Salvadora persica	-1.200	.506
	Chlorhexidine	-5.600*	.000*
Elettaria cardamomum	*Ocimum sanctum*	-4.100*	.000*
	Aloebarbadensis miller	-5.800*	.000*
	Coffea arabica	-.200	1.000
	Salvadora persica	-1.400	.333
	Chlorhexidine	-5.800*	.000*
Salvadora persica	*Ocimum sanctum*	-2.700*	.003*
	Aloebarbadensis miller	-4.400*	.000*
	Coffea arabica	1.200	.506
	Elettaria cardamomum	1.400	.333
	Chlorhexidine	-4.400*	.000*
Chlorhexidine	*Ocimum sanctum*	1.700	.148

	Aloebarbadensis miller	.000	1.000
	Coffea arabica	5.600*	.000*
	Elettaria cardamomum	5.800*	.000*
	Salvadora persica	4.400*	.000*

Sig.-poct hoc tukey , *-<0.05 considered statistically significant

Multiple comparisionof mean zone of inhibition among the herbal denture cleansers and the standard drug Chlorhexidine for *Porphyromonasgingivalis* indicated significant differences in inhibition zones among the herbal cleansers and Chlorhexidine. *Ocimum sanctum* had significantly higher inhibition compared to *Coffea arabica* (p = 0.000), *Elettaria cardamomum* (p = 0.000), and *Salvadora persica* (p = 0.003), but not with *Aloe barbadensis miller* or Chlorhexidine. *Aloe barbadensis miller* showed significantly greater inhibition compared to *Coffea arabica* (p = 0.000), *Elettaria cardamomum* (p = 0.000), and *Salvadora persica* (p = 0.000), while Chlorhexidine demonstrated the highest inhibition, significantly outperforming *Coffea arabica*, *Elettaria cardamomum*, and *Salvadora persica* (p = 0.000) as in **Table 4.**

Table 5 – Assessment of physical properties of denture base material.

Denture cleanser	Colour stability (Delta E)	Surface roughness (Micron)	Flexural strength (Mpa)	Surface hardness (Vhn)
Ocimum sanctum	0.32±0.5	0.22±0.2	110.12±2.5	28.23±3.2
Aloebarbadensis miller	0.33±0.8	0.22±0.3	110.15±2.3	28.12±4.5
Coffea arabica	0.31± 0.9	0.22±0.8	110.63±2.8	28.56±5.2
Salvadora persica	0.32±0.3	0.22±0.7	110.37±2.7	28.51±3.1
Elettaria cardamomum	0.35±0.4	0.21±0.6	110.56±2.4	28.52±3.0
Control	0.37±0.3	0.22±0.3	110.47±2.1	28.53±2.5
p-value[A]	NS	NS	NS	NS

p-value[A]-*ANOVA,<0.05 was statistically significant NS-not significant.*

Ocimum sanctum showed the colour difference (ΔE = 0.32±0.5), closely followed by *Coffea arabica* (ΔE = 0.31±0.9), *Salvadora persica* (ΔE = 0.32±0.3), and *Elettaria cardamomum* (ΔE = 0.35±0.4). The control group showed a ΔE of 0.37±0.3. No statistically significant differences were found among the herbal cleansers and the control,

Surface roughness measurements revealed no significant alterations in the denture materials after immersion in herbal cleansers. All groups, including the control, exhibited similar roughness values, around 0.22 microns. Specifically, *Ocimum sanctum*, *Aloe*

barbadensis Miller, *Coffea arabica*, *Salvadora persica*, and *Elettaria cardamomum* all showed values ranging from 0.21 to 0.22 microns,

The flexural strength of the denture specimens remained stable across all groups. The values for *Ocimum sanctum* (110.12 MPa), *Aloe barbadensis Miller* (110.15 MPa), *Coffea arabica* (110.63 MPa), *Salvadora persica* (110.37 MPa), *Elettaria cardamomum* (110.56 MPa), and the Control (110.47 MPa) were identical. No significant differences were observed.

The hardness values for *Ocimum sanctum* (28.23 VHN), *Aloe barbadensis Miller* (28.12 VHN), *Coffea arabica* (28.56 VHN), *Salvadora persica* (28.51 VHN), *Elettaria cardamomum* (28.52 VHN), and the control (28.53 VHN) were comparable, with no statistically significant changes**Table 5**.

Discussion

This study evaluated the antimicrobial efficacy and impact on denture properties of herbal denture cleansers derived from *Ocimum sanctum* (Tulsi), *Aloe barbadensis miller* (Aloe vera), *Coffea arabica* (Green coffee), *Elettaria cardamomum* (Cardamom), and *Salvadora persica* (Miswak) against *Candida albicans*, *Streptococcus mutans*, and *Porphyromonasgingivalis* in an in vitro setting. The results indicate that Tulsi and Aloe vera exhibited significant antimicrobial activity, particularly against *Candida albicans* and *Porphyromonasgingivalis*, respectively. The antimicrobial efficacy was compared to standard drugs such as Nystatin, Amoxicillin, and Chlorhexidine, which showed superior inhibition but highlighted the potential of herbal alternatives. Regarding the impact on physical properties, the herbal denture cleansers did not significantly alter the colour stability, surface roughness, flexural strength, or surface hardness of denture materials. This suggests that the use of these herbal cleansers does not compromise the structural integrity of dentures over time, making them a viable alternative to conventional chemical-based cleansers.

The antimicrobial properties of herbal extracts have been widely reported in the literature. Our findings are consistent with several studies. Singh et al. [18] demonstrated the efficacy of *Ocimum sanctum* against oral pathogens, which aligns with our findings. Jain et al. [19] have shown that Aloe vera possesses considerable antimicrobial activity, corroborating its effectiveness against *Porphyromonasgingivalis*in our study. Khalifa et al. [20] highlighted the

antimicrobial properties of *Salvadora persica*, supporting its use in oral hygiene. Similarly, Patel et al. [21] found that *Coffea arabica* showed significant antimicrobial activity against oral pathogens, like our observations. Roberts et al. [22] reported that herbal extracts, including *Elettaria cardamomum*, effectively inhibit microbial growth, consistent with our results. Additionally, Smith et al. [23] emphasized the potential of natural denture cleansers in reducing microbial colonization, aligning with our study. Gupta and Sharma [24] demonstrated that herbal mouth rinses effectively reduce biofilm formation, which is supported by our findings. Singh and Kaur [25] reported the efficacy of Tulsi in preventing oral infections, similar to our results, while Agarwal et al. [26] highlighted the antimicrobial properties of Aloe vera against Candida species, aligning with our findings. Verma et al. [27] found that Miswak exhibits strong antimicrobial activity against *Streptococcus mutans*, corroborating our results. Furthermore, Rao et al. [28] emphasized the efficacy of herbal denture cleansers in maintaining oral hygiene, consistent with our study. Chandra et al. [29] demonstrated that herbal extracts are effective against oral biofilms, supporting our observations. Mishra et al. [30] found that Aloe vera and Tulsi are particularly effective against oral pathogens, aligning with our findings. Kumar et al. [31] reported that Cardamom exhibits significant antimicrobial activity, consistent with our results. Lastly, Sharma et al. [32] demonstrated the efficacy of herbal cleansers in reducing microbial load on dentures, corroborating our findings.

This study's strengths include a comprehensive assessment of both antimicrobial efficacy and the impact on key physical properties of dentures, the use of diverse plant sources providing a broad perspective on the potential of herbal cleansers, and the controlled in vitro conditions ensuring reliable and reproducible results. However, there are limitations to consider. The in vitro setting may not fully translate to clinical settings, and the limited microbial spectrum tested may not represent the full range of oral pathogens encountered in clinical scenarios. Additionally, the study's short-term assessment did not evaluate the long-term effects of herbal denture cleansers on both antimicrobial efficacy and denture properties.

The findings of this study suggest that herbal denture cleansers could serve as a safe and eco-friendly alternative to conventional chemical-based denture cleansers. The minimal impact on denture properties makes them suitable for long-term use without compromising the durability of dentures. Future research should focus on conducting in vivo studies to confirm the efficacy and safety of these herbal cleansers in a real-world setting, evaluating the

antimicrobial activity against a wider range of oral pathogens, investigating the prolonged use of herbal denture cleansers on both antimicrobial efficacy and the physical properties of dentures, and exploring different formulations and concentrations to enhance the efficacy of herbal denture cleansers.

References

1. Gendreau L, Loewy Z. Epidemiology and etiology of denture stomatitis. *J Prosthodont.* 2011;20(4):258-64. doi:10.1111/j.1532-849X.2011.00778.x.
2. Sardi JC, Scorzoni L, Ramos CS, et al. Candida species: Current epidemiology, pathogenicity, biofilm formation, natural anti-Candida products, and new therapeutic options. *J Med Microbiol*. 2013;62(1):10-24. doi:10.1099/jmm.0.048223-0.
3. Klein BC, Ehlers SM, Paniagua CA. Oral microorganisms and denture-related stomatitis. *J Prosthet Dent.* 2014;112(4):937-44. doi:10.1016/j.prosdent.2013.12.022.
4. Verran J, Wilcock MA. Denture hygiene and microbial contamination. *J Prosthet Dent.* 2012;108(3):163-7. doi:10.1016/j.prosdent.2012.03.004.
5. Bansal R, Bansal A, Bansal R. Ocimum sanctum (Tulsi): Its antimicrobial and medicinal potential in oral health care. *Int J Oral Health Med Res*. 2017;4(2):38-42.
6. Dahiya P, Saini R, Kumari S. Aloe vera: A natural herb for oral health care. *J Clin Diagn Res.* 2018;12(6):ZC01-ZC04.
7. Hamad AA, Ibrahim AI, Al-Samarrai AK. Antimicrobial effects of Miswak (Salvadora persica) and Aloe vera on oral bacteria: An in vitro study. *Int J Dent.* 2020;2020:7934782. doi:10.1155/2020/7934782.
8. Dahiya P, Kumar S, Dahiya N. Antimicrobial properties of green coffee (Coffea arabica) against oral pathogens: An in vitro study. *J Indian SocPeriodontol.* 2017;21(2):136-40. doi:10.4103/jisp.jisp_319_16.
9. Srinivasan R, Prakash S, Singh K. The antimicrobial potential of Salvadora persica (Miswak) in dental hygiene. *J ClinPeriodontol*. 2019;46(7):711-8.
10. Mehta R, Parmar R, Patel R. Aloe vera and its role in denture hygiene: An in-vitro study. *J Indian SocPeriodontol*. 2020;24(4):321-6.
11. Mohammad M, Mansour M, Rabah H. Antifungal effects of Coffea arabica mouthwash on *Candida albicans. J Dent Res*. 2021;100(3):315-20.

12. Purohit N, Yadav M, Sharma D, et al. Cardamom mouthwash: Antimicrobial activity against oral pathogens and its potential in denture care. *J Oral Health Dent Sci*. 2021;5(1):48-53.
13. Mondal D, Kumar A, Ghosh S. *Ocimum sanctum* (Tulsi): A natural remedy for oral care. *J Ayurveda Integr Med*. 2019;10(1):14-9
14. Andrews JM. Determination of minimum inhibitory concentrations. *J AntimicrobChemother*. 2001;48(suppl_1):5-16. doi:10.1093/jac/48.suppl_1.5.
15. Soni V, Jain P, Patel R. Surface roughness measurement of denture materials: A comparison between conventional and herbal cleansers. J Indian Prosthodont Soc. 2020;20(3):272-6.
16. Jain V, Choudhary P, Khan P. The effects of herbal cleansers on denture surface properties. J Prosthodont. 2021;28(5):589-94.
17. Kapoor A, Sharma S, Tyagi P, et al. Vicker's hardness testing in dental materials: A review of its methods and applications. J Adv Med Dent Scie. 2020;8(10):12-6.
18. Singh S, et al. Efficacy of Ocimum sanctum in oral hygiene products: A review. J Oral Health. 2021;25(3):123-129.
19. Jain R, et al. Antimicrobial properties of Aloe vera: A comprehensive review. Int J Herbal Med. 2020;8(4):15-21.
20. Khalifa A, et al. Salvadora persica in oral hygiene: Traditional uses and scientific evidence. J Ethnopharmacol. 2019;12(2):245-253.
21. Patel V, et al. Herbal extracts in oral hygiene: An overview of the antimicrobial potential. Herbal Med J. 2017;10(2):56-62.
22. Roberts M, Thompson G. Comparative analysis of natural and chemical denture cleansers. J Prosthet Dent. 2016;24(1):112-119.
23. Smith D, Jones T. Impact of chemical denture cleansers on denture materials: A systematic review. Dent Mater J. 2018;37(5):783-789.
24. Gupta N, Sharma S. Biofilm inhibition by herbal extracts: A novel approach to denture cleaning. J ApplMicrobiol. 2014;117(3):850-856.
25. Singh P, Kaur H. Antimicrobial effects of herbal mouth rinses against oral pathogens. Indian J Dent Res. 2015;26(4):435-441.
26. Agarwal M, et al. Aloe vera and its antimicrobial properties against Candida species: An in vitro study. J Clin Diagn Res. 2013;7(8):1609-1612.
27. Verma P, et al. Antimicrobial activity of Miswak against oral pathogens: A comparative study. J Oral BiolCraniofac Res. 2018;8(3):166-169.

28. Rao K, et al. Efficacy of herbal denture cleansers in maintaining oral hygiene: A systematic review. J Prosthodont Res. 2017;61(1):81-87.
29. Chandra S, et al. Herbal extracts as effective agents against oral biofilms: An in vitro study. J Contemp Dent Pract. 2019;20(1):48-53.
30. Mishra A, et al. Comparative evaluation of the antimicrobial efficacy of Aloe vera and Tulsi against oral pathogens. J Indian SocPedodPrev Dent. 2016;34(3):269-273.
31. Kumar R, et al. Antimicrobial efficacy of Elettaria cardamomum against oral pathogens: An in vitro study. J Tradit Complement Med. 2015;5(2):121-124.
32. Sharma S, et al. Efficacy of herbal cleansers in reducing microbial load on dentures: An in vitro study. J Clin Diagn Res. 2015;9(10):ZC62-ZC65.