

## Original Research

Received 09 June 2026

Revised 21 July 2026

Accepted 04 Aug 2026

Natural Resources for Human Health 2026, Vol. 6 (15s):182–192

### KEYWORDS:

oral biofilm, medicinal plants, chlorhexidine, *Azadirachta indica*, *Ocimum sanctum*, *Syzygium aromaticum*, *Curcuma longa*, antimicrobial activity.

## Comparative Antibiofilm Activity of Indigenous Medicinal Plants and Chlorhexidine against Oral Pathogens: An In Vitro Experimental Study

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### ABSTRACT:

**Background:** Oral biofilms contribute substantially to dental caries, periodontal disease, and other oral infections, creating interest in plant-derived antibiofilm agents.

**Aim:** To compare the antibiofilm activity of *Azadirachta indica*, *Ocimum sanctum*, *Syzygium aromaticum*, and *Curcuma longa* with 0.12% chlorhexidine against selected oral pathogens.

**Methods:** This in-vitro experimental study evaluated 200 observations involving *Streptococcus mutans*, *Streptococcus sanguinis*, *Enterococcus faecalis*, and *Candida albicans*. Biofilm inhibition was assessed using optical density at 595 nm, while antimicrobial activity was additionally evaluated using changes in log<sub>10</sub> CFU/mL. Six treatment groups were evaluated: 0.12% chlorhexidine, *A. indica*, *O. sanctum*, *S. aromaticum*, *C. longa*, and a negative control.

**Results:** Mean biofilm inhibition was 79.58% with chlorhexidine, 72.99% with *S. aromaticum*, 68.39% with *A. indica*, 63.67% with *O. sanctum*, 53.84% with *C. longa*, and 5.59% with the negative control. Treatment significantly affected biofilm inhibition (two-way

ANOVA,  $p < 0.001$ ) and  $\log_{10}$  CFU reduction ( $p < 0.001$ ), while pathogen type also showed significant effects. The treatment-by-pathogen interaction was not statistically significant for either outcome.

**Conclusion:** All four indigenous medicinal plants demonstrated measurable antibiofilm activity compared with the negative control, although their activity differed from 0.12% chlorhexidine. *S. aromaticum* demonstrated the highest antibiofilm activity among the tested plants, supporting further investigation of standardized clove preparations as potential oral antibiofilm agents.

## INTRODUCTION

Dental biofilms are structured microbial communities embedded within an extracellular matrix that supports microbial adhesion, spatial organization, and persistence [1]. Dental caries and periodontal disease are associated with changes in the composition and activity of dental plaque, making biofilm control an important objective of preventive and therapeutic oral care [2]. The matrix and interactions among microorganisms also contribute to the reduced antimicrobial susceptibility of established oral biofilms [1, 2]. Chlorhexidine remains one of the most extensively investigated chemical antiplaque agents. A Cochrane review found strong evidence of plaque reduction when chlorhexidine mouthrinse was used alongside mechanical oral hygiene, while also reporting increased extrinsic tooth staining [3]. Taste disturbances and other tolerability concerns further motivate investigation of plant-derived products as potential adjunctive antimicrobial agents [3].

Medicinal plants contain biologically active compounds, including polyphenols, alkaloids, and essential oils, that have been investigated for oral antimicrobial and antibiofilm applications [4, 5]. Systematic reviews identify promising botanical interventions but also emphasize differences in plant species, extraction procedures, concentrations, formulations, and biofilm models [5, 6]. Essential-oil toothpaste studies illustrate the importance of testing formulated products rather than assuming that the activity of an isolated ingredient will be preserved after incorporation into an oral-care preparation [7]. *Azadirachta indica* (neem) has been investigated for antiplaque activity in a clinical gel study and for antimicrobial activity against multispecies dentinal biofilms [8, 9]. A systematic review of neem mouthrinses found promising plaque and gingivitis outcomes, but the small number of trials and limitations in reporting prevented firm clinical conclusions [10]. *Ocimum sanctum* (Tulsi) has similarly shown activity against a periodontal pathogen in vitro and has been evaluated in a randomized mouthwash trial assessing plaque and gingival inflammation [11, 12].

*Syzygium aromaticum* (clove) is rich in eugenol, a phenolic compound with documented antimicrobial properties [13, 14]. Clove essential oil has been evaluated against a multispecies oral biofilm [15]. Eugenol has also demonstrated activity against *Candida albicans* biofilms and against single and mixed biofilms involving *C. albicans* and *Streptococcus mutans* [16, 17]. These experimental findings provide a rationale for evaluating clove preparations against both bacterial and fungal oral microorganisms, while their clinical effectiveness remains to be established. *Curcuma longa* (turmeric) contains curcuminoids with experimental oral antibiofilm activity. Hu et al. reported that curcumin inhibited *S. mutans* sortase A activity and reduced biofilm formation [18]. Li et al. subsequently demonstrated effects on *S. mutans* biofilm metabolism, bacterial viability, and extracellular polysaccharides [19]. Turmeric-containing mouthwash preparations have also been evaluated in a randomized clinical study of salivary bacterial levels, although these outcomes differ from direct measurements of experimental biofilm inhibition [20]. Despite increasing interest in botanical products, direct comparisons involving several indigenous medicinal plants against multiple oral pathogens under a common experimental framework remain limited. Therefore, the present study compared the antibiofilm activity of *A. indica*, *O. sanctum*, *S. aromaticum*, and *C. longa* with 0.12% chlorhexidine against four clinically relevant oral microorganisms.

## Objective

The primary objective was to compare the percentage inhibition of biofilm formation produced by four indigenous medicinal plants and 0.12% chlorhexidine. The secondary objective was to compare the corresponding reduction in viable microbial load, expressed as  $\log_{10}$  CFU/mL.

## Hypothesis

The null hypothesis was that there would be no significant difference in biofilm inhibition or log<sub>10</sub> CFU reduction among the tested treatment groups.

## MATERIALS AND METHODS

### Study Design

An in-vitro experimental comparative study was conducted using 200 experimental observations. The dataset comprised six treatment groups evaluated against four oral pathogens.

The study did not involve human participants; therefore, the observations represent experimental specimens or microbial preparations rather than enrolled patients.

### Experimental Groups

Six treatment groups were represented in the dataset:

1. 0.12% chlorhexidine — reference treatment
2. *Azadirachta indica* (Neem)
3. *Ocimum sanctum* (Tulsi)
4. *Syzygium aromaticum* (Clove)
5. *Curcuma longa* (Turmeric)
6. Negative control

The four microorganisms evaluated were:

- *Streptococcus mutans*
- *Streptococcus sanguinis*
- *Enterococcus faecalis*
- *Candida albicans*

The dataset contained 33–34 observations per treatment group and 8–9 observations for each treatment-pathogen combination.

### Preparation of Test Materials

The experimental preparations were evaluated according to the laboratory protocol used to generate the dataset. The reference group consisted of 0.12% chlorhexidine, whereas the negative-control group received the corresponding control preparation specified in the original laboratory protocol.

### Biofilm Assessment

Biofilm formation was quantified using optical density measurements at 595 nm. Baseline biofilm optical density was recorded before treatment, followed by post-treatment optical density measurement.

The percentage biofilm inhibition was calculated as:

$$\text{Biofilm inhibition (\%)} = [(\text{Baseline OD} - \text{Post-treatment OD}) / \text{Baseline OD}] \times 100$$

Higher inhibition percentages represented greater suppression of biofilm biomass.

### Microbial Viability Assessment

Microbial viability was assessed using baseline and post-treatment microbial counts expressed as log<sub>10</sub> CFU/mL.

The reduction in viable microbial load was calculated as:

$$\text{Log}_{10} \text{ CFU reduction} = \text{Baseline log}_{10} \text{ CFU/mL} - \text{Post-treatment log}_{10} \text{ CFU/mL}$$

A larger positive value represented greater reduction in viable microorganisms.

## Statistical Analysis

The dataset was analyzed using descriptive and inferential statistical methods. Continuous variables were expressed as mean and standard deviation. The primary outcome was percentage biofilm inhibition, while log<sub>10</sub> CFU reduction was considered the secondary microbiological outcome. A two-way analysis of variance (ANOVA) was used to evaluate the independent effects of treatment group and pathogen and their interaction. When significant overall treatment differences were identified, Tukey's honestly significant difference post-hoc test was used for pairwise comparisons. Statistical significance was established at  $p < 0.05$ .

## RESULTS

### Overall Treatment Effect

A total of 200 experimental observations were included in the analysis. The highest overall mean biofilm inhibition was observed with 0.12% chlorhexidine ( $79.58 \pm 5.54\%$ ), followed by *S. aromaticum* ( $72.99 \pm 6.32\%$ ), *A. indica* ( $68.39 \pm 5.94\%$ ), *O. sanctum* ( $63.67 \pm 5.80\%$ ), and *C. longa* ( $53.84 \pm 6.64\%$ ). The negative control produced only  $5.59 \pm 4.62\%$  inhibition (Table 1; Figure 1). The corresponding mean log<sub>10</sub> CFU reductions were  $1.23 \pm 0.12$  for chlorhexidine,  $1.12 \pm 0.16$  for *S. aromaticum*,  $1.06 \pm 0.15$  for *A. indica*,  $1.04 \pm 0.20$  for *O. sanctum*,  $0.86 \pm 0.17$  for *C. longa*, and  $0.14 \pm 0.14$  for the negative control (Table 1).

**Table 1.** Overall antibiofilm activity according to treatment group

| Treatment                          | n  | Biofilm inhibition, mean $\pm$ SD (%) | 95% CI      | Log <sub>10</sub> CFU reduction, mean $\pm$ SD |
|------------------------------------|----|---------------------------------------|-------------|------------------------------------------------|
| 0.12% Chlorhexidine                | 34 | $79.58 \pm 5.54$                      | 77.65–81.52 | $1.23 \pm 0.12$                                |
| <i>Syzygium aromaticum</i> (Clove) | 33 | $72.99 \pm 6.32$                      | 70.75–75.24 | $1.12 \pm 0.16$                                |
| <i>Azadirachta indica</i> (Neem)   | 34 | $68.39 \pm 5.94$                      | 66.31–70.47 | $1.06 \pm 0.15$                                |
| <i>Ocimum sanctum</i> (Tulsi)      | 33 | $63.67 \pm 5.80$                      | 61.60–65.73 | $1.04 \pm 0.20$                                |
| <i>Curcuma longa</i> (Turmeric)    | 33 | $53.84 \pm 6.64$                      | 51.49–56.19 | $0.86 \pm 0.17$                                |
| Negative control                   | 33 | $5.59 \pm 4.62$                       | 3.96–7.23   | $0.14 \pm 0.14$                                |

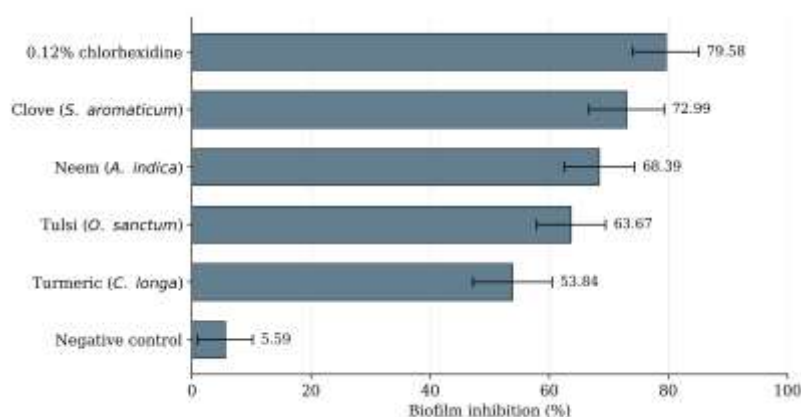


**Figure 1.** Overall biofilm inhibition by treatment group. Bars represent means and error bars represent  $\pm 1$  standard deviation, pooled across the four pathogens. Values are from Table 1;  $n = 33$ – $34$  observations per treatment group.

## Pathogen-Specific Findings

The mean biofilm inhibition across treatment groups was highest for *Streptococcus mutans* ( $60.31 \pm 25.80\%$ ), followed by *S. sanguinis* ( $59.75 \pm 24.67\%$ ), *E. faecalis* ( $56.28 \pm 25.21\%$ ), and *C. albicans* ( $53.26 \pm 24.91\%$ ). The treatment-specific data demonstrated that chlorhexidine consistently produced high inhibition across all four organisms. Among the botanical preparations, clove generally produced the highest inhibition (Table 2; Figure 2). For *S. mutans*, mean inhibition was 83.65% with chlorhexidine, 75.41% with clove, 72.12% with neem, 67.27% with Tulsi, and 55.05% with turmeric. For *S. sanguinis*, mean inhibition was 79.65% with chlorhexidine and 77.30% with clove, while neem, Tulsi, and turmeric produced 68.64%, 65.60%, and 55.96% inhibition, respectively. For *E. faecalis*, inhibition was 78.47% with chlorhexidine, 71.24% with clove, 68.43% with neem, 61.43% with Tulsi, and 54.17% with turmeric. For *C. albicans*, inhibition was 76.03% with chlorhexidine, 67.72% with clove, 63.85% with neem, 59.90% with Tulsi, and 50.02% with turmeric.

**Table 2.** Biofilm inhibition (%) according to treatment and pathogen

| Pathogen            | Chlorhexidine | Neem         | Tulsi        | Clove        | Turmeric     | Negative control |
|---------------------|---------------|--------------|--------------|--------------|--------------|------------------|
| <i>S. mutans</i>    | 83.65 ± 5.86  | 72.12 ± 7.03 | 67.27 ± 5.37 | 75.41 ± 5.59 | 55.05 ± 9.16 | 8.37 ± 6.01      |
| <i>S. sanguinis</i> | 79.65 ± 4.31  | 68.64 ± 5.39 | 65.60 ± 6.37 | 77.30 ± 4.86 | 55.96 ± 4.41 | 7.73 ± 3.22      |
| <i>E. faecalis</i>  | 78.47 ± 3.80  | 68.43 ± 3.80 | 61.43 ± 2.85 | 71.24 ± 5.03 | 54.17 ± 6.36 | 3.92 ± 3.16      |
| <i>C. albicans</i>  | 76.03 ± 5.73  | 63.85 ± 4.53 | 59.90 ± 5.41 | 67.72 ± 5.83 | 50.02 ± 4.70 | 2.01 ± 1.69      |

Values are mean ± SD.

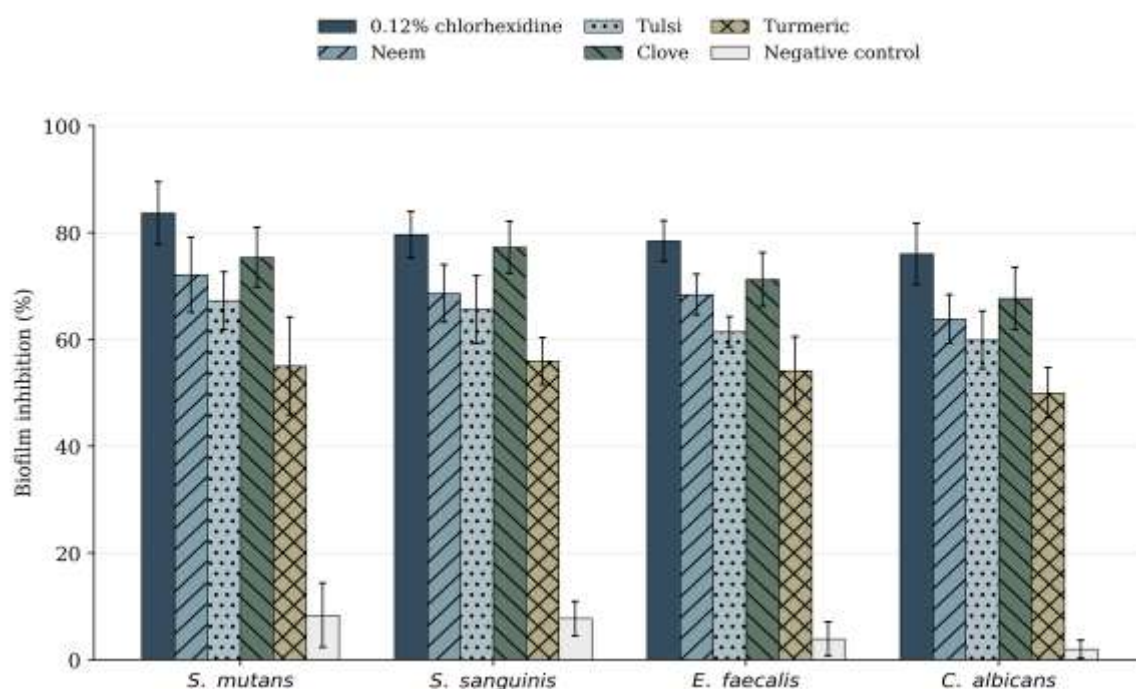


**Figure 2.** Biofilm inhibition by treatment group and pathogen. Bars represent means and error bars represent  $\pm 1$  standard deviation. Values are from Table 2;  $n = 8-9$  observations per treatment–pathogen combination. Neem: *A. indica*; Tulsi: *O. sanctum*; clove: *S. aromaticum*; turmeric: *C. longa*.

## Analysis of Variance

Two-way ANOVA demonstrated a highly significant effect of treatment on biofilm inhibition ( $F = \text{approximately } 846$ ;  $p < 0.001$ ). Pathogen type also had a statistically significant effect ( $p < 0.001$ ) (Table 3). However, the treatment  $\times$  pathogen interaction was not statistically significant ( $p = 0.934$ ), indicating that the relative treatment pattern did not differ significantly according to the

microorganism tested. For log<sub>10</sub> CFU reduction, treatment again demonstrated a highly significant effect ( $F=206.88$ ;  $p<0.001$ ), while pathogen type also showed a significant effect ( $F=5.70$ ;  $p<0.001$ ). The treatment  $\times$  pathogen interaction was not significant ( $p=0.873$ ) (Table 3).

**Table 3.** Two-way ANOVA

| Outcome                         | Factor                      | F value | p value |
|---------------------------------|-----------------------------|---------|---------|
| Biofilm inhibition              | Treatment                   | ~846    | <0.001  |
|                                 | Pathogen                    | ~17.7   | <0.001  |
|                                 | Treatment $\times$ pathogen | ~0.51   | 0.934   |
| Log <sub>10</sub> CFU reduction | Treatment                   | 206.88  | <0.001  |
|                                 | Pathogen                    | 5.70    | <0.001  |
|                                 | Treatment $\times$ pathogen | 0.60    | 0.873   |

## Post-hoc Analysis

Tukey's multiple-comparison analysis demonstrated significant differences between all treatment groups for percentage biofilm inhibition. Compared with neem, chlorhexidine produced 11.19 percentage points greater inhibition ( $p<0.001$ ), whereas clove produced 4.61 percentage points greater inhibition ( $p=0.018$ ). Clove also demonstrated significantly greater inhibition than Tulsi (difference 9.33 percentage points,  $p<0.001$ ) and turmeric (difference 19.15 percentage points,  $p<0.001$ ). For log<sub>10</sub> CFU reduction, chlorhexidine produced significantly greater reduction than neem (mean difference 0.173 log<sub>10</sub> CFU/mL,  $p<0.001$ ), Tulsi (0.233,  $p<0.001$ ), and turmeric (0.373,  $p<0.001$ ). The difference between chlorhexidine and clove was not statistically significant for log<sub>10</sub> CFU reduction (mean difference 0.109 log<sub>10</sub> CFU/mL,  $p=0.063$ ).

## DISCUSSION

The present in-vitro study demonstrated significant differences in antibiofilm activity among the tested agents. Chlorhexidine produced the highest mean biofilm inhibition (79.58%), followed by *Syzygium aromaticum* (72.99%), *Azadirachta indica* (68.39%), *Ocimum sanctum* (63.67%), and *Curcuma longa* (53.84%), whereas the negative control showed only 5.59% inhibition. The significant treatment effect observed by two-way ANOVA ( $p<0.001$ ) indicates that the tested agents differed substantially in their ability to inhibit biofilm formation. The absence of a significant treatment  $\times$  pathogen interaction further indicates that the relative pattern of treatment activity remained broadly consistent across the microorganisms evaluated.

The findings are in agreement with the growing evidence supporting medicinal plants as potential antibiofilm agents. Cardoso et al. (2021) [5] conducted a systematic review of 47 randomized clinical trials and reported that several medicinal plants demonstrated beneficial effects against oral biofilm-associated conditions. Notably, *Azadirachta indica* extracts showed efficacy similar to chlorhexidine for improvement of periodontal parameters, including plaque and gingival indices. This observation is consistent with the present finding that neem produced substantial antibiofilm activity (68.39%), although its mean inhibition remained below that of chlorhexidine. The difference between the clinical evidence and the present laboratory findings should be interpreted cautiously because the former assessed clinical periodontal outcomes, whereas the present study directly quantified experimental biofilm inhibition. Dhingra and Vandana (2017) also identified promising neem mouthrinse outcomes, but emphasized the limited evidence and methodological weaknesses of the included trials [10]. Consequently, comparable plaque or gingival index outcomes should not be interpreted as proof that neem and chlorhexidine have equivalent effects in the present laboratory model.

The relatively high activity of *Syzygium aromaticum* was one of the important findings of the present study. Clove demonstrated 72.99% mean biofilm inhibition and was the most active botanical preparation investigated. This result is strongly supported by Vasconcelos et al. (2022), [15] who evaluated *S. aromaticum* essential oil against *Candida* species and a multispecies oral biofilm. The authors reported reduced CFU counts and biofilm metabolic activity following treatment, with the essential oil not significantly different from chlorhexidine for some biofilm outcomes. The comparable performance observed in the present study may be associated with the high concentration of eugenol and other bioactive constituents present in clove. Importantly,

the current study extends these observations by demonstrating activity against both bacterial and fungal oral pathogens within the same experimental framework.

Further evidence for the antibiofilm potential of clove was provided by Kačániová et al. (2021) [21], who investigated the chemical composition and antimicrobial and antibiofilm activity of *S. aromaticum* essential oil. Eugenol constituted 82.4% of the essential oil, and the preparation demonstrated antimicrobial activity against several bacterial and fungal organisms, with experimental observations indicating effects on established biofilms. These findings provide a plausible chemical basis for the relatively strong activity of clove observed in the present investigation. Nevertheless, differences in extraction procedures, eugenol concentration, microbial species, biofilm maturity, and exposure conditions mean that numerical inhibition values cannot be directly compared between the studies. Broader investigations of eugenol and clove essential oil also report antimicrobial or antibiofilm activity, including experiments against *Helicobacter pylori*; these findings provide complementary context but cannot establish efficacy against oral biofilms [13, 14, 22].

More directly relevant to the present fungal and bacterial outcomes, He et al. (2007) investigated eugenol against preformed *C. albicans* biofilms and subsequent biofilm formation [16]. Jafri et al. (2020) evaluated eugenol against single and mixed *C. albicans*–*S. mutans* biofilms and reported antibiofilm effects, including interactions with antimicrobial drugs [17]. Together, these studies support the experimental rationale for clove-derived compounds. However, purified eugenol, clove essential oil, and an unspecified botanical extract differ in composition and concentration, so their effects should not be treated as interchangeable.

The present results for *Curcuma longa* also correspond with previous experimental evidence. Suwal et al. (2021) [23] investigated *C. longa* rhizome extract against biofilm-producing *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Their microtiter-plate assay demonstrated significant inhibition of biofilm formation, while phytochemical analysis identified curcuminoids, phenolics, flavonoids, alkaloids, and terpenoids. In the present study, turmeric produced 53.84% mean biofilm inhibition and 0.86-log<sub>10</sub> CFU reduction. Although its activity was lower than that of the other botanical preparations, the substantial difference from the negative control confirms measurable antibiofilm activity. The lower activity may reflect differences in extract composition, concentration, curcuminoid availability, and interaction with the biofilm matrix.

Evidence involving oral microorganisms further supports investigation of turmeric-derived compounds. Hu et al. (2013) demonstrated inhibition of *S. mutans* sortase A and biofilm formation [18], while Li et al. (2018) reported reductions in biofilm metabolism, bacterial viability, and extracellular polysaccharides [19]. Sainudeen et al. (2020) also evaluated a turmeric preparation against *E. faecalis* biofilms on tooth substrate, reporting antibacterial activity under an endodontic experimental model [24]. These findings are consistent with measurable activity in the present study, but do not explain the observed ranking of turmeric because the extract composition and exposure conditions have not been adequately specified.

More recent evidence further supports the potential of turmeric-derived preparations. Beshiru et al. (2024) [25] evaluated *C. longa* rhizome extract for antibiofilm activity and identified multiple bioactive constituents using GC-MS and FTIR analyses. The extract demonstrated concentration- and time-dependent antimicrobial effects and reduced biofilm cells and extracellular polymeric substances. Although their study involved foodborne antibiotic-resistant pathogens rather than oral microorganisms, the demonstrated ability of turmeric constituents to interfere with established biofilm architecture provides mechanistic support for the activity observed in the current study.

The present results should also be interpreted in relation to chlorhexidine. Chlorhexidine produced the highest overall biofilm inhibition (79.58%) and the greatest mean log<sub>10</sub> CFU reduction (1.23), confirming its strong antimicrobial performance under the experimental conditions used. However, the relatively small difference between chlorhexidine and clove is noteworthy. The mean difference in biofilm inhibition was approximately 6.59 percentage points, while the difference in viable microbial reduction was approximately 0.11 log<sub>10</sub> CFU/mL. This pattern suggests that clove may warrant further investigation as a source of antibiofilm compounds, but it does not establish clinical equivalence with chlorhexidine. The clinical evidence for chlorhexidine also provides an important benchmark: James et al. (2017) reported substantial plaque reduction with adjunctive mouthrinse use, together with increased tooth staining [3]. Accordingly, comparisons with plant preparations should consider both antimicrobial performance and tolerability, rather than laboratory inhibition alone.

An additional important finding was the significant effect of pathogen type. Mean biofilm inhibition differed among *S. mutans*, *S. sanguinis*, *E. faecalis*, and *C. albicans*. Such variation is expected because bacterial and fungal microorganisms differ in cell-wall or cell-membrane characteristics, adhesion mechanisms, extracellular polymeric substance composition, and susceptibility to phytochemicals. However, the absence of a significant treatment × pathogen interaction suggests that the relative efficacy

pattern of the tested agents was not substantially altered by the pathogen. Bowen et al. (2018) describe the contribution of the extracellular matrix and polymicrobial interactions to oral biofilm behaviour [1]. Marsh (2006) further emphasizes that dental plaque is an ecological community [2]. Therefore, suppression of the four organisms tested here cannot by itself establish a beneficial effect on the broader oral microbiota.

The findings collectively suggest that the tested medicinal plants possess measurable antibiofilm activity, but their performance varied considerably. Clove showed the greatest activity among the botanical preparations, while turmeric demonstrated the lowest activity. These differences may be attributable to variations in phytochemical composition, concentration of active constituents, extraction method, biofilm penetration, and susceptibility of individual microorganisms. Therefore, standardization of plant extracts is essential before comparisons can be translated into clinical applications. This need for standardization is consistent with the concerns raised by Freires and Rosalen and by Karygianni et al. regarding inconsistent preclinical methods and the translation of botanical findings into oral-care products [4, 6].

A major strength of the present study is the simultaneous comparison of four indigenous medicinal plants with 0.12% chlorhexidine against four oral pathogens using both biofilm inhibition and viable microbial reduction as outcomes. This provides a broader comparative assessment than studies evaluating a single plant or microorganism. However, the study remains limited by its in-vitro design. The results cannot establish clinical effectiveness, safety, substantivity, cytotoxicity, taste acceptability, or staining potential. Furthermore, differences in plant species, extraction techniques, concentrations, and experimental models across the comparative literature limit direct numerical comparison. Karygianni et al. specifically reviewed herbal interventions against multispecies oral biofilms, supporting the use of these more complex models in subsequent validation [6].

Future studies should therefore focus on standardized botanical preparations, characterization of their active phytochemicals, minimum biofilm inhibitory and eradication concentrations, mature multispecies oral biofilms, cytotoxicity against oral epithelial and fibroblast cells, and appropriately designed clinical trials. Within these limitations, the present findings provide experimental evidence that indigenous medicinal plants, particularly *S. aromaticum*, possess substantial antibiofilm activity and may serve as candidates for further development of plant-derived oral antimicrobial formulations. Eugenol-based polymeric materials have also been investigated for antibacterial applications, offering a possible formulation research direction rather than evidence of an effective clinical dental product [26].

## LIMITATIONS

Several limitations should be acknowledged. First, this was an in-vitro study, and the findings cannot be directly extrapolated to clinical effectiveness. Second, the dataset does not contain sufficient information regarding the botanical authentication, plant part, extraction procedure, concentration, solvent, preparation standardization, exposure time, or storage conditions. These methodological variables can substantially influence botanical antimicrobial activity and should therefore be reported from the original laboratory protocol. Third, the sample sizes varied slightly between treatment-pathogen combinations. Fourth, the analysis is based on experimental observations rather than independent human participants. Finally, the study did not evaluate cytotoxicity, substantivity, sensory acceptability, staining potential, or long-term effects. Future investigations should use chemically standardized extracts, determine minimum inhibitory and minimum biofilm eradication concentrations, assess mature multispecies oral biofilms, evaluate cytotoxicity against oral tissues, and subsequently conduct well-designed clinical trials.

## CONCLUSION

Within the limitations of this in-vitro experimental model, all four tested medicinal plants demonstrated substantially greater antibiofilm activity than the negative control. *Syzygium aromaticum* exhibited the highest mean biofilm inhibition among the plant preparations, followed by *Azadirachta indica*, *Ocimum sanctum*, and *Curcuma longa*. Chlorhexidine produced the highest overall biofilm inhibition and viable microbial reduction.

The findings support further laboratory investigation of standardized indigenous plant preparations, particularly clove, as potential sources of oral antibiofilm compounds. However, clinical studies are required before these experimental findings can be translated into recommendations for routine oral healthcare.

## DECLARATIONS

### Ethics Approval

Not applicable. The present study was an in-vitro experimental investigation and did not involve human participants or identifiable human biological samples.

### Consent for Participation

Not applicable.

### Consent for Publication

Not applicable.

### Availability of Data

The data underlying the findings of this study are available from the corresponding author upon reasonable request.

### Conflict of Interest

The authors declare that they have no conflicts of interest related to this study.

### Funding

No external funding was reported for this study.

### Author Contributions

All authors contributed to the conception and design of the study, data analysis, interpretation of findings, manuscript preparation, critical revision, and approval of the final manuscript.

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