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Original Research Article

Comparison of antibacterial efficacy of a non-herbal toothpaste with the polyherbal preparation on the growth of *Aggregatibacter actinomycetemcomitans*: an *in vitro* study

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ABSTRACT

Background: *Aggregatibacter actinomycetemcomitans* is an important oral pathogen implicated in periodontal disease and is commonly detected in pediatric populations. Chemical dentifrices are effective against oral bacteria but may cause adverse effects, whereas herbal preparations may offer safer alternatives.
Methods: An in vitro experimental study was conducted to compare the antibacterial efficacy of a non-herbal toothpaste (Kidodent™ Indoco Pharmaceuticals Ltd., India) and a polyherbal preparation containing Neem (*Azadirachta indica*), Tulsi (*Ocimum sanctum*), and Ginger (*Zingiber officinale*). Antibacterial activity was evaluated using agar well diffusion assay at concentrations of 20, 50, and 100 mg/ml, and minimum inhibitory concentration (MIC) was determined by broth microdilution. All tests were performed in triplicate. Statistical analysis was carried out using one-way ANOVA, independent Student's t-test, and Mann–Whitney U test as appropriate.
Results: The non-herbal toothpaste demonstrated significantly greater zones of inhibition compared to the polyherbal preparation ($p<0.001$). The polyherbal formulation showed a concentration-dependent antibacterial effect, with the highest mean zone of inhibition observed at 100 mg/ml. The MIC value of the polyherbal preparation was 31.25 µg/mL, whereas the non-herbal toothpaste exhibited a lower MIC, indicating higher antibacterial potency.
Conclusions: The non-herbal toothpaste showed superior antibacterial efficacy against A. actinomycetemcomitans; however, the polyherbal preparation demonstrated significant, concentration-dependent antimicrobial activity. Polyherbal formulations may serve as safer adjuncts in pediatric oral care, though further clinical studies are warranted.

Keywords: Polyherbal preparation, Non-herbal toothpaste, *A. actinomycetemcomitans*, Antibacterial activity, Pediatric dentistry

INTRODUCTION

The oral cavity harbors a diverse microbiota comprising bacteria, fungi, protozoa, and viruses, many of which play a crucial role in maintaining oral health, while others contribute to the development of oral diseases. Dental caries and periodontal diseases remain among the most prevalent chronic conditions affecting children worldwide, with microorganisms such as *Streptococcus* mutans, Actinomyces species, and *A. actinomycetemcomitans* being commonly implicated.[1] Toothpastes constitute the cornerstone of daily oral hygiene practices. Conventional non-herbal dentifrices contain active chemical agents such as fluoride compounds, detergents, abrasives, and preservatives that effectively reduce oral microbial load.

However, prolonged use of chemical dentifrices has been associated with adverse effects including mucosal irritation, altered taste sensation, and hypersensitivity reactions, particularly in pediatric populations.[2] In recent years, there has been growing interest in herbal and polyherbal formulations as safer alternatives for oral health maintenance. Medicinal plants such as Neem

(*Azadirachta indica*), Tulsi (*Ocimum sanctum*), and Ginger (*Zingiber officinale*) possess well-documented antibacterial, anti-inflammatory, and antioxidant properties. These herbs have traditionally been used in oral care products and are considered biocompatible with minimal side effects.[3-5]

A. actinomycetemcomitans is a Gram-negative facultative anaerobe associated with aggressive periodontitis and is frequently detected in children and adolescents. Given its role as a keystone periodontal pathogen, evaluation of antimicrobial agents against this organism is of particular relevance in pediatric dentistry.

The present in vitro study was therefore undertaken to compare the antibacterial efficacy of a commercially available non-herbal toothpaste and a polyherbal preparation containing Neem, Tulsi, and Ginger against A. actinomycetemcomitans, with the aim of exploring the potential of herbal formulations as safer adjuncts in pediatric oral care.

METHODS

Study design

This in vitro experimental study was conducted to evaluate and compare the antibacterial efficacy of a non-herbal toothpaste and a polyherbal preparation against *A. actinomycetemcomitans*.

Ethical approval

The study protocol was reviewed and approved by the Scientific Review Board of Seema Dental College & Hospital, Rishikesh (EC/NEW/ INST/2025/UA/0615). As this was an in vitro laboratory-based study, no human or animal subjects were directly involved.

Microorganism and culture conditions

A standard strain of *A. actinomycetemcomitans* (ATCC 29522) was procured from a certified diagnostic supplier and stored at 2–4°C as per manufacturer's instructions. The organism was revived within 48–72 hours and cultured in Mueller-Hinton broth. For antimicrobial testing, Mueller–Hinton agar plates were prepared under sterile conditions.

Preparation of polyherbal extract

Fresh Neem leaves and Tulsi leaves were thoroughly washed with distilled water, shade-dried for 10–14 days, and ground into a fine powder (Figure 1a, b). Ginger rhizomes were washed, grated, shade-dried, and powdered (Figure 1c). The powdered plant materials were mixed in equal proportions and subjected to cold percolation using acetone as solvent in a ratio of 1:10. The mixture was filtered using Whatman filter paper, and the solvent was evaporated under reduced pressure. The dried extract was reconstituted in 10% dimethyl sulfoxide (DMSO) to obtain stock solutions of 20 mg/ml, 50 mg/ml, and 100 mg/ml (Figure 2).

Control preparation

A commercially available non-herbal toothpaste (Kidodent™, Indoco Pharmaceuticals Ltd., India) was used as the positive control. Ten percent DMSO served as the negative control.

Agar well diffusion assay

The antibacterial activity was assessed using the agar well diffusion method. Mueller–Hinton agar plates were inoculated with A. actinomycetemcomitans using a sterile cotton swab to obtain a uniform lawn culture. Wells of 6 mm diameter were prepared using a sterile cork borer, and 50 µl of the test solutions at different concentrations were dispensed into the respective wells. Plates were incubated at 37°C for 24 hours. Zones of inhibition were measured in millimeters. All experiments were performed in triplicate.

Minimum inhibitory concentration assay

The minimum inhibitory concentration (MIC) was determined using the broth microdilution method. Two-fold serial dilutions of the extracts (1000–1.95 µg/ml) were prepared in Mueller–Hinton broth in 96-well microtiter plates. Each well received 100 µl of extract and 100 µl of bacterial suspension adjusted to 10^5 CFU/ml. After incubation at 37°C for 24 hours, microbial growth was assessed visually and confirmed using resazurin dye. The MIC was defined as the lowest concentration showing no visible color change.

Statistical analysis

Data were analyzed using SPSS version 23.0 (IBM Corp., Armonk, NY, USA) and expressed as mean±standard deviation. One-way analysis of variance (ANOVA) was used to compare zones of inhibition across different concentrations of the polyherbal preparation. Independent Student's t-test was applied for intergroup comparison between the polyherbal preparation and the non-herbal toothpaste. MIC values were compared using the Mann–Whitney U test. A p value <0.05 was considered statistically significant.

RESULTS

The antibacterial activity of the polyherbal preparation and the non-herbal toothpaste against *A. actinomycetemcomitans* was evaluated using zone of inhibition and minimum inhibitory concentration assays. The polyherbal preparation exhibited a concentration-dependent increase in antibacterial activity. At 20 mg/ml, the mean zone of inhibition was 8.67±0.58 mm, which increased to 11.00±1.00 mm at 50 mg/ml and 14.33±0.58

mm at 100 mg/ml. One-way analysis of variance demonstrated a statistically significant difference in zones of inhibition across the three concentrations ($p<0.05$) (Figure 3, Table 1).

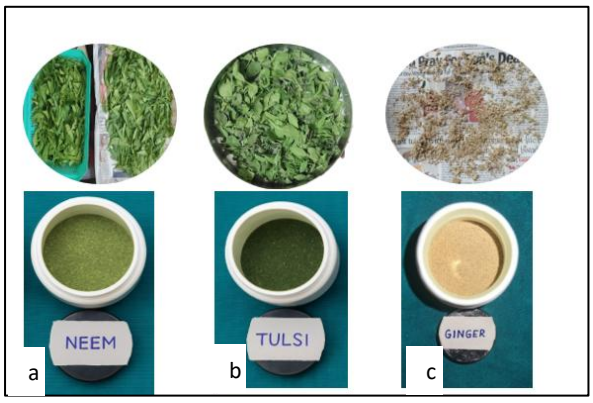

Figure 1 (a-c): Fresh neem leaves and its powder, fresh Tulsi leaves and its powder, grated ginger and its powder.

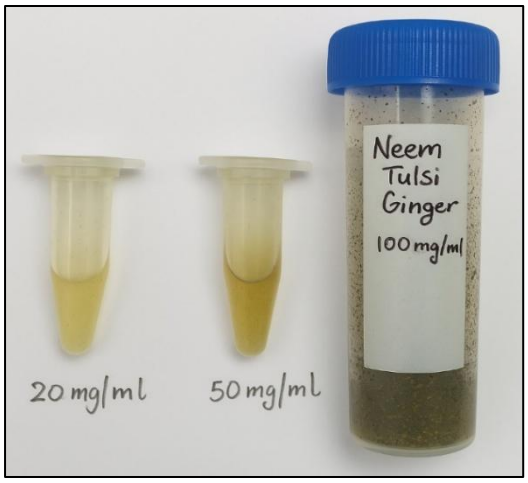

Figure 2: Serial concentration of herbal preparation at 20 mg/ml, 50 mg/ml and 1000 mg/ml.

Figure 3: Zones of inhibition produced by polyherbal and non- herbal toothpaste formulations against *A. actinomycetemcomitans*.

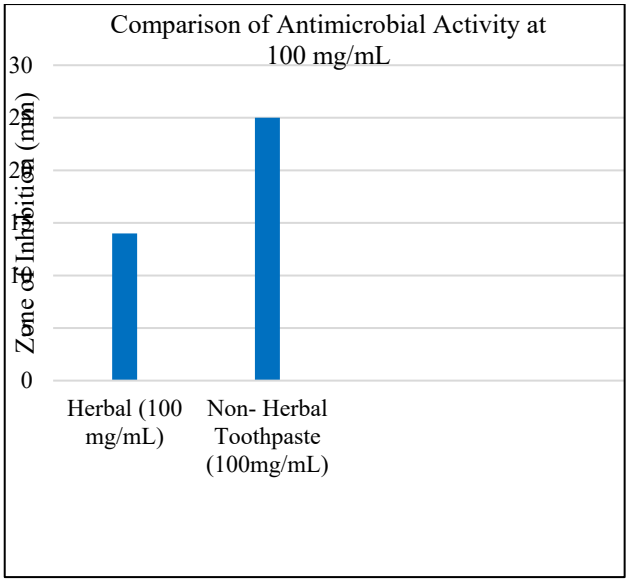

Figure 4: Comparison of zones of inhibition produced by herbal preparation and non-herbal toothpaste at 100 mg/ml.

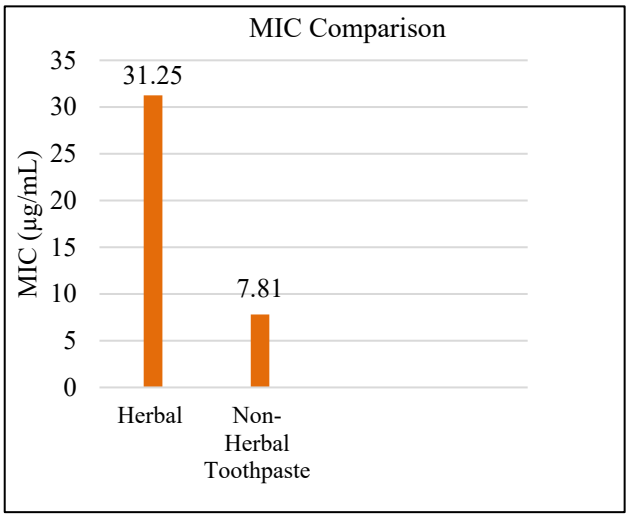

Figure 5: Comparison of minimum inhibitory concentration (MIC) values of the herbal preparation and non-herbal toothpaste.

When compared at a concentration of 100 mg/ml, the non-herbal toothpaste produced a significantly larger mean zone of inhibition (25.00±1.00 mm) than the polyherbal preparation (14.33±0.58 mm). Independent Student's t-test revealed this difference to be highly significant ($p<0.001$) (Table 2, Figure 4).

The minimum inhibitory concentration assay showed that the polyherbal preparation inhibited bacterial growth at a concentration of 31.25 µg/ml, whereas the non-herbal toothpaste demonstrated inhibition at a lower concentration of 7.81 µg/ml. Comparison of MIC values using the Mann–Whitney U test indicated a statistically significant difference between the two groups ($p=0.002$) (Table 3, Figure 5).

Table 1: Zone of inhibition of polyherbal preparation at different concentrations.

Group	Concentration (mg/ml)	Mean ZOI (mm)	Statistical comparison	P value
Polyherbal preparation	100	14.0	Independent Student's t-test	<0.001* highly significant difference
Non-herbal toothpaste	100	25.0		

One-way analysis of variance (ANOVA); * indicates significant difference at p≤0.05.

Table 2: Comparison of zone of inhibition between polyherbal and non-herbal toothpaste.

Concentration (mg/ml)	Mean ZOI (mm)	Statistical comparison	P value
20	8.6	One-way ANOVA	<0.05* significant difference
50	11.0		
100	14.2		

Independent Student's t-test; * indicates highly significant difference at p<0.001.

Table 3: Comparison of MIC between polyherbal preparation and non-herbal toothpaste.

Group	MIC (µg/ml)	Statistical comparison	P value
Polyherbal preparation	31.25	Mann–Whitney U test	0.002*
Non-herbal toothpaste	7.81		

Mann–Whitney U test * Statistically significant difference at p≤0.05

DISCUSSION

The present in vitro study evaluated the antibacterial efficacy of a polyherbal dentifrice containing Neem (*Azadirachta indica*), Tulsi (*Ocimum sanctum),* and Ginger (*Zingiber officinale*) against *A. actinomycetemcomitans* and compared it with a non-herbal chemical dentifrice. The findings demonstrated a clear concentration-dependent antibacterial effect of the polyherbal formulation; however, the non-herbal dentifrice exhibited significantly greater inhibitory activity. These results are consistent with previous studies reporting measurable antibacterial effects of plant-based formulations, albeit inferior to conventional chemical agents. Shah et al using disk diffusion and colony-forming unit assays, reported that neem exhibited the highest antibacterial activity among tested herbal irrigants against *Actinomyces radicidentis*, although sodium hypochlorite remained the most potent agent overall.[6] This supports the current observation that herbal agents can exert meaningful antibacterial action, but chemical formulations continue to demonstrate superior efficacy under in vitro conditions. Tulsi, a key component of the polyherbal formulation, has been documented to possess significant antibacterial activity against oral pathogens. Mallikarjun et al showed that ethanolic extracts of Tulsi at concentrations of 5–10% significantly inhibited *A. actinomycetemcomitans*, with efficacy comparable to doxycycline, although reduced activity was noted against Prevotella intermedia and *P. gingivalis*. These findings align with the present study, reinforcing the concentration-dependent antibacterial response observed with the polyherbal dentifrice.[7] Ginger has also been shown to possess antimicrobial properties against both Gram-positive and Gram-negative oral microorganisms. Ahmed et al and Fatalla et al reported that aqueous ginger extracts inhibited anaerobic peri-implant pathogens, with higher concentrations producing greater inhibition. The present findings are consistent with these observations and may be attributed to gingerols and related phenolic compounds, which are recognized as the principal antimicrobial constituents of Ginger.[7] A broader comparative analysis by Motallaei et al highlighted the advantages of herbal agents, including superior biocompatibility, anti-inflammatory and antioxidant properties, and greater patient acceptance.[9] Nevertheless, most studies reported a slower onset of action and reduced antibacterial potency of herbal agents compared with chemical formulations at equivalent concentrations. This provides a plausible explanation for the superior inhibitory effect of the non-herbal dentifrice observed in the current investigation, despite the demonstrable antibacterial activity of the polyherbal formulation. From a pediatric perspective, *A. actinomycetemcomitans* is of particular clinical relevance. Although often detected in periodontally healthy individuals, it is considered a keystone pathogen in aggressive periodontitis among adolescents and has been reported in various pediatric populations, including preschool children, children with Down syndrome, and individuals with special healthcare needs.[10,11] Its role in early colonization and potential periodontal breakdown underscores the importance of evaluating both chemical and herbal antimicrobial agents intended for pediatric oral healthcare products.

Limitations

The present study has certain limitations. As an in vitro investigation, it does not replicate the complex oral environment, including salivary dynamics, host immune responses, pH variations, and microbial interactions.[12]

The use of a single bacterial species limits extrapolation to the polymicrobial nature of dental plaque.[13] Additionally, variability in herbal extract composition, absence of cytotoxicity and biocompatibility assessment, and evaluation of only short-term antibacterial effects further restrict clinical translation.[2,14,15]

Future directions and clinical implications

Despite these limitations, the study highlights the potential of polyherbal dentifrices as adjuncts in oral healthcare. The demonstrated dose-dependent antibacterial effect suggests scope for optimization of herbal formulations to enhance efficacy. Given their additional anti-inflammatory and antioxidant properties, herbal dentifrices may offer particular advantages in pediatric populations where long-term safety and patient acceptability are paramount. Future studies should focus on biofilm models, cytotoxicity assessment, and well-designed clinical trials to better establish the therapeutic role of polyherbal dentifrices in pediatric dentistry.

CONCLUSION

The non-herbal toothpaste showed noticeably more antibacterial action against *A. actinomycetemcomitans* than the polyherbal formulation, within the constraints of this in vitro study. Nonetheless, the herbal formulation's concentration-dependent inhibitory action was evident, demonstrating the plant extracts' antibacterial properties. Polyherbal formulations could be viable natural adjuncts or substitutes for traditional dentifrices due to their safety, biocompatibility, and lower risk of side effects. To enhance herbal combinations and assess their clinical effectiveness in preventing Actinomyces-associated dental problems, more research is necessary.

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Ethical approval: The study was approved by the Institutional Ethics Committee

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