Comparative Evaluation of *Mangifera indica* Leaf Mouthwash with Chlorhexidine on Plaque Accumulation, Gingival Inflammation, and Salivary Streptococcal Growth

**Abstract**

**Aims:** This study aims to compare the efficacy of a mango (*Mangifera indica*) leaf mouthwash with chlorhexidine on plaque status, gingival status, and salivary *Streptococcus mutans* count. **Materials and Methods:** A sample of twenty children, aged 8–14 years, Mangalore Residential School, Karnataka, India, was allocated into two groups. Group A (10) and Group B (10) were given test mouthwash “mango leaf mouthwash” and chlorhexidine, respectively. The clinical trial was carried out for ½ h after rinsing and after 5 days, during which children were asked to rinse once daily, with 10 ml of the given mouthwash ½ h after tooth brushing. **Results:** There was a significant reduction in microbial count, improved plaque control and gingival health in mango leaf and chlorhexidine mouthwash groups with higher reduction in microbial count, and better plaque control and gingival health seen in chlorhexidine group. **Conclusion:** Herbal alternatives proved to be an effective and safe alternative to conventional modes of treatment.

**Keywords:** Chlorhexidine, gingival status, herbal mouthwash, plaque status, *Streptococcus mutans*

**Introduction**

With the exponential advancement in the field of dentistry, various preventive measures have emerged targeting the causative factors of the oral diseases.[1] Plaque accumulation is one such factor which predisposes the individual to both dental caries and periodontal disease.[2-4] Salivary microflora such as *Streptococcus mutans* and other predisposing factors play an important role in the initiation and progression of dental diseases such as dental caries.[1,6] Chemotherapeutic and antimicrobial agents aiming at these predisposing factors, therefore, play a significant role in the prevention of these oral diseases and have a dramatic impact on improving the oral health of the individual.[7]

Among the plethora of oral hygiene products available, chlorhexidine has been the mouthwash of choice owing to its dramatic therapeutic effect, but its various side effects such as taste alteration, supragingival calculus formation, and desquamation of oral mucosa have restricted its usage in pediatric age group.[4,8] It also causes extrinsic staining by attaching to the polyphenolic and tannin group of beverages such as tea and coffee. Alternative agents based on herbal extracts are therefore of particular interest.

*Mangifera indica* (mango) leaf has various biomedical applications including antioxidative or free radical scavenging, anti-inflammation, anti-allergic cardioprotective, anticancer, hepatoprotective, analgesic, and immune modulator activities. It is a rich source of various biologically active compounds. Phytochemical studies of different parts of *M. indica* have demonstrated the presence of phenol constituents, triterpenes, flavonoids, phytosterols, and polyphenols.[9] The decoction of the leaves functions as anthelmintics and can also be used to gargle in the prevention of halitosis. It has been reported that an ethanolic extract of mango seed kernel possessed an antimicrobial activity against food-borne pathogenic bacteria.[10]

Therefore, this study aims to clinically evaluate the effect of herbal mouthwash prepared from *M. indica* leaves with chlorhexidine mouthwash on plaque accumulation, gingival health, and salivary *Streptococcus* count.

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Materials and Methods

Children aged 8–14 years in Mangalore Residential School, Karnataka, were allocated into two groups. The entire sample size of 20 was randomly divided into Group A: (ten individuals) who were given herbal mouthwash prepared from mango leaves and Group B: (ten individuals) who were given chlorhexidine and served as control.

Individuals with good general health, a minimum of 12 gradable teeth, agreement to delay any elective dental treatment including oral prophylaxis, and agreement to comply with the study visits were included in the study. Exclusion criteria involved individuals with relevant medical condition, history of early-onset periodontitis, acute necrotizing ulcerative gingivitis, gross oral pathology, history of steroid or antimicrobial therapy in the last 3 months and those developing severe gingival inflammation during the study.

The clinical trial was carried out for ½ h after rinsing and after 5 days, during which children were asked to rinse once daily, with 10 ml of the given mouthwash ½ h after tooth brushing.

For the preparation of herbal mouthwash, the collected plant’s leaves were washed with tap water, followed by distilled water and shade dried [Figure 1]. The dried samples were made into a coarse powder [Figure 2]. Around 500 g of M. indica coarse powder was used for preparing the 90% ethanolic extracts in Soxhlet apparatus [Figure 3].

After 48 h of extraction, the ethanol was removed by Rotavapor apparatus [Figure 4]. The semisolid paste, which was obtained after removal of ethanol, was constituted in sterile double distilled water. Sweetening agent (20%) was added to obtain the final mouthwash [Figure 5].

Plaque index given by Silness and Loe and gingival index given by Loe were used to assess the plaque accumulation and gingivitis. To assess the effect of mouthwashes on caries susceptibility, S. mutans, Streptococcus mitis, and Streptococcus salivarius in the saliva were counted and compared.

For this, children were asked to rinse once daily with 10 ml of given mouthwash ½ h after brushing, for a clinical trial period of 5 days. 0.5 mL of the unstimulated saliva was collected in closed containers just before the usage of mouthwashes, after ½ h, and at the end of clinical trial period of 5 days. The collected saliva was incubated within 30 min to avoid use of any transportation media. Culture media used were mitis salivarius agar which is a selective medium of S. mutans, S. mitis, and S. salivarius.[12] 0.004 ml of sample was inoculated into agar and culture plates were incubated at 37°C for 72 h and the microbial count was done. Baseline data were collected just before the usage of mouthwashes and then after ½ h after rinsing, and the final value was collected at the end of clinical trial period of 5 days.[12]

Results

There was a significant reduction in microbial count and improved plaque control and gingival health in both groups, but with higher reduction in microbial count, better improvement in plaque control and gingival health was seen in chlorhexidine group.

Results of all the three parameters, plaque status, gingival status, and Streptococcus count, have been depicted in Table 1.

There was significant difference in all the parameters. Chlorhexidine showed higher difference in Streptococcus count and higher difference in plaque and gingival values than mango leaf mouthwash after ½ h and after 5 days of usage of mouthwashes. Higher reduction in streptococcal count, better plaque control, and improved gingival health were seen in chlorhexidine group.

There was significant difference in Streptococcus count in both groups, but higher difference in count was seen
in chlorhexidine group, ½ h and 5 days after rinsing with mouthwash [Graph 1].

Chlorhexidine showed higher difference in *Streptococcus* count than mango leaf mouthwash when *Streptococcus* level in saliva was counted and compared just before, after ½ h, and after 5 days of usage of mouthwashes [Graph 2].

There was significant reduction in *Streptococcus* count, after ½ h and after 5 days usage of mouthwash for both groups, but with increased difference in reduction seen after 5 days for chlorhexidine group.

Chlorhexidine shows higher difference in plaque and gingival values than mango leaf mouthwash after ½ h and after 5 days of usage of mouthwashes [Graph 3], so better plaque control and improved gingival health were seen in chlorhexidine group.

| Table 1: Independent *t*-test comparing chlorhexidine and mango |
|---------------------------------------------------------------|
| **Group** | **n** | **Mean** | **SD** | **t** | **P** |
| --- | --- | --- | --- | --- | --- |
| Reduction in streptococcal count after ½ h | CHX | 10 | 1773 | 339.067 | 5.07 | <0.001 |
|  | Mango leaf | 10 | 1203.1 | 106.703 |  |
| Reduction in streptococcal count after 5 days | CHX | 10 | 2860.4 | 422.196 | 6.545 | <0.001 |
|  | Mango leaf | 10 | 1879.6 | 215.238 |  |
| Count difference | CHX | 10 | −1087.4 | 328.4784 | 3.584 | 0.002 |
|  | Mango leaf | 10 | −676.5 | 153.4284 |  |
| Difference in plaque index | CHX | 10 | 1.0699 | 0.030784 | 5.086 | 0.001 |
|  | Mango leaf | 10 | 0.8066 | 0.160782 |  |
| Difference in gingival index | CHX | 10 | 1.042 | 0.042895 | 7.551 | <0.001 |
|  | Mango leaf | 10 | 0.747 | 0.115859 |  |

*P* * Statistical significance, *n*=Number of students, SD=Standard deviation, CHX=Chlorhexidine
Discussion

The present study was designed to determine the efficacy of an herbal mouth rinse with *M. indica* leaves with chlorhexidine on plaque accumulation, gingival health, and streptococcal count.

All the individuals taken for the study were residing in the same residential school, thereby eliminating the bias occurring due to different eating patterns. Results show that the chlorhexidine mouthwash had better antimicrobial effect on salivary *Streptococcus* and effect on plaque control and gingival health than mouthwash with *M. indica* leaves.

Chlorhexidine is a cationic bisbiguanide with excellent antimicrobial action. It is active against a wide range of microorganisms because it is bacteriostatic at low concentrations and bactericidal at higher concentrations. However, it was found to be highly cytotoxic to human periodontal ligament cells by inhibiting double-stranded nucleic acid content, protein synthesis, and mitochondrial activity. An *in vitro* study done by Arabaci *et al.* showed that they also have genotoxic and cytotoxic effects on human lymphocytes.[13]

Similarly, the study conducted by Park *et al.* showed that the treatment with chlorhexidine decreased the viability of the cell growth. The stem cells derived from the buccal fat pad were sensitive to chlorhexidine and a reduction in cellular viability was observed.[14]

*M. indica* leaves can be used to gargle in the prevention of halitosis. Mangiferin is a phenolic compound which possesses antimicrobial, antioxidant, radioprotective, immunomodulatory, anti-tumor, anti-allergic, anti-inflammatory, and antidiabetic properties. It also demonstrated promising therapeutic potential both in the prevention and treatment of periodontitis. In a study done by Ling and Yap, a standardized aqueous extract from the leaves of *M. indica* was reported to contain anti-inflammatory activity.[15] Another study done by Maisuthisakul mouthwash containing mango kernel exhibited inhibition zone against *Staphylococcus aureus* and *S. mutans* and also showed inflammatory inhibitory properties.[16]

In our study, there was a definitive reduction in oral salivary microbial population, reduction in plaque growth, and improved gingival health by the usage 2% of *M. indica* mouthwash when exposed for 5 days. Since there is no evidence for severe adverse effects by the use of mango leaf mouthwash, they may be less harmful for patients compared to usage of antimicrobial agents such as chlorhexidine.

Conclusion

Both the mango leaf mouthwash (2%) and chlorhexidine mouthwash (0.12%) were effective against salivary microbial population, plaque inhibition, and improved gingival health. Chlorhexidine mouthwash showed higher efficacy than mango leaf mouthwash. However, mango leaf mouthwash has antimicrobial activity with no side effects such as dental staining and other cytotoxic effects when compared to chlorhexidine mouthwash. Hence, *M. indica* mouthwash can be a promising alternative for the patients. Further clinical investigations are needed for standardization and preparation of mango leaves containing toothpaste and mouthwashes for the prevention of oral microbial diseases.

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Conflicts of interest

There are no conflicts of interest.

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