



Assessment of Antiplaque and Anti-Gingivitis Efficacy of Mouthwashes Prepared from Neem and Mango Extracts

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ABSTRACT

Objectives: This study aimed to determine the minimum inhibitory concentration (MIC) of mouthwashes prepared from neem and mango extracts against *Streptococcus mutans* (*S. mutans*) in vitro. Their taste acceptability and effects on plaque index (PI), gingival index (GI) and salivary pH were also evaluated and compared with chlorhexidine (CHX) in children.

Materials and Methods: Dry extracts of neem and mango twigs were prepared and their MIC against *S. mutans* was determined. The effective MIC was used to prepare mouthwashes from the two extracts. Three parallel groups of children (n=30) used either neem, mango or 0.2% CHX mouthwash for 21 days. The PI and GI were recorded at baseline and at 7 and 21 days. The salivary pH and taste acceptability were also assessed.

Results: The MIC of both extracts was achieved at 25% concentration. There was a significant difference between the GI score of mango group compared with neem and CHX at the three time points ($P < 0.001$). There was no significant difference between the neem and mango groups in PI ($P = 0.674$). There was no significant difference among the three groups in salivary pH either ($P = 0.817$). Intragroup comparison showed significant reductions in PI, GI and salivary pH in all the three groups after 21 days ($P < 0.001$).

Conclusion: Neem and mango mouthwashes can be used as effective alternatives to CHX in children.

Keywords: Chlorhexidine; Oral Hygiene; Herbal Medicine; Saliva

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INTRODUCTION

Dental plaque is the prime causative agent of dental caries and gingivitis in individuals. Eradication of plaque would help in improvement of gingival and overall dental health of the patient. Chlorhexidine (CHX) has earned its reputation as a gold standard in chemical plaque control [1]; however, long-term use of CHX may lead to various complications such as altered taste perception, metallic taste, and staining of teeth [2]. Thus, the quest for an ideal mouthwash

with beneficial properties of CHX without its side effects continues.

Since evolution, humans have been dependent on plants for their basic needs of food and shelter. Plants have been used by humans for various diseases due to their medicinal properties and presence of bioactive compounds. Neem [3], mango [4], turmeric [5], aloe-vera [6], alum [7], pomegranate [8], green tea [9], triphala [10] and cloves [11] have been researched upon in the field of dentistry.

Neem twigs have been used as an oral hygiene aid since ancient times [12]. Neem has been researched extensively in various fields of medicine due to its antihyperglycemic, immunomodulatory, anti-inflammatory, antimalarial, antioxidant, antiviral, antimutagenic and anticarcinogenic properties [3]. The beneficial properties of neem have been attributed to the presence of various bioactive ingredients such as azadirachtin, nimbin and nimbidin [13]. Evidence shows the use of mango chewing sticks in countries such as India, Pakistan and Panama [14]. It has been shown to possess antioxidant, radioprotective, immunomodulatory, antitumor, anti-allergic, anti-inflammatory, antidiabetic, and antimicrobial properties [15]. Mango has been shown to possess various components such as natural C-glucoside xanthone mangiferin, along with tannins and resins [14]. Sharma et al. [16] used 50% extracts of neem and mango and showed promising results as antiplaque and anti-gingivitis agents. However, the color and bitter taste of the neem extract made it unacceptable to children [17].

Hence, a lower concentration could possibly be more acceptable to children. Thus, the aim of the present study was to assess the in vitro antibacterial effect of 25% and 50% neem and mango twig extracts on *Streptococcus mutans* (*S. mutans*) and to prepare mouthwashes from the extracts. The antiplaque and anti-gingivitis efficacy of the mouthwashes and their effect on the salivary pH were also assessed along with their taste acceptability. The null hypothesis was that the two herbal mouthwashes would have no significant effect on gingival health and oral hygiene status in comparison with CHX.

MATERIALS AND METHODS

The present study was conducted in a residential school in Mumbai, India. The study was approved by the Institutional Ethical Committee (DYPUSOD/SS-PG- Pedo.-Ethical/672-A/of 2016). The study was divided into three phases:

Phase I: Preparation of neem and mango

extracts and in vitro antibacterial assessment of the extracts.

Phase II: Preparation of neem and mango mouthwashes

Phase III: In vivo assessment of the effect of prepared mouthwashes on plaque, gingivitis and salivary pH. Their taste acceptability was also evaluated in comparison with CHX in children.

Written informed consent was obtained from the authorities of the residential school and the parents of children participating in the study. The procedure, its advantages and possible limitations were explained to the authorities giving consent. They were also informed that they were free to withdraw from the study at any given point.

Phase I: Preparation of neem and mango extracts and mouthwashes:

The neem and mango twigs were collected from the neem and mango trees and submitted to Total Herb Solution Private Limited botanical laboratory for taxonomical verification. Once the species were verified, dry extracts of neem and mango were prepared according to the guidelines suggested by Sharma et al [16].

Phase II: Determining the minimum inhibitory concentration (MIC) of neem and mango extracts:

The prepared neem and mango extracts were diluted to two concentrations of 25% and 50% with sterile distilled water. Next, *S. mutans* (MTCC 497) was procured from the Microbial Type Culture Collection and Gene Bank, CSIR Institute of Microbial Technology, Chandigarh. The MIC of the prepared extracts was determined using agar well-diffusion method. This was performed by mixing 30 mL of sterile nutrient agar with 1 mL of standardized inoculum of *S. mutans*.

The plates were poured and allowed to solidify. A sterile 6-mm-diameter well borer was used to punch wells at a distance of 4 cm on the agar plates. Next, 30 μ L volume of each concentration of neem and mango extract was added aseptically to the desired well. CHX mouthwash (0.2%) (ICPA Health Products Ltd., Mumbai, India) and sterile distilled water were used as positive and negative control,

respectively.

The plates were kept in a refrigerator at 2-8°C for 15 minutes to allow the antibacterial agent to diffuse in the medium. The plates were then incubated in upright position at 37°C for 24 hours in 5% CO₂. After incubation, the plates were evaluated for zone of growth inhibition around the wells. The diameter of the growth inhibition zone for 25% and 50% neem was 18.5 mm and 19 mm, respectively. The diameter of the growth inhibition zone for 25% and 50% mango was 20 mm and 23 mm, respectively. Hence, 25% concentration of both neem and mango extracts was used to prepare the mouthwashes.

Preparation of mouthwashes:

The MIC of the prepared extracts was given to the Department of Pharmacy, NCRD's Sterling Institute of Pharmacy, Navi Mumbai for the mouthwash formulation. The formulation of mouthwashes was based on the guidelines suggested by Sharma et al [16]. Cold maceration technique was employed where neem and mango powders were allowed to soak in 100 mL of sterile deionized distilled water for 48 hours in a refrigerator at 4°C. The mixture was then filtered followed by addition of sweetening agent (30% sucralose, code E955) and preservative (0.05% sodium benzoate, code 211 and 0.01% sodium methyl paraben, code 218). Thus, the final mouthwashes of neem and mango were prepared.

Phase III: Evaluation of in vivo efficacy of the mouthwash:

A total of 300 children between 8-13 years were examined for this study and their dmft/DMFT [18], plaque index (PI) [19], gingival index (GI) [20] and salivary pH were recorded. A total of 90 children with the habit of brushing twice daily, with dmft/DMFT scores between 3 and 6, fair plaque scores and moderate gingival scores were selected for the study.

Children suffering from systemic diseases affecting salivary flow, those with a history of antibiotic use for the past 1 month, those who had undergone orthodontic treatment, and those who required emergency dental treatment or had allergy to any of the

materials used in the mouthwash were excluded from the study.

The 90 subjects were further divided into three groups namely: Group I: Children who used neem mouthwash

Group II: Children who used mango mouthwash

Group III: Children who used CHX mouthwash. They were provided with 250 mL of respective mouthwashes in opaque bottles. The children were instructed to swish 10 mL of the provided mouthwash twice daily under professional supervision for 30 seconds for a total of 21 days. After 12 days, the bottles were refilled with 250 mL of the respective mouthwash. PI and GI were measured at baseline, at 7 days and at 21 days. The assessment of indices was done 2 hours after the breakfast by a single examiner who was blinded to the subject allocation to the mouthwashes.

The pH measurement:

The pH of the saliva was measured by using commercially available Indikrom pH strips i.e. indikrom papers ranging from 2-4.5 to 5.0-7.5. The pH strips were kept in the patients' saliva for 1 minute. The color change of the pH strips was noted and matched with the color of standardized color chart given by the manufacturer to determine the pH of the saliva [17].

Assessment of taste acceptability of the prepared mouthwashes:

The taste acceptability of the mouthwashes was assessed with the help of a questionnaire proposed by Mali et al [21]. The questionnaire consisted of questions assessing subjective criteria that included taste acceptability, burning sensation and dryness/soreness and objective criteria including ulcer formation, staining of teeth, staining of tongue and allergy. These questions were scored as follows:

0: Acceptable

1: Tolerable

2: Unacceptable

Descriptive and inferential statistical analyses were carried out in the present study.

The results of continuous measurements were presented as mean \pm standard deviation and

the results of categorical measurements were presented in numbers (percentage). Level of significance was set at $P=0.05$ and any value less than or equal to 0.05 was considered statistically significant.

One-way ANOVA was used to find significant change in study parameters for intragroup and intergroup analyses. The post-hoc analysis by the Tukey's test was carried out if ANOVA revealed a significant difference. SPSS version 20.0 (SPSS Inc., IL, USA) was used for the analyses of the data and Microsoft Word and Excel were used to generate tables.

RESULTS

Table 1 presents the intragroup mean plaque score reduction from baseline to 21 days in all groups. It shows that there was a highly significant reduction in all three groups with significant differences between them ($P<0.001$). Table 2 presents the intragroup mean gingival score reduction from baseline to 21 days in all the groups. It shows that there was a highly significant reduction in all three groups with significant differences between them ($P<0.001$). Table 3 shows the alterations of the mean pH values from baseline to 21 days in the neem, mango and CHX groups using

ANOVA.

There was a reduction in the pH values from baseline to 21 days and this difference was highly significant ($P<0.001$). There was a reduction in the pH values from baseline to 21 days and this difference was highly significant ($P<0.001$). Table 4 shows that there was a highly significant correlation between different time points in terms of reduction in the mean plaque score, gingival score and salivary pH in all three study groups ($P<0.001$).

When the pH values were compared at different time points using the Tukey's post-hoc test, there was a highly significant difference in the pH values from baseline to day 7, day 7 to day 21 and from baseline to day 21 in all the three groups; however, there was no statistically significant reduction from baseline to day 7 ($P=0.093$) in the CHX group. Table 5 shows that there was a highly significant difference in reduction of the mean plaque scores among neem, mango and CHX groups ($P<0.05$). However, the maximum mean reduction was shown by neem (mean=0.336) group followed by mango (mean=0.313) and lastly by CHX (mean=0.130) ($P<0.001$).

Table 1. Intragroup mean reduction in plaque score from baseline to 21 days in neem, mango and CHX groups using ANOVA (n=30)

| Groups | Plaque index | Mean | Standard Deviation | F value | P value |
|--------|--------------|-------|--------------------|---------|----------|
| Neem | Baseline | 0.593 | 0.10 | 90.068 | <0.001** |
| | 7th day | 0.483 | 0.09 | | |
| | 21st day | 0.257 | 0.10 | | |
| | Total | 0.444 | 0.17 | | |
| Mango | Baseline | 0.550 | 0.07 | 95.552 | <0.001** |
| | 7th day | 0.450 | 0.09 | | |
| | 21st day | 0.237 | 0.09 | | |
| | Total | 0.412 | 0.15 | | |
| CHX | Baseline | 0.537 | 0.112 | 10.102 | <0.001** |
| | 7th day | 0.503 | 0.096 | | |
| | 21st day | 0.407 | 0.136 | | |
| | Total | 0.482 | 0.127 | | |

** Highly significant

Table 2. Intragroup mean reduction in gingival score from baseline to 21 days in neem, mango and CHX groups using ANOVA (n=30)

| Groups | Gingival index | Mean | Standard Deviation | F value | P value |
|--------|----------------|-------|--------------------|---------|----------|
| Neem | Baseline | 1.250 | 0.16 | 50.391 | <0.001** |
| | 7th day | 1.047 | 0.19 | | |
| | 21st day | 0.780 | 0.18 | | |
| | Total | 1.026 | 0.26 | | |
| Mango | Baseline | 1.240 | 0.13 | 164.220 | <0.001** |
| | 7th day | 0.960 | 0.13 | | |
| | 21st day | 0.597 | 0.14 | | |
| | Total | 0.932 | 0.29 | | |
| CHX | Baseline | 1.223 | 0.113 | 54.383 | <0.001** |
| | 7th day | 1.053 | 0.116 | | |
| | 21st day | 0.893 | 0.136 | | |
| | Total | 1.057 | 0.181 | | |

** Highly significant

Table 3. Intragroup comparison of the pH values at different time points in Neem, Mango and CHX groups using ANOVA (n=30)

| Groups | pH | Mean | Standard Deviation | F value | P value |
|--------|----------|------|--------------------|---------|----------|
| Neem | Baseline | 6.03 | 0.41 | 49.985 | <0.001** |
| | 7th day | 6.57 | 0.50 | | |
| | 21st day | 7.13 | 0.34 | | |
| | Total | 6.58 | 0.61 | | |
| Mango | Baseline | 5.93 | 0.36 | 65.373 | <0.001** |
| | 7th day | 6.40 | 0.49 | | |
| | 21st day | 7.10 | 0.30 | | |
| | Total | 6.48 | 0.62 | | |
| CHX | Baseline | 5.93 | 0.52 | 58.790 | <0.001** |
| | 7th day | 6.17 | 0.46 | | |
| | 21st day | 7.07 | 0.25 | | |
| | Total | 6.39 | 0.64 | | |

** Highly significant

Table 4. Intragroup comparison of plaque score and gingival score in neem, mango and CHX groups at different time points using Tukey's post hoc test

| Time interval | Plaque Index | Neem | | | Mango | | | Chlorhexidine | | |
|-------------------|--------------|----------------|-------------|--------------|----------------|-------------|--------------|----------------|-------------|--|
| | | Gingival Index | Salivary pH | Plaque Index | Gingival Index | Salivary pH | Plaque Index | Gingival Index | Salivary pH | |
| Baseline - day 7 | <0.001** | <0.001** | <0.001** | <0.001** | <0.001** | <0.001** | <0.001** | <0.001** | 0.093 | |
| Day 7 - day 21 | <0.001** | <0.001** | <0.001** | <0.001** | <0.001** | <0.001** | <0.001** | <0.001** | <0.001** | |
| Baseline - day 21 | <0.001** | <0.001** | <0.001** | <0.001** | <0.001** | <0.001** | <0.001** | <0.001** | <0.001** | |

** Highly significant

Table 5. Intergroup comparison for mean reduction in plaque scores from baseline to 21 days using ANOVA (n=30)

| Group | Mean | SD | F-value | P value |
|-------|-------|-------|---------|----------|
| Neem | 0.336 | 0.080 | 33.866 | <0.001** |
| Mango | 0.313 | 0.107 | | |
| CHX | 0.130 | 0.126 | | |
| Total | 0.260 | 0.140 | | |

** Highly significant; SD: Standard Deviation

Table 6 showed that there was a highly significant difference in reduction of the mean gingival score among the neem, mango and CHX groups ($P < 0.05$). The maximum mean reduction was shown by mango (mean=0.643) followed by neem (mean=0.470) and lastly by CHX (mean=0.330) ($P < 0.001$).

Table 7 shows the mean pH values from baseline to 21 days assessed by ANOVA. There was a reduction in the pH scores of all three study groups from baseline to 21 days; however, this difference was not statistically significant ($P = 0.817$). Table 8 shows that there were highly significant differences between the three groups with respect to plaque scores and gingival scores from baseline to 21 days ($P < 0.001$) except for the mean plaque score reduction between the neem and mango groups ($P > 0.05$).

Table 6. Intergroup comparison for mean reduction in gingival scores from baseline to 21 days using ANOVA (n=30)

| Group | Mean | SD | F value | P value |
|-------|-------|-------|---------|----------|
| Neem | 0.470 | 0.148 | 32.813 | <0.001** |
| Mango | 0.643 | 0.135 | | |
| CHX | 0.330 | 0.164 | | |
| Total | 0.481 | 0.196 | | |

** Highly significant; SD: Standard Deviation

DISCUSSION

The World Health Organization estimates that over 100 million Europeans are currently users of traditional medicine [22]. Trees with medicinal properties such as neem [23] and

mango [24] are mainly cultivated in India. Thus, they are readily available which makes them economical [25]. Hence, they are affordable even for the lower socioeconomic population. Along with this, the various properties of both neem and mango have shown protective effects against dental caries [26]. An extract is interpreted to be highly effective against a microorganism if the diameter of the growth inhibition zone is more than 18 mm; it is not effective if the diameter of the growth inhibition zone is less than 13 mm and intermediate if it is in-between [27].

Table 7. Intergroup comparison of the mean difference of pH values (Baseline – 21 days) in all 3 groups using ANOVA (n=30)

| | Mean | SD | F value | P value |
|-------|------|-------|---------|---------|
| Neem | 1.10 | 0.402 | 0.202 | 0.817 |
| Mango | 1.16 | 0.379 | | |
| CHX | 1.13 | 0.434 | | |
| Total | 1.13 | 0.402 | | |

** Highly significant; SD: Standard Deviation

Table 8. Intergroup comparison with regard to the mean reduction in plaque score and gingival score from baseline to 21 days using Tukey's post hoc test

| | Plaque score | Gingival score |
|------------|--------------|----------------|
| Neem-Mango | 0.674 | <0.001** |
| Neem-CHX | <0.001** | <0.001** |
| Mango-CHX | <0.001** | <0.001** |

** Highly significant

In vitro evaluation of the extracts in our study revealed an 18.5 mm growth inhibition zone of *S. mutans* caused by neem extract and 19 mm caused by mango extract at 25% concentration. The findings of our study were in contrast to those of Prashant et al, [28] who reported a zone of inhibition of 3.8 mm using 50% neem and 2.9 mm using 50% mango extract. Their study did not assess 25% concentration of the extracts. Elangovan et al. [26] reported a zone of inhibition of 4 mm with 25% neem and 3 mm with 25% mango extract. Kankariya et al. [29] reported that concentrations of 40% and 50% of neem

extract showed better antibacterial efficacy against *S. mutans* isolated from dental plaque with a zone of inhibition of 19 mm and 20 mm, respectively than their lower concentrations, which was contradictory to our study.

In the present study, the age group of 8-13 years was selected since increased accumulation of plaque is seen on partially erupted teeth than on completely erupted ones [30]. Also, because of the change in dietary habits and lifestyle, children in this age group are at high risk of developing dental caries as well as periodontal problems [31]. Our study was conducted in a residential school where the diet was the same for all children for the period of investigation. The diet mainly consisted of fiber-rich food along with less sticky and sugary food substances which are among the important causes of dental caries [32]. Hence, the type of diet did not affect the factors examined in the present study. The results of the present study revealed that there was a highly significant reduction in plaque score and gingival score in both neem and mango groups from baseline to 21 days. These findings were in accordance to the study by Balappanavar et al, [17] who observed a reduction in plaque and gingival scores of the subjects after the use of neem mouth rinse. Also, our findings were similar to those of a study by Sharma et al, [16] who observed a reduction in plaque and gingival scores with 50% concentration of both neem and mango extracts compared with CHX. However, the results of our study were in contrast to those of Bhat et al, [33] who found that CHX was more effective than mango mouth rinse prepared at 2% concentration in their study in reducing the plaque and gingival scores. This study was conducted for a trial period of only 5 days while the present study was conducted for 21 days, which could be one of the reasons for such contradictory results. Evaluation of salivary pH plays an important role in assessment of an individual's risk of caries. Saliva contains a variety of host defense factors and is also known for its buffering action which in turn increases the salivary pH. It has been shown that increase in the

salivary pH leads to increase in plaque pH [34]. Hence, in the present study, the salivary pH of the patients was recorded. The pH values were assessed using the Indikrom pH strips. Intragroup evaluation of the pH values in all three groups at baseline, 7 days and 21 days revealed that there was a highly significant increase in the pH values. When intergroup comparisons were made between the three groups at baseline, 7 days and 21 days, the increase in pH values was found to be non-significant. These results were in accordance with the study done by Balappanavar et al, [17] and Hegazy and Awad [35].

Assessment of taste acceptability of the prepared mouthwashes revealed that both herbal mouthwashes were comparatively more acceptable by children than CHX. The neem mouthwash was acceptable to 21 subjects (70%), tolerable by 8 subjects (26.7%) and unacceptable to 1 subject (3.3%). While the mango mouthwash was acceptable to 27 subjects (90%) and tolerable to 3 subjects (10%). The CHX mouthwash was acceptable to 23 subjects (76.7%) and tolerable to 7 subjects (23.3%). However, no previous study is available evaluating the taste acceptability of neem and mango mouthwashes to compare our results with. One limitation of the present study was that staining of tongue was present in 6.7% of subjects using neem and mango mouthwashes. Follow-up evaluation until the disappearance of the stain was not performed. Further long-term studies are required to assess the substantivity of the effect of mouthwashes after their withdrawal.

CONCLUSION

From the present study, it can be concluded that neem and mango extracts were equally effective against *S. mutans* comparable with CHX. Highly significant reductions in plaque and gingival scores and salivary pH in both neem and mango groups were noted compared with CHX group. Hence, neem and mango mouthwashes could be effectively used as alternatives to CHX in children to improve their gingival health and decrease plaque formation.

CONFLICT OF INTEREST STATEMENT

None declared.

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