

## Original Article

## Comparative Evaluation of Effectiveness of Curry-leaf Mouthwash, Cinnamon Mouthwash, and Chlorhexidine Mouthwash in Maintaining Salivary and Tongue pH

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### ABSTRACT

**Background & Aim:** Saliva plays a critical role in maintaining oral health through various defensive mechanisms and salivary pH is a fair indicator of various dental diseases. The purpose of the study was to compare the effectiveness of curry leaf and cinnamon mouthwashes in maintaining salivary and tongue pH as compared to chlorhexidine mouthwash. **Methodology:** A randomized parallel-group study was conducted among 60 participants who were randomly allocated to the three groups (curry leaf, cinnamon, and chlorhexidine). The participants were asked to rinse their mouth with respective mouthwashes. Stimulated saliva was collected before and after rinsing with respective mouthwashes and the salivary and tongue coating pH were measured by a digital pH meter and coloured pH indicators. Data were analyzed statistically using one way ANOVA and Student t-test. **Results:** There was no statistically significant difference between the three groups in relation to salivary and tongue pH ( $p > 0.05$ ). Within the cinnamon mouthwash group, the mean salivary pH was only found significant between baseline and 30 minutes after rinsing ( $p < 0.05$ ), whereas, it was found significant at all the point intervals ( $p < 0.05$ ) within the curry leaf and chlorhexidine mouthwash groups. The mean tongue pH difference was found significant only between baseline and immediately after rinsing and between baseline and half an hour after rinsing with the curry leaf mouthwash ( $p < 0.05$ ) and non-significant within cinnamon and chlorhexidine mouthwash groups. **Conclusion:** Curry leaf and cinnamon mouthwashes can be considered as safe, effective, and economical agent and as an alternative to the commercially available mouthwash.

**Key words:** Chlorhexidine, Herbal medicine, Mouthwash, Saliva, Tongue

Oral diseases starting from cavities to cancer are recognized as major public health problems throughout the world. Among them, the most common one is dental caries. It is an infectious microbiologic disease of the tooth with multifactorial aetiology and factors. One of the recognised and contributing factor for the causation or prevention of dental caries is saliva. The defensive system of saliva includes salivary pH, buffering capacity, salivary flow, etc.[1].

Salivary pH is of great relevance to oral cavity more specifically to dental caries as many of the pathological conditions are strongly dependant on the pH changes [2,3]. A lot of commercial agents are available in the market for improving the oral health of the individual by stabilising the salivary pH through anti-cariogenic effects of the agents, but they also have several undesirable side-effects such as allergic reactions, vomiting, diarrhoea, and tooth staining [4]. In order to overcome these side effects, World Health Organization (WHO) has

advised to incorporate natural products as an alternate to these commercial agents as they are safe, effective and economical.

Among those natural food products, one of them is extremely popular ingredient of Indian cuisine and is used in every home to give flavour and aroma to most of the recipes, it is known as curry leaf (*Murraya koenigii*; Rutaceae) or “Magical plant of Indian Spice”. The official name of curry leaf in India is *Saurabhanimba*. The plant has a monography at the Ayurvedic Pharmacopoeia of India. It is a green leafy vegetable which is easily available, commonly known as *Kurrypattu* or *meethi neem*. [5] Chowdhury et al. reported that alkaloids present in curry leaf have antimicrobial activity against gram positive and negative bacteria and fungi. [6]

Another natural agent is Cinnamon (*Cinnamomum zeylanicum*) which is a member of Lauraceae family used in dry or ground form. Previous studies have proved that cinnamon possesses antibacterial, anti-inflammatory, and antifungal property. [7] Based on this evidence, cinnamon extract has been previously evaluated as a mouthrinse for the treatment of gingivitis and promoted the gingival health.

Few studies related to curry leaf and cinnamon were conducted in past but no study has compared the effects of curry leaf mouthwash and cinnamon mouthwash with a commercial available mouthwash. [8-12] Therefore there is a need to evaluate the effectiveness of curry leaf and cinnamon mouthwashes on salivary and tongue coating pH and its comparison with the gold standard mouthwash i.e., the 0.2% chlorhexidine gluconate mouthwash.

## MATERIALS AND METHODS

**Study design:** A randomized controlled trial consisting of parallel groups (curry leaf mouthwash group, cinnamon mouthwash, and chlorhexidine group) was conducted to assess the effectiveness of curry leaf and cinnamon mouthwashes on salivary and tongue pH as compared to gold standard chlorhexidine mouthwash.

**Study population, duration and setting:** College students aged 18-24 years of Bangalore City, without the history of systemic diseases and

antibiotics are included in the study. The study was carried out from September 2016 to October 2016 in a dental college and the study duration was one week.

**Ethical approval:** The study protocol was reviewed by the Ethical committee of Institutional Review Board and was granted ethical clearance

**Sample size:** Based on the previous papers, a final sample size was 60 students was taken (20 students in each group).

The study protocol was described to the students and then consent form was distributed. Sixty college students aged 18 years and above and who signed the informed consent form, were included in the study. After the inclusion of the participants in the study, randomization of the students to their respective groups was done using table of random numbers by a different investigator.

### Preparation of Mouthwashes: 2.5% curry leaves mouth wash [8]

- Fresh curry leaves was obtained and an aqueous extract was prepared by using 100 g of fresh curry leaves and 100 ml of distilled water, all of which were processed in an electric mixer for 10 minutes.
- The resulting extract was filtered and sterilised by paper filter.
- All solid and liquid residues were weighed and attained a concentration of 25%. (250gm of curry leaves paste in 1000 ml or 1 litre).
- It was again diluted with distilled water to attain a final concentration of 2.5%.

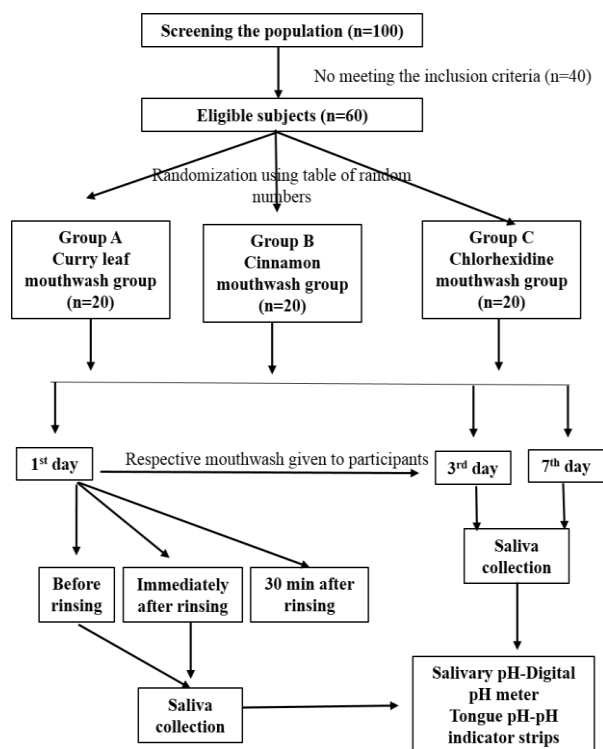
### Cinnamon mouthwash preparation [12]

- Fresh cinnamon bark was taken from the botanical garden. It was ground to a fine powder in a mechanical grinder. Ten grams of this finely powdered cinnamon was mixed with 100 ml of sterile deionized water and kept in a water bath in a round-bottomed flask at 55°C–60°C for 5 h and then filtered through sterile filter paper (Whatman®, UK). The resulting extract was filtered and sterilised by paper filter. It was suspended in polyethylene glycol 400 (20% w: v) and sterile distilled water to give a final concentration of 20% w/v. The entire

procedure was performed under proper aseptic conditions.

**Collection of saliva:** On the day of study, the participants were asked to perform their normal oral hygiene procedure, but refrain from eating or drinking up to one hour prior to saliva collection. Participants were seated comfortably in a dental chair and were instructed to bend the head forward to collect stimulated saliva into a paper cup with the help of chewable rubber bands.

Figure 1 showing the CONSORT flow diagram of the participants. Saliva collection was done on three days over a period of one week (1<sup>st</sup> day, 3<sup>rd</sup> day and 7<sup>th</sup> day) and on first day at three time intervals (before rinsing, immediately after rinsing and half an hour after rinsing). Stimulated saliva was collected by the investigator on the first day before rinsing with the respective mouthwash and then tongue coating pH was recorded with the help of pH strips. The participants were then asked to rinse the mouth with the respective mouthwashes (10 ml for 1 minute) and then again saliva was collected and tongue pH was recorded. After half an hour of rinsing, same procedure was repeated for the salivary pH and tongue pH assessment.



**Figure 1: CONSORT flow diagram showing the enrollment, intervention, and follow-up**

None were allowed to eat or drink during the phases. Mouthwashes were handed over to the students for seven days as per the group respectively (10 ml mouthwash, twice daily for 1 minute). Salivary samples were collected on third and seventh day and estimation of salivary and tongue pH was done. Salivary pH was assessed using digital salivary pH meter and tongue pH with the help of pH indicator strips (pH 0-14)

**Statistical analysis:** The Statistical software Statistical Package for Social Science (SPSS) version 22.0 was used for the analysis of the data and Microsoft Excel have been used to generate graphs, tables, etc. Descriptive statistics with frequency and percentage was obtained. Repeated measures analysis of variance (ANOVA) and Student's *t*-test were used for phase and group comparisons, respectively. The *p* value was taken as significant when less than 0.05.

## RESULTS

Sixty (100%) college students of age 18 years and above were included in the study with a mean ( $\pm$  S.D.) age of 20.46 ( $\pm$  2.16) years. Forty three (71.6%) of them were females and 17 (28.4%) were males. Mean salivary pH was compared between curry leaf, cinnamon, and chlorhexidine mouthwashes group at different time intervals within one week. There was no significant difference found between groups ( $p > 0.05$ ) (Table 1).

Table 2 showing the mean tongue pH between the three mouthwashes groups. It was also not found statistically significant ( $p > 0.05$ ). The mean salivary pH and tongue difference was calculated by comparing the mean pH of various intervals with the baseline pH to evaluate the deviation from the mean pH at various points of study intervals. Figure 2 shows the mean salivary pH difference and mean tongue pH difference within the cinnamon mouthwash group. Mean salivary pH was found significant between baseline and 30 minutes after rinsing ( $p < 0.05$ ) but the tongue pH was non-significant at all the time intervals.

Within the curry leaf mouthwash group, the mean salivary pH was found significant at all the point intervals ( $p < 0.05$ ), whereas mean tongue pH difference was found significant only between baseline and immediately after rinsing and between baseline and half an hour after rinsing with the

curry leaf mouthwash ( $p < 0.05$ ) (Figure 3). Figure 4 shows the mean salivary pH difference and mean tongue pH difference within the chlorhexidine mouthwash group. Mean salivary pH difference was found significant at all the point intervals ( $p < 0.05$ ), whereas mean tongue pH difference was found non-significant ( $p > 0.05$ ).

## DISCUSSION

Dental diseases are highly prevalent in developing countries due to negligence, lack of awareness and

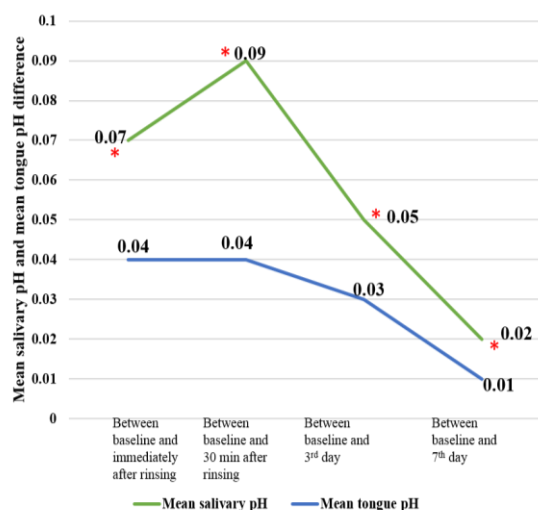
scarcity of treatment as dental care services are often too costly and totally absent in the rural areas and concentrated mainly in urban areas. So, there is a great demand for alternate methods of prevention of dental diseases that are natural, safe, economical and effective. One such method used in this study was curry leaf in mouthwash form as mouthwashes are widely used nowadays and gaining attention in the market because of their easy to use method and the presence of active ingredients [13].

**Table 1: Estimation of mean salivary pH at different time intervals for the three groups (n=60)**

Group	Before rinsing	Immediately after rinsing	After 30 minutes of rinsing	Third day	Seventh day	P value
Curry leaf mouthwash	7.43	7.62	7.67	7.6	7.56	P=0.06
Cinnamon mouthwash	7.48	7.55	7.57	7.53	7.5	
Chlorhexidine mouthwash	7.49	7.56	7.59	7.61	7.54	

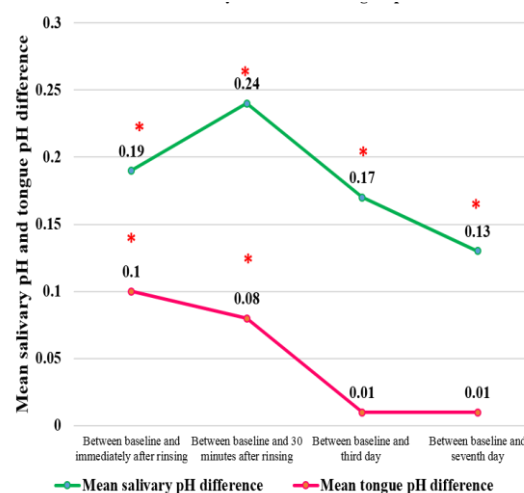
**Table 2: Estimation of mean tongue pH at different time intervals for the three groups (n=60)**

Group	Before rinsing	Immediately after rinsing	After 30 minutes of rinsing	Third day	Seventh day	P value
Curry leaf mouthwash	7.18	7.28	7.26	7.19	7.19	0.076
Cinnamon mouthwash	7.19	7.23	7.23	7.22	7.20	
chlorhexidine mouthwash	7.21	7.23	7.22	7.22	7.22	



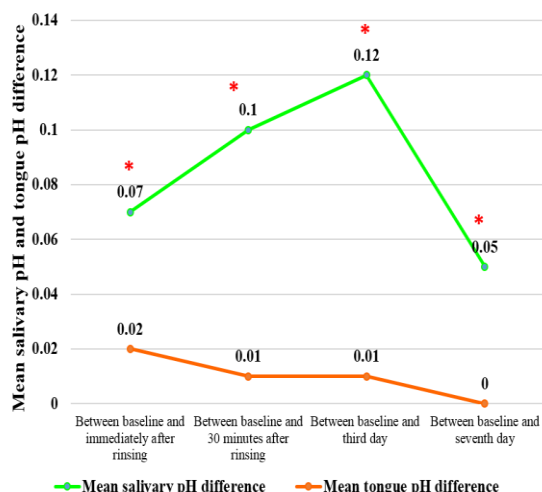
(Paired t test, \* denotes statistical significance)

**Figure 2: Comparison of mean salivary pH difference and mean tongue pH difference at different point intervals within cinnamon mouthwash group**



(Paired t test, \* denotes statistical significance)

**Figure 3: Comparison of mean salivary pH difference and mean tongue pH difference at different point intervals within curry leaf mouthwash group**



(Paired t test, \* denotes statistical significance)

**Figure 4: Comparison of mean salivary pH difference and mean tongue pH difference at different point intervals within chlorhexidine mouthwash group**

In the present study, three varieties of mouthwashes were taken, two were natural and the third was synthetic. First was curry leaf mouthwash (2.5%), second was cinnamon mouthwash (20% w/w), and the third one was chlorhexidine mouthwash (0.2%). Curry leaf or *Murraya Koenigii*, that belongs to Family Rutaceae is a very popular plant for its characteristic aroma and medicinal value. According to Math MV[14], chewing 2 to 4 fresh curry leaves with 10 to 15mls water, swishing for 5 to 7 minutes and then rinsing the mouth with water is very helpful in maintaining good oral hygiene. This method was same as using mouthwash. Cinnamon with scientific name of *Cinnamomum zeylanicum* is a shrub of Lauraceae and of Laurales species [15]. The third mouthwash used was chlorhexidine mouthwash (0.2%). Chlorhexidine is considered as the 'gold standard' mouthwash. It also has evidence related to be used as anti-caries and anti-plaque agent but also has some side effects such as alteration of taste, tooth staining and desquamation of the oral mucosa.[16] Chlorhexidine mouthwash was available in two concentrations (0.12% and 0.2%). Franco NetoCA et al. [17] stated that there is no difference in the efficacy of chlorhexidine between 0.12 to 0.2%. Hence, In the present study, 0.2% concentration of chlorhexidine mouthwash was used.

The present study was a parallel group randomised controlled trial using three different

types of mouthwash. In the present study, stimulated saliva was used to measure salivary pH. Stimulated saliva contains more calcium and bicarbonate and has a higher pH than unstimulated saliva, making it even more effective at remineralizing the enamel crystals. The outcome variables were mean salivary pH and mean tongue pH compared between and within the groups at different point intervals of the study. The mean salivary pH and tongue pH was compared at different time intervals of the study between the groups and it was found non-significant between the three groups, it implies that all the three mouthwashes did not have much difference regarding alteration in salivary and tongue pH. Similar results were obtained by Varghese A et al in their study. They assessed the effectiveness of *M. koenigii* mouthwash in reduction of plaque and gingivitis in comparison with commercially available chlorhexidine (CHX) mouthwash and found that *M. koenigii* mouthwash is equally effective as CHX, in treating plaque-induced gingivitis.<sup>10</sup>

Molania T et al also found no significant difference between groups in plaque index and gingival index between cinnamaldehyde group and chlorhexidine mouthwash ( $P < 0.001$ ). They concluded that cinnamaldehyde mouthwash was helpful in improving gingival status and its effects were comparable to that of chlorhexidine without its significant side effects.[11] The results of our study are in contrary to a study conducted by Ramesh G et al in which a significant difference was observed between the three groups (curry leaf, mint leaf and tulsi leaf group) ( $p < 0.05$ ) and also between the tulsi and curry leaf groups on the last assessment or final day with respect to mean tongue pH ( $p < 0.05$ ).[10] Gupta and Jain evaluated the effect of chlorhexidine and cinnamon extract mouthrinses on gingival status and dental plaque levels. They found that the chlorhexidine group showed the maximum decrease in both plaque and gingival scores, followed by cinnamon extract, but the result was statistically insignificant.[12]

Mean salivary pH difference was compared within the three mouthwashes group and it was found significant at all the point intervals. The results are in accordance with the study conducted by Ramesh G et al in which mean salivary pH difference between baseline and further intervals was found significant ( $p < 0.05$ ) within curry leaf

mouthwash group.[8] Same results were obtained by Ashwini Y et al in which 0.2% chlorhexidine mouthwash was compared with neem and green tea mouthwash and the salivary pH was significant within the chlorhexidine group.[18] The increased value of mean salivary pH was found immediately after rinsing and 30 minutes after rinsing in both the groups. This can be explained by the fact that rinsing mouth with the mouthwash may increase salivary flow and in turn stimulates salivation which increases the saliva's bicarbonate concentration and thus increases salivary pH.[19] Increased salivary pH has a lot of benefits in maintaining oral health as increased salivary pH actively reduces demineralisation and increases remineralisation of the enamel crystals damaged by an acid attack and in turn less probabilities of dental decay.

The mean tongue pH difference was found significant only at two point intervals (between baseline and immediately after rinsing and between baseline and 30 minutes after rinsing) in curry leaf mouthwash group whereas, non-significant in chlorhexidine and cinnamon mouthwash group. Tongue pH is a major indicating factor for the bad breath forming elements as increased tongue pH or alkalinity favours the production of odours while acidity or decreased tongue pH inhibit the production of such substances.[20] Bad breath forming elements that contributes to the alkalinity of tongue pH and hence halitosis includes odorivores, which are the final products of proteolysis such as amines, ammonia, and urea.[19]

The antimicrobial effects of curry leaf and cinnamon observed in the present study could be due to the presence of active substances in curry leaf and cinnamon. According to previous research, curry leaves are used as calcium source to those having calcium deficiency and are rich source of Vitamin A, Vitamin B and B2, Vitamin C, iron, carbohydrates, proteins, amino acids and minerals.[10] The fresh curry leaves contain 2.65% volatile essential oils like sesquiterpenes and monoterpenes which have broad anti-microbial effects.[14] Curry leaf also contains chlorophyll, which was thought to be the anticariogenic agent and reduces halitosis.[14] Cinnamon's skin contains 0.5 to 2.5 percent essential oil, containing more than 50 different compounds, 80-65 percent of which are cinnamaldehydes. Other compounds include cinnamic acid, phenolic compounds such as

eugenol, fiedron and safrole, terpene compounds such as limonene and lanolol, trans-cinnamaldehyde, tannin, coumarin, resin, phenylpropane compounds such as hydroxycinnamaldehyde and mannitol, whose sweet taste is due to manythol. According to previous studies, the active substances of cinnamon are cinnamic aldehyde, an aromatic aldehyde, and eugenol.[12,15,21] Cinnamon bark is rich in cinnamaldehyde (50.5%), which is highly electronegative and interferes in biological processes involving electron transfer, and reacts with nitrogen-containing components, for example, proteins and nucleic acids, thereby inhibiting the growth of the microorganisms.[22]

We have tried our best to compare the antimicrobial efficacy of two natural mouthwashes with a commercially available synthetic mouthwash but despite our efforts, some limitations are present in the study. Since the sample size of the present study was sixty and the study duration was only 1 week, more studies with larger sample size and with longer duration on curry leaf mouthwash should be encouraged to assess the long-term effectiveness.

## CONCLUSION

In the present study, there was no significance regarding mean salivary pH and mean tongue pH between the three mouthwash groups. Within the curry leaf mouthwash group, the mean salivary pH and mean tongue pH difference was found significant at various point intervals but within the cinnamon and chlorhexidine mouthwashes group, the mean salivary pH difference was found significant and non-significant with respect to mean tongue pH difference. More and more natural products with a wide biological activity should be searched as they have immense antimicrobial potential. Therefore, further clinical trials are required in this field to ascertain their use in dentistry.

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