Comparative evaluation of the antimicrobial efficacy of four chewing sticks commonly used in South India: An in vitro study

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ABSTRACT

Background and Aim: The use of chewing sticks has been well documented since ancient times in India. Chewing sticks are a good alternative to the toothbrush for maintaining oral hygiene. The present study was designed and conducted to compare and evaluate the antimicrobial effects of the aqueous extracts of neem, miswak, mango, and banyan chewing sticks against two bacterial species considered the most important in the initiation and progression of dental caries, namely Streptococcus mutans and Lactobacillus acidophilus, respectively.

Materials and Methods: Twigs of the above mentioned chewing sticks were sun dried and powdered, and sterile aqueous solutions of 10%, 25% and 50% concentrations were prepared. Culture plates for S mutans and L acidophilus were prepared and the growth was transferred to nutrient agar and Mueller-Hinton agar; antimicrobial activity of the extracts was tested after 72 h, using the disc diffusion method. Normal saline was used as control.

Results: The antimicrobial activity of neem, miswak, and mango extracts increased as their concentrations increased. Both banyan extract and saline showed no antimicrobial activity against the organisms tested.

Conclusion and Recommendations: Based on the zones of inhibition, aqueous extracts of neem showed the most antimicrobial activity against S mutans, while miswak extracts showed superior antimicrobial activity against L acidophilus. We recommend further phytochemical and pharmacological studies to discover newer nonsynthetic tooth pastes and mouthwashes.

Key words: Chewing sticks, mango, miswak, neem

Oral health is a vital component of the general health of an individual. Dental caries is one of the most common dental diseases, which is a matter of concern. With increased awareness among people, oral hygiene practices have improved, although there are still some villages where people still use the twigs of certain trees to clean their teeth. Surveys among these people show that the caries prevalence is either low or comparable to that of their counterparts in rural areas.[1,2] This could be due to their food practices or the effect of the natural ingredients present in the chewing sticks they use. Chewing sticks can be a good alternative to the toothbrush as a means of preventing oral and dental disease.

Aims and objective

This in vitro study was designed to evaluate and compare the antimicrobial effects of aqueous extracts of four naturally available chewing sticks used in South India [miswak (arak; Salvadora persica), neem (vembu; Azadirachta indica), mango (ma; Mangifera indica), and banyan (alam; Ficus bengalensis)] against Streptococcus mutans and Lactobacillus acidophilus.

MATERIALS AND METHODS

The following materials were used in this study
- Dried chewing sticks of
  - Mango (Mangifera indica)
  - Neem (Azadirachta indica)
  - Miswak (Salvadora persica)
  - Banyan (Ficus bengalensis)
- Sterile plastic containers
- Sterile deionised water
• Two species of bacterial microorganisms
  ▪  *Streptococcus mutans* (MTCC 890)
  ▪  *Lactobacillus acidophilus* (MTCC 447)

• Blood agar plates
• Chocolate agar plates
• Incubator
• Nutrient agar medium
• Mueller-Hinton agar medium
• Inoculating wire loop
• Sterilized 6 mm paper discs
• Antibiotic zone scale

**Preparation of chewing stick extracts**
Twigs of locally available mango, neem, banyan, and miswak were bought from the open market and were identified by their color and scent [Figure 1]. They were cut into pieces of approximately 15 cm length. All the sticks were authenticated by the Department of Botany, National College of Arts and Science, Tiruchirapalli. The twigs were sun dried for 2 days and were ground separately into coarse powders. Aqueous extracts of all the four chewing sticks were prepared by adding 10 gm, 25 gm, and 50 gm of each chewing stick powder to 100 ml of deionised distilled water. The mixture was then shaken well by hand and allowed to soak for 48 h at 4°C. It was then filtered to get extracts of 5%, 10%, and 50% concentration of each type of chewing stick.

The group distribution was as follows:
- Group 1 (miswak)
  ▪  1A- 10% miswak
  ▪  1B- 25% miswak
  ▪  1C- 50% miswak
- Group 2 (neem)
  ▪  2A- 10% neem
  ▪  2B- 25% neem
  ▪  2C- 50% neem
- Group 3 (mango)
  ▪  3A- 10% Mango
  ▪  3B- 25% Mango
  ▪  3C- 50% Mango
- Group 4 (banyan)
  ▪  4A- 10% banyan
  ▪  4B- 25% banyan
  ▪  4C- 50% banyan
- Group 5 (control; normal saline)

**Preparation of culture media**
Ampoules containing pure forms of *S mutans* (MTCC 890) and *L acidophilus* (MTCC 447) in liquid transporting media were obtained from HiMedia Laboratories Pvt. Ltd, Mumbai. Culture plates for *S mutans* and *L acidophilus* were prepared separately in the Department of Microbiology, Ragas Dental College, by inoculating the contents of the ampoules in chocolate agar and blood agar plates for *S mutans* and *L acidophilus*, respectively, at 37°C for 12 h. Growth obtained from agar plates was transferred to nutrient agar and Mueller-Hinton agar for testing the antimicrobial activity of the extracts.

**Testing antimicrobial efficacy (disc diffusion method)**
Standard 6 mm sterile discs were dipped in the prepared extracts (i.e., concentrations of 10%, 25%, and 50% of each type of chewing stick) and placed over the agar plates, which were then incubated at 37°C for 72 h. Normal saline was used as a control. Zones of inhibition were measured using the antibiotic zone scale (HiMedia). The same experiment was repeated thrice for each extract and the mean values of the zones of inhibition for different concentrations against *S mutans* and *L acidophilus* were calculated [Figures 2 and 3].

**RESULTS**
The mean values of the zones of inhibition of the extracts against *S mutans* were calculated. For statistical analysis used we used one-way ANOVA and the Mann-Whitney U-test. The mean values of the zones of inhibition and intra-group comparisons of the action of the extracts against *S mutans* are...
are listed in Table 1. Inter-group comparisons are listed in Table 2.

Similarly, the mean values of the zones of inhibition of the aqueous extracts against *L. acidophilus* and the intra-group comparisons are listed in Table 3; inter-group comparisons are listed in Table 4.

DISCUSSION

Dental caries, one of the most common dental diseases seen in Indians, can be controlled by various preventive and therapeutic measures. One preventive measure that can be practiced by the individual at home is maintenance of good oral hygiene, which can be achieved by regular brushing with a toothbrush or a natural cleaning aid such as a chewing stick. Due to increased dental health awareness among the public and the continuous media publicity, there has been an increase in the proportion of people using modern facilities for cleaning their teeth; however, in many rural areas, people still depend on natural materials to clean their teeth. Chewing sticks, which are even now being widely used, are effective for cleaning the teeth, costs little, possess various medicinal properties, and are easily available in the rural areas of the developing countries. Studies done to compare the oral health status of people using chewing sticks with that of people using toothbrushes show that the oral hygiene was comparable between the two groups. The present study was designed to evaluate and compare the antimicrobial action against *S. mutans* and *L. acidophilus* of aqueous extracts of four chewing sticks, namely miswak, *Neem*, *Banyan*, and *Mango*.

Table 1: Mean zones of inhibition against *S. mutans* after 72 h (diameter in millimeters) and intra-group comparison

| Concentration used | Miswak | Neem | Mango | Banyan | Control |
|--------------------|--------|------|-------|--------|---------|
| 10%                | 4      | 9    | 0     | 0      | 0       |
| 25%                | 8      | 12   | 1     | 0      | 0       |
| 50%                | 11     | 14   | 0     | 0      | 0       |
| *P* value: 10% vs 25% | 0.02*  | 0.12* | 0.83* | 1.0    | --      |
| *P* value: 25% vs 50% | 0.03*  | 0.32* | 0.00* | 1.0    | --      |
| *P* value: 50% vs 10% | 0.00*  | 0.01* | 0.00* | 1.0    | --      |

*P* ≤ 0.05 indicates significant difference in the zones of inhibition; *P* > 0.05 indicates nonsignificant difference in the zones of inhibition

Table 2: Inter-group comparisons of the antimicrobial activity of chewing stick extracts against *S. mutans*

| Agents             | Antimicrobial efficacy of chewing sticks at different concentrations |
|--------------------|---------------------------------------------------------------|
|                    | 10% concentration | 25% concentration | 50% concentration |
| Miswak vs neem     | 0.00*             | 0.04*             | 0.28              |
| Miswak vs mango    | 0.00*             | 0.82              | 1.0               |
| Miswak vs banyan   | 0.00*             | 0.00*             | 0.00*             |
| Neem vs mango      | 0.00*             | 0.12              | 0.28              |
| Neem vs banyan     | 0.00*             | 0.00*             | 0.00*             |
| Mango vs banyan    | 0.845             | 0.00*             | 0.00*             |
| Mango vs control   | 0.00*             | 0.00*             | 0.00*             |
| Miswak vs control  | 0.00*             | 0.00*             | 0.00*             |
| Neem vs control    | 0.00*             | 0.00*             | 0.00*             |
| Banyan vs control  | 1.0               | 1.0               | 1.0               |

*P* ≤ 0.05 indicates significant difference in the antimicrobial activity; *P* > 0.05 indicates nonsignificant difference in the antimicrobial activity

Table 3: Mean zones of inhibition against *L. acidophilus* after 72 h (diameter in millimeters) and intra-group comparisons

| Concentration used | Miswak | Neem | Mango | Banyan | Control |
|--------------------|--------|------|-------|--------|---------|
| 10%                | 2      | 0    | 0     | 0      | 0       |
| 25%                | 6      | 4    | 3     | 0      | 0       |
| 50%                | 11     | 9    | 11    | 0      | 0       |
| *P* value: 10% vs 25% | 0.01*  | 0.00* | 0.00* | 1.0    | --      |
| *P* value: 25% vs 50% | 0.02*  | 0.04* | 0.02* | 1.0    | --      |
| *P* value: 50% vs 10% | 0.00*  | 0.00* | 0.00* | 1.0    | --      |

*P* ≤ 0.05 indicates significant difference in the zones of inhibition; *P* > 0.05 indicates nonsignificant difference in the zones of inhibition

Table 4: Inter-group comparisons of the antimicrobial activity of chewing stick extracts against *L. acidophilus*

| AGENTS                  | Antimicrobial efficacy of chewing sticks at different concentrations |
|-------------------------|---------------------------------------------------------------|
|                        | 10% concentration | 25% concentration | 50% concentration |
| Miswak vs neem          | 0.00*             | 0.43              | 0.91              |
| Miswak vs mango         | 0.00*             | 0.00*             | 0.58              |
| Miswak vs banyan        | 0.00*             | 0.00*             | 0.00*             |
| Neem vs banyan          | 0.00*             | 0.00*             | 0.27              |
| Neem vs mango           | 0.00*             | 0.00*             | 0.00*             |
| Neem vs banyan          | 0.00*             | 0.00*             | 0.00*             |
| Mango vs banyan         | 1.0               | 0.03*             | 0.00*             |
| Mango vs control        | 1.0               | 0.00*             | 0.00*             |
| Neem vs control         | 0.04*             | 0.00*             | 0.00*             |
| Neem and banyan         | 1.0               | 0.00*             | 0.00*             |
| Banyan vs control       | 1.0               | 1.0               | 1.0               |

*P* ≤ 0.05 indicates significant difference in the antimicrobial activity; *P* > 0.05 indicates nonsignificant difference in the antimicrobial activity
neem, mango, and banyan, that are commonly used in South India.

Aqueous extracts of the chewing sticks to be tested were prepared at 10%, 25%, and 50% concentrations. Normal saline was used as control. Culture plates of S. mutans and L. acidophilus were prepared using pure cultures and the disc diffusion method was used to find out the antimicrobial activity of each extract at different concentrations.[6] The zone of inhibition was measured using the antibiotic zone scale.

The results show that extracts of neem, miswak, and mango have antimicrobial activity in descending order against S. mutans, and the activity was greater at higher concentrations. Neem showed greater antimicrobial activity against S. mutans than the other extracts, and the maximum antimicrobial activity was observed at 50% concentration. Similar findings were observed in the studies done by Wolinsky et al.,[5] Prasanth et al.,[6] and Khalid.[7] The antimicrobial activity of neem can be attributed to the presence of fluoride (1.0 µg/g) and silica; the former is known to exert an anticariogenic action, and the latter is an abrasive and prevents accumulation of plaque. Alkaloids, known to exert an analgesic action, also contribute towards dental well-being.[8] The presence of the alkaloid margeosine, resins, gum, chloride, fluoride, silica, sulfur, tannins, oils, saponins, flavanoids, sterols, calcium, triterpenoids, phenolic compounds, carotenoids, steroids, valvenoids, ketones, and the tetranortriterpenoid azadirachtin confers various medicinal and antimicrobial properties to neem extract.[9] The oils have carminative, antiseptic, and analgesic effects. Tannins exert an astringent effect and form a coat over the enamel, thus protecting against tooth decay.

Miswak extract showed better antimicrobial activity against L. acidophilus than against S. mutans when compared to neem and mango extracts. Similar findings were reported by Abdel Rahman et al.[10] The antimicrobial activity of miswak extracts increased with the concentration. The antimicrobial effect of aqueous extract of miswak is believed to be due to its high content of chlorides, tannins, trimethylamine, salvadora, nitrate, thiocyanate, and sulphur. Benzyl isothiocyanate is a natural component of miswak and acts as an inhibitor of bacterial multiplication.[7]

Mango aqueous extract was effective against both S. mutans and L. acidophilus only at high concentrations (50%). Similar results were shown by Prasanth et al.[6] and Muhammed and Muhammed.[11] Mango contains tannins, bitter gum, and resins that give it antimicrobial activity against a few microorganisms.[11]

Though banyan extract showed no antimicrobial effect at any concentration against the organisms tested in this study, the wide use of banyan chewing sticks may be because it is a good mechanical plaque remover.

CONCLUSION

- The antimicrobial activity of neem, miswak, and mango extract against S. mutans and L. acidophilus was directly proportional to the concentration.
- Among the agents used in the present study, neem aqueous extracts showed the most antimicrobial activity against S. mutans.
- Miswak aqueous extracts showed better antimicrobial activity than the other extracts against L. acidophilus.
- Though banyan chewing sticks are widely used in many parts of South India, it was found to have no antimicrobial effect against the organisms tested in this study. Likewise, no antimicrobial activity was shown by normal saline.

This in vitro evaluation of antibacterial action is an attempt to encourage phytochemical and pharmaceutical studies to discover newer nonsynthetic antibiotics and nonsynthetic toothpastes/ mouthwashes.

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