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Investigation of antibacterial efficacy of *Acacia nilotica* against salivary mutans streptococci: a randomized control trial

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This double-blind, randomized control trial sought to evaluate the clinical effects of 3 mouthrinses against salivary mutans streptococci (MS). Ninety high-caries risk volunteers were randomly assigned to 3 groups, each group using a selected mouthrinse BID for 30 days. Subjects in Group 1 rinsed with 10 ml of 50% *Acacia nilotica*, Group 2 subjects rinsed with 10 ml of 0.2% chlorhexidine (active control), and subjects in Group 3 rinsed with saline water (passive control). Unstimulated saliva samples were collected at baseline, 30, and 60 days. MS were cultured on *mitis salivarius* bacitracin agar, and colony counts were obtained. The margin of error was fixed at 5%. ANOVA and *post hoc* least significant difference tests were performed. There were significant decreases in the MS colony count in the *A. nilotica* and chlorhexidine groups at 30 days (85% and 83%, respectively) and at 60 days (65% and 63%, respectively) ($P < 0.0001$). The antibacterial action of *A. nilotica* against MS was similar to that of chlorhexidine.

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Dental caries is one of the most prevalent infectious diseases in humans worldwide.[1] *Caries* is defined as a localized, progressive demineralization of the hard tissues of the crown and/or root surfaces of teeth. This demineralization is caused by acids produced by bacteria, particularly mutans streptococci (MS), which ferment dietary carbohydrates. This occurs within dental plaque, a bacteria-laden gelatinous material that adheres to tooth surfaces and becomes colonized by bacteria. Thus, caries results from the interplay of 3 factors over time: dietary carbohydrates, cariogenic bacteria within dental plaque, and susceptible hard tooth surfaces.[1] If left untreated, caries may lead to pain, infection, and tooth loss. During the past few decades, changes have been observed in the prevalence and epidemiology of dental caries.[1]

Mouthrinses are adjuncts to mechanical plaque control and serve as delivery vehicles for antimicrobial agents. For decades, chlorhexidine has been considered the 'gold standard' among the different antimicrobial mouthrinsees commercially available.[2] Although chlorhexidine is effective in reducing the number of MS, it has inherent side effects, such as staining of teeth and composite restorations, altered taste perception, metallic taste, and burning sensation.[3] Plant compounds can be therapeutic substitutes for synthetically created antimicrobial agents.[4]

Acacia species—commonly known as *Babool* (or *babul*), *Egyptian mimosa, Egyptian thorn, kikar, Indian gum,* and *red thorn*—have long been used for the treatment of various ailments and for other practical uses. The wood of *A. nilotica* was used by ancient Egyptians to make statues and furniture. Its use has been reported since early Egyptian dynasties. Dioscorides, the Greek physician considered to be the father of botany, described the use of *A. nilotica* (as a preparation extracted from the leaves and fruit pods) in his *De Materia Medica*.[5] He named it *akakia*, and it is from this word that the modern name, *acacia*, is derived. The origin of the word, *acacia*, is "spiny," which is a typical feature of the species. The species is widely spread in Africa, with a range extending from Egypt to Mauritania southwards to South Africa, and also in Asia, ranging eastwards to Pakistan and India. It has been introduced in China, Australia (where it is considered to be a pest plant of national importance), Caribbean and Indian Ocean islands, United States, Central America, and South America. It has been introduced as a medicinal, forage, and fuel wood plant in many parts of world.

A. nilotica has been proven as an effective medicine in the treatment of malaria, sore throat (aerial part), toothache (bark), acute diarrhea, colds, bronchitis, diarrhea, bleeding hemorrhoids, and leucoderma.[6] *A. nilotica* twigs have been used as toothbrushes.[6]

Considerable efforts have been made to find an active agent against *Streptococcus mutans*, as it is found to be resistant to many antibacterial agents, such as penicillin, amoxicillin, cefuroxime, and erythromycin.[7] Thus, there is a growing need to investigate natural antimicrobial agents that are effective and safe for patients.

A. nilotica mouthrinses have demonstrated effective antibacterial effects against halitosis-inducing bacteria on the tongue, and has also been used in the treatment of gingival bleeding and mouth ulcers.[8] The antimicrobial efficacy of *A. nilotica* against MS has been ascertained in previous in vitro studies.[9,10] However, no in vivo studies have been carried out to assess the antibacterial efficacy of *A. nilotica* against MS in comparison with chlorhexidine. Hence, the current study was conducted.

Materials and methods

This double-blind, randomized control trial was conducted on undergraduate student volunteers in the Department of Public Health Dentistry, Teerthankar Mahaveer Dental College and Research Centre, India. The protocol was approved by the Institutional Review Board (IRB) of Teerthankar Mahaveer University. All subjects signed an IRB-approved consent form. A pilot study was done on 10 patients from each of 3 test groups to check the feasibility of the study; those results are not included in the study.

Table 1. Baseline background of the subjects.

Baseline characteristics	*A. nilotica* n = 30	Chlorhexidine n = 30	Placebo control n = 30	*P* value
No. of men/women	15/15	10/20	12/18	0.698
Range of age (years); mean (SD)	20-24; 22.16 (2.01)	19-25; 21.42 (2.07)	20-25; 22.74 (2.28)	0.362
Number of times toothbrushing	Once–25, Twice–5	Once–24, Twice–6	Once–25, Twice–5	0.897
Additional oral hygiene aids	None	None	None	
DMFT, mean (SD)	3.52 (3.39)	3.67 (2.43)	3.18 (2.85)	0.759
Incipient lesions, mean (SD)	5.58 (3.48)	5.42 (4.08)	5.63 (4.64)	1.098

Abbreviations: *A. nilotica, Acacia nilotica*; DMFT, decayed/missing/filled teeth; SD, standard deviation.

Table 2. ANOVA results for the 3 study groups.

		Sum of squares	df	Mean square	*F* value	*P* value
MS (baseline)	Between groups	21709.972	2	10854.986	0.542	1.3120
	Within groups	1589356.737	54	29432.532		
MS (Day 30)	Between groups	468912.035	2	234456.017	15.825	0.0001
	Within groups	789459.474	54	14619.619		
MS (Day 60)	Between groups	389590.877	2	194795.438	9.361	0.0001
	Within groups	929856.000	54	17219.555		

Abbreviations: df, degree of freedom; MS, mutans streptococci.

Preparation of extract

A water-washed section of *A. nilotica* bark was subjected to coarse grating (sieve No. 44) to produce a coarse powder of uniform texture. A hot solid-liquid (Kumagawa) extraction procedure was applied to obtain the extract of *A. nilotica*. The powder was subjected to 50% ethanol for 48 hours at 60°C-65°C. The resulting separate 50% extract was then concentrated and the ethanol solvent was completely removed under reduced pressure by a Lyotrap dryer (LTE Scientific Ltd.). The extract was stored at 4°C in a tightly closed container to preserve it from any contamination, deterioration, and/or decomposition.

Inclusion and exclusion criteria

Volunteers who had 1 or more active incipient lesions and/or frank carious lesions were considered to be at high risk for dental caries and were included in the study. Participants having a baseline plaque score >2 and a baseline DMFT index of 2-5 were included in the study. Volunteers who had used antibiotics or any mouthrinse for 7 consecutive days, or taken corticosteroids in the past 15 days were excluded from the study. Subjects with a history of sensitivity to any mouthrinse, and those who had used removable prostheses or an orthodontic appliance, were excluded from the study.

All volunteers were subjected to clinical examination, and a sampling frame (n = 90) was prepared of those who fulfilled the inclusion and exclusion criteria. Subjects were instructed to refrain from any drug and alcohol consumption for the study period of 60 days and to report any consumption of these products. The subjects were divided into 3 groups (n = 30). This sample size was chosen as the minimum size required due to the calculations for error used in this study: α error <5% ($P < 0.05$), β error 20%, expected mean difference 3.257, and standard deviation 2.715.

The volunteers were randomly allocated into 3 study groups through computer-generated numbers. Individuals were identified by code numbers throughout the study. The clinical trial was conducted according to American Dental Association's *Adjunctive Dental Therapies for the Reduction of Plaque and Gingivitis* guidelines.[11] All eligible subjects participated in the study.

For the study, all subjects were asked to rinse with 10 ml of their designated mouthrinse BID for 30 days. Group 1 subjects were given a 50% *A. nilotica* mouthrinse, Group 2 subjects were given a 0.2% chlorhexidine mouthrinse, and Group 3 (control) was given a saline water mouthrinse (placebo).

Methodology

DMF scores and incipient lesion scores were recorded at baseline. The unstimulated salivary samples were collected from the participants and inoculated onto mitis

Table 3. *Post hoc* significant difference test for multiple comparisons.

Dependent variable	Group (I)	Group (J)	Standard error	*P* value	95% confidence interval	
					Lower limit	Upper limit
MS (baseline)	*A. nilotica*	Chlorhexidine	52.68	0.4220	-149.90	47.80
	A. nilotica	Placebo control	52.68	0.6980	-138.12	62.17
	Chlorhexidine	Placebo control	52.68	0.8970	-69.28	114.01
MS (Day 30)	*A. nilotica*	Chlorhexidine	33.23	0.9810	-69.23	53.23
	A. nilotica	Placebo control	33.23	0.0001	-212.18	-99.72
	Chlorhexidine	Placebo control	33.23	0.0001	-211.18	-98.72
MS (Day 60)	*A. nilotica*	Chlorhexidine	40.18	0.8560	-87.64	79.69
	A. nilotica	Placebo control	40.18	0.0001	-224.33	-79.99
	Chlorhexidine	Placebo control	40.18	0.0001	-216.85	-66.52

(I) and (J) designations according to *post hoc* analysis.
Abbreviations: *A. nilotica, Acacia nilotica*; MS, mutans streptococci.

salivarius bacitracin (MSB) agar (M259, HiMedia Laboratories). The MS colony counts were obtained by a microbiologist who was blinded to the groups. Each participant was given the same brand of toothbrush and toothpaste to minimize bias.

All 3 solutions were made in the university's pharmacy department. Each mouthrinse was the same color, and kept in a coded container. Study subjects were instructed to rinse with 10 ml of mouthrinse for 60 seconds BID, post-breakfast and post-lunch, for 30 days. They were not to rinse with water afterward. They were also instructed not to consume any solid or liquid for a half hour following mouthrinse use. Except for the BID mouth rinsing, the volunteers were asked to maintain their normal oral hygiene practices. All subjects lived in the same student housing, so they all shared the same diet. A compliance diary was given to each study participant; they were asked to make an entry of each usage and side effects experienced, if any. Unstimulated saliva samples were collected and inoculated on MSB agar (MS1) before the study began (baseline). Unstimulated saliva samples were collected from subjects of all 3 groups at the end of 30 days and inoculated onto MSB agar (MS2); colony counts were obtained after 48 hours incubation. On Day 31, the subjects were instructed to stop using the mouthrinse and continue with their routine oral hygiene care. At Day 60, unstimulated saliva samples were collected again, inoculated onto MSB agar (MS3), and colony counts were obtained after incubation.

Collection of saliva sample

The unstimulated saliva samples were collected during the study in the mornings after the use of mouthrinse. The study subjects were asked not to swallow for 60 seconds, after which time the pooled saliva on the floor of the mouth was aspirated with a syringe. The syringes were coded and the saliva samples were diluted in distilled water. The sample was inoculated within 30 minutes after collection. All the microbiological procedures were carried out in the microbiology lab of the university's medical college.

Statistical analysis

SPSS version 21 (SPSS, Inc.) was used for data analysis. Repeated ANOVA and ANOVA followed by *post hoc* least significant difference (LSD) tests were used for analysis. A *P* value of 0.05 was taken to be significant.

Results

All 90 participants completed the study. Descriptive statistics are presented in Table 1. No statistically significant difference was found in the baseline data between the 3 groups. Compliance with mouthrinse use was determined to be acceptable for both the experimental groups. Mean compliance in the *A. nilotica* group was 90.1% (range 87% to 95%), while that of the chlorhexidine group was 86.3% (range 82% to 96%). ANOVA was used to analyze the reduction in colony counts in the 3 groups. There was a significant decrease in the MS colony count in both the *A. nilotica* and chlorhexidine groups at Day 30 (85% and 83%, respectively) and at Day 60 (65% and 63%, respectively) ($P < 0.0001$). The colony counts obtained at Day 60 showed a slight increase compared to counts obtained at Day 30, but an overall reduction to the baseline colony count was seen ($P < 0.0001$). The control group showed a slight decrease at Day 30 and a slight increase at Day 60 (3% and 7%, respectively). This variation, however, was not statistically significant ($P = 0.201$). ANOVA was carried out to assess the intra- and intergroup variations (Table 2). There was no significant difference in the baseline colony count between the 3 groups ($P = 1.312$), while the difference at Day 30 and Day 60 was statistically significant ($P = 0.0001$). Post-hoc LSD was performed to obtain multiple comparisons (Table 3). The difference in the decrease in colony counts between *A. nilotica* and chlorhexidine groups was not statistically significant ($P = 0.981$ and $P = 0.856$ at Days 30 and 60, respectively). However, the differences between both Group 1 and Group 2 vs Group 3 (control) were highly significant ($P < 0.0001$).

Adverse events

The most common adverse event reported was a mild burning sensation in both the *A. nilotica* and chlorhexidine groups. The chlorhexidine group reported altered taste and brown staining of the teeth (50% and 67%, respectively). Such side effects were not recorded in the *A. nilotica* group.

Discussion

The present study was conducted to assess the antibacterial action of a 50% *A. nilotica* mouthrinse against salivary MS in comparison with the 'gold standard' 0.2% chlorhexidine mouthrinse and a placebo (saline water).

Research has been focused in recent years on herbal medicines as alternatives to synthetically created antimicrobial agents, due to their wide range of biological and medicinal activities, potentially higher safety margins, and lower costs. Several antibacterial agents—such as chlorhexidine, fluorides, and various antibiotics—are commercially available that can be used to prevent dental caries. However, some of these have been reported to have undesirable side effects, including nausea, vomiting, tooth staining, and metallic taste.[4] *A. nilotica* is considered safe for human use.[12]

Research on *A. nilotica*-containing products has demonstrated its oral health benefits. Acacia gum has the potential to inhibit early plaque formation, although there is no proven long-term benefit. For centuries, *A. nilotica* gum has been used for oral hygiene in the Middle East and North Africa.[13] In a 2010 study, a gel containing *A. nilotica* significantly improved clinical gingival and plaque index scores over a period of 6 weeks.[14] In a comparison study of other herbal remedies, Dhanya Kumar & Sidhu indicated that an *A. nilotica* extract (concentration 50%) showed the highest antimicrobial activity against *S. mutans*.[15] Thus, a 50% extract concentration was chosen for this study. Following the use of mouthrinse for 30 days, the MS colony counts in the saliva decreased by 85% and 83% in Groups 1 and 2, respectively. This reduction was statistically significant. The MS colony counts at Day 60 showed a reduction in Groups 1 and 2 (65% and 63%, respectively). This suggests that the antibacterial efficacy of *A. nilotica* against salivary MS parallels that of chlorhexidine. In contrast, Group 3 showed a slight variation in MS colony count. For both Groups 1 and 2, there was a slight decrease in colony counts at Day 30 and a slight increase at Day 60. This variation was not statistically significant and it was possibly due to physiological changes.

A. nilotica stem bark extracts contain alkaloids, saponins, cardiac glycosides, tannins, flavonoids, and anthraquinones which might be responsible for its antibacterial properties.[9] A review of the available literature revealed that some authors have reported in vivo antibacterial activity of herbs such as *Terminalia chebula* and Triphala against salivary MS, and *Aloe vera* against dental plaque, but to date, no studies have been conducted to assess the effect of *A. nilotica* on salivary MS.[16-20] The results of 50% *A. nilotica* extract mouthrinse on salivary MS could not be compared with other studies, as no in vivo studies that have tried to assess the same effect have been reported in the literature. However, studies have been reported that suggest that *A. nilotica* possesses other beneficial properties for general and oral health.[21]

Compliance with mouthrinse use was acceptable in both Groups 1 and 2. Mean compliance in the *A. nilotica* group was 93.6% while that in the chlorhexidine group was 91.2%. The taste of *A. nilotica* mouthrinse was acceptable to all the subjects of the group. The astringent action of *A. nilotica* resulted in the drying of the oral cavity, and subjects reported that it acted as a breath freshener. Side effect profiles were also checked at the end of the trial. No staining of the teeth or altered taste perception was reported by the volunteers in the *A. nilotica* group. Volunteers using chlorhexidine reported a yellowish discolouration of the teeth and a metallic taste.

Cost effectiveness

Commercially available 0.2% chlorhexidine mouthrinse (100 ml) ranges in cost from 55 to 100 rupees (or 0.90 to 1.63 USD). In India, this is very expensive for people of lower economic means. However, India's rural population has the option to dry and powder the bark of an *A. nilotica* tree, and then mix it with 2 parts water to 1 part powder. This mixture can then be heated and allowed to simmer until the water is reduced by 75%. The extract can then be used as a mouthrinse. This method is the prevailing oral hygiene practice in rural parts of India. Alternatively, purified *A. nilotica* is commercially available in powder form. At 50 rupees (0.82 USD) for 500 g, the powder is very cost efficient and can be used instead of bark. For a family of 4, 10 mg of powder can be used to make 100 ml of mouthrinse—enough for the entire family to use for 4 days. The cost per 10 ml of mouthrinse use is estimated to be approximately 1 rupee (0.02 USD). Our data show that a mouthrinse made from *A. nilotica* is just as effective in combating caries as chlorhexidine. *A. nilotica* can be considered a viable substitute for chlorhexidine, especially among populations of lower socioeconomic means.

Conclusion

As *S. mutans* is generally considered the main oral pathogen responsible for dental caries, the fact that *A. nilotica* inhibited the growth of *S. mutans* provides some scientific rationale for the use of this plant for the treatment of dental caries. The results of the present study clearly indicate the use of *A. nilotica* as a viable mouthrinse among rural communities of lower economic means, where it is easily accessible. However, as this is the first attempt to assess the effects of *A. nilotica* on salivary *S. mutans,* a clinical trial of longer duration with a larger sample size is necessary in the consideration of a commercially available *A. nilotica* mouthrinse.

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References

1. Bagramian RA, Garcia-Godoy F, Volpe AR. The global increase in caries. A pending health crisis. *Am J Dent.* 2009;22(1):3-8.
2. Jones CG. Chlorhexidine: is it still the gold standard? *Periodontol 2000.* 1997;15:55-62.
3. McCoy LC, Wehler CJ, Rich SE, Garcia RI, Miller DR, Jones JA. Adverse events associated with chlorhexidine use: results from the Department of Veterans Affairs Dental Diabetes Study. *J Am Dent Assoc.* 2008; 139(2):178-183.

4. Gupta D, Bhaskar DJ, Gupta RK, et al. Effect of *Terminalia chebula* extract and chlorhexidine on salivary pH and periodontal health: 2 weeks randomized control trial. *Phytotherapy Res.* 2013;28(7):992-998.
5. Dioscorides. *De Materia Medica.* Available at: http://penelope.uchicago.edu/~grout/encyclopaedia_romana/aconite/materiamedica.html. Accessed October 1, 2014.
6. Gupta D, Gupta RK, Bhaskar DJ, et al. Comparative evaluation of *Terminalia chebula* extract mouthwash and chlorhexidine mouthwash on plaque and gingival inflammation - 4-week randomised control trial. *Oral Health Prev Dent.* 2014. [Epub ahead of print]
7. Jarvinen H, Tenevuo J, Huovinen P. Susceptibility of *Streptococcus mutans* to chlorohexidine and six other antimicrobial agents. *Antimicrob Agents Chemother.* 1993;37(5):1158-1159.
8. Dhinahar S, Lakshmi T. Role of botanicals as antimicrobial agents in management of dental infections – a review. *Int J Pharm Biosci.* 2011;2(4):8690-8704.
9. Deshpande SN, Kadam DG. Phytochemical analysis and antibacterial activity of *Acacia nilotica* against *Streptococcus mutans. Int J Pharm Biosci.* 2013;5(1):236-238.
10. Xavier TF, Vijayalakshmi P. Screening of antibiotic resistant inhibitors from Indian traditional medicinal plants against *Streptococcus mutans. J Plant Sci.* 2007. Available at: http://www.docsdrive.com/pdfs/academicjournals/jps/2007/370-373.pdf. Accessed September 30, 2014.
11. American Dental Association Council on Scientific Affairs. *Adjunctive Dental Therapies for the Reduction of Plaque and Gingivitis.* Available at: http://www.ada.org/~/media/ADA/Science%20and%20Research/Files/guide_adjunctive.ashx. Accessed October 2, 2014.
12. Kannan N, Sakthivel KM, Guruvayoorappan C. Protective effect of *Acacia nilotica* (L.) against acetaminophen-induced hepatocellular damage in Wistar rats. *Adv Pharmacol Sci.* 2013. Available at: http://www.hindawi.com/journals/aps/2013/987692/. Accessed September 30, 2014.
13. Gazi MI. The finding of antiplaque features in Acacia arabica type of chewing gum. *J Clin Periodontol.* 1991;18(1):75-77.
14. Pradeep AR, Happy D, Garg G. Short-term clinical effects of commercially available gel containing *Acacia arabica:* a randomized controlled clinical trial. *Aust Dent J.* 2010;55(1):65-69.
15. Dhanya Kumar NM, Sidhu P. The antimicrobial activity of *Azardirachta Indica, Glycyrrhiza glabra, Cinnamum zeylanicum, Syzygium aromaticum, Accacia nilotica* on *Streptococcus mutans* and *Enterococcus faecalis:* an in vitro study. *Endodontology.* 2011;23:18-25.
16. Jagtap AG, Karkera SG. Potential of the aqueous extract of *Terminalia chebula* as an anticaries agent. *J Ethnopharmacol.* 1999;68(1-3):299-306.
17. Nayak SS, Kumar BR, Ankola AV, Hebbal M. The efficacy of *Terminalia chebula* rinse on *Streptococcus mutans* count in saliva and its effect on salivary pH. *Oral Health Prev Dent.* 2010;8(1):55-58.
18. Srinagesh J, Pushpanjali K. Assessment of antibacterial efficacy of triphala against mutans streptococci: a randomised control trial. *Oral Health Prev Dent.* 2011;9(4):387-393.
19. Karim B, Bhaskar D.J., Agali C., et al. Effect of *Aloe vera* mouthrinse on periodontal health: triple blind randomized control trial. *Oral Health Dent Manag.* 2014;13(1):14-19.
20. Gupta D, Bhaskar DJ, Gupta RK, et al. A randomized controlled clinical trial of *Ocimum sanctum* and chlorhexidine mouthwash on dental plaque and gingival inflammation. *J Ayurveda Integr Med.* 2014;5(2):109-116.
21. Pradeep AR, Happy D, Garg G. Short-term clinical effects of commercially available gel containing *Acacia arabica:* a randomized controlled clinical trial. *Aust Dent J.* 2010;55(1):65-69.

Manufacturers

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