



Pesquisa Brasileira em Odontopediatria e
Clínica Integrada

ISSN: 1519-0501

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Brasil

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Pesquisa Brasileira em Odontopediatria e Clínica Integrada, vol. 9, núm. 3, septiembre-diciembre,
2009, pp. 259-263
Universidade Federal da Paraíba
Paraíba, Brasil

Available in: <http://www.redalyc.org/articulo.oa?id=63712843001>

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Effect of Curry Leaves, Garlic and Tea Tree Oil on Streptococcus Mutans and Lactobacilli in Children: A Clinical and Microbiological Study

Efeito de Folhas de Curry, Alho e Óleo de Malaleuca sobre Streptococcus mutans e Lactobacilli em Crianças: Um Estudo Clínico e Microbiológico

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RESUMO

Objetivo: Avaliar e comparar a eficácia antibacteriana de folhas de curry, alho e óleo de malaleuca sobre Streptococcus mutans e Lactobacilli.

Método: O estudo teve duração de 14 dias. Trinta e seis crianças foram selecionadas e divididas aleatoriamente em quatro grupos: Grupo I (solução de bochecho placebo), Grupo II (solução de bochecho de folhas de curry a 2,5%), Grupo III (solução de bochecho de alho a 2,5%), Grupo IV (solução de bochecho de óleo de malaleuca a 0,2%). As amostras iniciais de saliva (dia "0") foram coletadas na manhã do 1º dia após a escovação dos dentes. Após meia hora, todos os grupos utilizaram 10 ml de solução de bochecho por 1 minuto, seguindo-se da coleta de amostra de saliva designada como amostra "1/2 hora". O uso da respectiva solução de bochecho duas vezes ao dia seguiu-se por 7 dias em cada grupo. As demais amostras de saliva foram coletadas na manhã do 3º e do 7º dia. Após o 7º dia, o regime foi interrompido e amostras de saliva foram coletadas no 14º dia a fim de analisar a substantividade de cada solução. Placas contendo agar Mitis Salivarius Bacitracin (MSB) e ágar Rogassa L foram inoculadas com as amostras de saliva dos sujeitos seguindo-se de contagem do número de colônias.

Resultados: Folhas de curry, alho e óleo de malaleuca apresentaram significativa atividade antimicrobiana sobre streptococcus mutans e lactobacilli. Significativa manutenção de níveis reduzidos de microorganismos foi observada somente para o alho e óleo de malaleuca no 14º dia. Gosto desagradável foi reportado (folhas de curry: 44,4%, alho: 88,9%, óleo de malaleuca: 66,6%), sensação de queimação (folhas de curry: 55,6%, alho: 88,9%, óleo de malaleuca: 77,8%), halitose (folhas de curry: 44,4%, alho: 100%, óleo de malaleuca: 22,2%), e náusea (folhas de curry: 0%, alho: 100%, óleo de malaleuca: 44,4%).

Conclusão: Soluções de bochecho a base de folhas de curry, alho e óleo de malaleuca mostraram-se eficiente contra bactérias cariogênicas e podem ser uma alternativa facilmente acessível e mais baratas às soluções de bochecho alopatícas.

ABSTRACT

Objective: To evaluate and compare the antimicrobial efficacy of Curry leaves, Garlic and Tea tree oil mouthwashes against Streptococcus mutans and Lactobacilli.

Method: The study was carried out for 14 days. Thirty six children were selected and randomly divided into four groups: group I (subjected to placebo mouthwash), Group II (subjected to 2.5% curry leaves mouthwash), group III (subjected to 2.5% garlic mouthwash), group IV (subjected to 0.2% tea tree oil mouthwash). Baseline samples ('0' day) were collected on the 1st day morning after brushing. After half an hour, all groups were subjected to 10 ml of mouthwash rinse for one minute as divided group wise followed by the collection of salivary sample designated as '1/2 hr' sample. Daily twice rinsing of mouthwashes was carried out for 7 days as per group respectively. The rest of salivary samples were collected on 3rd day and 7th day morning. After 7th day, the regimen was discontinued and saliva sample was collected on the 14th day to observe the substantivity. Dishes containing Mitis Salivarius Bacitracin agar (MSB) and Rogassa L agar were inoculated with the subject's saliva followed by colony counting respectively.

Results: Curry leaves, Garlic and Tea tree oil showed significant antimicrobial activity against streptococcus mutans and lactobacilli. Significant maintenance of reduced levels of microorganisms was observed only for garlic and tea tree oil on the 14th day. Unpleasant taste (curry leaves 44.4%, garlic 88.9%, tea tree oil 66.6%), burning sensation (curry leaves 55.6%, garlic 88.9%, tea tree oil 77.8%), bad breath (curry leaves 44.4%, garlic 100%, tea tree oil 22.2%), and nausea (curry leaves 0%, garlic 100%, tea tree oil 44.4%), were reported.

Conclusion: Tea tree oil, Garlic and Curry leaves mouthwashes are effective against cariogenic bacteria and might be an easily accessible and cheaper alternative to allopathic mouthwashes.

DESCRIPTORES

Streptococcus mutans; Alho; Óleo de Malaleuca; Enxaguatório.

KEYWORDS

Mutans streptococci; Garlic; Tea Tree Oil; Mouthwashes.

INTRODUCTION

Dental diseases are recognized as major public health problems throughout the world. Numerous epidemiological studies showed that tooth decay is the most common affliction of mankind¹.

Forty years ago, it was shown in animal studies that dental caries is an infectious and transmissible disease. Since that time, acidogenic bacteria especially mutans streptococcus and lactobacillus have been associated with this disease in humans².

Various synthetic chemical agents have been evaluated over the years with respect to their antimicrobial effects against dental caries, however all are associated with various side effects; thus patient are going away of modern day medicines and they prefer using herbal ayurvedic preparations which are efficient with least possible side effects³.

Ayurveda is a science of life. It is to with healing through herbs and natural means. Ayurveda is an ancient Indian medical science, the origin of which can be traced back to the Vedas, which are the oldest available classics of the world. The term "Danta-shastra" i.e. dentistry in ayurveda is not new; it all started with chewing sticks and has come of age to mouthwashes⁴.

The curry leaf tree (*Murraya Koenigii* spreng – a green leafy vegetable) is grown all over India and other countries for its aromatic leaves which are used daily as an ingredient in Indian cuisine. The fresh curry leaves contain 2.65 volatile essential oils like sesquiterpenes and monoterpenes which have broad antimicrobial effects; it also contains chlorophyll which is proposed as an anticariogenic agent⁵.

Garlic (*Allium sativum*) is one of the most extensively researched medicinal plants and its typical odor and antibacterial activity depends on allicin produced by enzymatic activity of allinase (a cysteine sulfoxide lyase) on alliin after crushing or cutting garlic clove. Allicin and other thiosulfinates have reported to inhibit growth of various gram-positive and gram-negative bacteria in the oral cavity⁶.

Tea tree oil (TTO), the volatile essential oil derived mainly from the Australian native plant, *Melaleuca alternifolia* is composed of Terpinen-4-ol chemotype, a terpinolene chemotype, and four 1,8-cineole chemotypes and the antimicrobial activity of TTO is attributed mainly to terpinen-4-ol⁷.

The aim of this study was to evaluate the effects and adverse effects of mouthwashes with 2.5% curry leaves, 2.5% garlic and 0.2% tea tree oil on *Streptococcus mutans* and *Lactobacilli*.

MATERIALS AND METHODS

Thirty six children, aged between 9 to 11 years were selected for the study from two schools of Davangere city, Karnataka, India. Permission from school authorities and informed written consent from parents were obtained to conduct the study. Ethical clearance was obtained from Institutional Review Board.

The criteria used for inclusion were: Children of both sexes; Age between 9 to 11 years; DMFT > 3; Child who had not received any antibiotic therapy for last one month; With no systemic disease; No history of fluoride/topical fluoride and Presenting no allergies to herbal products.

Preparation of Mouthwashes⁸

- 2.5 percent curry leaves mouthwash: Fresh curry leaves were obtained in a local market 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 by paper filter and sterilized through a 0.2 µm membrane filter by using a vacuum pump. All solid and liquid residues were weighed and concentration of final solution was 25% (w/v) or 250 mg/ml. It was diluted to 2.5% using vehicle solution (distilled water).

- 2.5 percent garlic mouthwash: White garlic bulbs were obtained in a local market and dry peel removed. Only perfect bulbs were used. Aqueous garlic extract was prepared by using 100 g of fresh rootless bulbs and 100 ml of distilled water, all of which processed in an electric mixer for 10 minutes. The resulting extract was filtered by paper filter and sterilized through a 0.2 µm membrane filter by using a vacuum pump. All solid and liquid residues were weighed and concentration of final solution was 25% (w/v) or 250 mg/ml. It was diluted to 2.5% using vehicle solution (distilled water).

- 0.2 percent tea tree oil mouthwash: 100 percent pure oil (Imported from Australia by Thursday plantations Ltd.) was analyzed through gas chromatography. Oil was diluted to 0.2% using vehicle solution and 0.5% Tween 80.

The study was carried out for 14 days. Thirty six children were selected and randomly divided into four groups of 9 each: Group I: Children subjected to placebo mouthwash; Group II: Children subjected to 2.5% curry leaves mouthwash; Group III: Children subjected to 2.5% garlic mouthwash; and Group IV: Children subjected to 0.2% tea tree oil mouthwash.

Collection of Saliva

Each child was seated comfortably, with head stable, back straight, eyes open and mandibular plane parallel to floor. Each child was then instructed to bend the head forward and after initial swallow, spit out into sterile glass tube. It was done for a period of 5 minutes, repeating spitting every 60 seconds⁹.

Baseline samples ('0' day) were collected on the 1st day morning 30 minutes after brushing. After half an hour, all groups were subjected to 10 ml of mouthwash rinse for one minute as divided group wise followed by the collection of salivary sample, designated as '1/2 hr' sample. Daily twice rinsing of mouthwashes was carried out after last tooth brushing for 7 days as per group respectively. The rest of salivary samples were collected on 3rd day and 7th day morning. After 7th day, mouthwash regimen was discontinued and salivary samples were collected on the 14th day to observe the substantivity. All salivary samples were sent to the laboratory within 30 minutes of the collection and were spread on small Petri dishes containing MSB agar and Rogasa SL agar separately. Dishes were placed in incubator at 37°C for 2 days to obtain culture of Streptococcus mutans and Lactobacilli. Individual plates were subsequently assayed for number of colony forming units (CFUs) of Streptococcus mutans and Lactobacillus using digital colony counter.

At each experimental day, all subjects were questioned about solution taste, breath alteration, burning sensation, nausea, tooth color alteration and systemic adverse effects. An analytical scale (10 cm) was used to assess the above mentioned parameters in which a line was drawn from 0 cm to 10 cm and subjects marked the scale with an 'x' and the length was measured. All reports were interpreted by using the following classifications⁸:

- 0 cm: None;
- 0 cm < L = 2.5 cm: Low
- 2.5 cm < L = 5.5 cm: Moderate
- 5.5 cm < L = 8.5 cm: Serious
- 8.5 cm < L = 10 cm: Severe

Wilcoxon rank test was used for intragroup comparison alternative to paired t test. Kruskal Wallis ANOVA was used for intergroup comparisons followed by Mann-Whitney test, Chi-square test was used to measure the categorical data for clinical parameters.

RESULTS

Figure 1 shows Streptococci mutans and Lactobacilli means (cfu/ml) for curry leaves group. Statistically significant reduction ($p = .008$) was seen between baseline, 1/2 hour, 3rd and 7th day samples for both

S.mutans and Lactobacilli. However, difference between baseline sample and 14th day sample was not significant showing poor substantivity of curry leaves mouthwash.

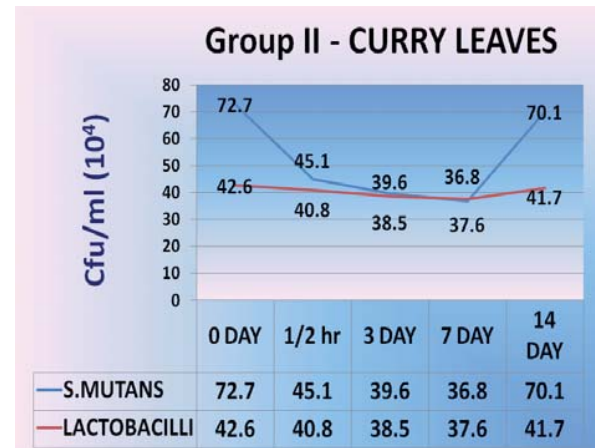


Figure 1. Streptococci mutans and Lactobacilli means (cfu/ml) for curry leaves group.

Figure 2 shows Streptococci mutans and Lactobacilli means (cfu/ml) for garlic group. Statistically significant reduction ($p=.008$) was seen between baseline, 1/2 hour, 3rd and 7th day samples for both S.mutans and Lactobacilli. The difference between baseline sample and 14th day sample was also statistically significant ($p=.008$) showing good substantivity of garlic mouthwash.

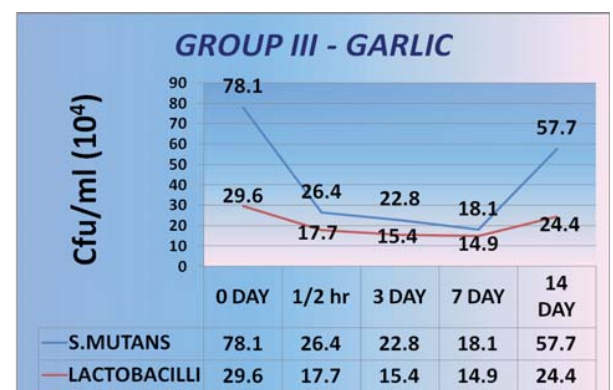


Figure 2. Streptococci mutans and Lactobacilli means (cfu/ml) for garlic group.

Figure 3 shows Streptococci mutans and Lactobacilli means (cfu/ml) for tea tree oil group. Statistically significant reduction ($p=.008$) was seen between baseline, 1/2 hour, 3rd and 7th day samples for both S.mutans and Lactobacilli. The difference between baseline sample and 14th day sample was also statistically significant ($p=.008$) showing good substantivity of tea tree oil mouthwash.

Figure 4 shows solution taste, bad breath, burning sensation, tooth color and systemic adverse effects.

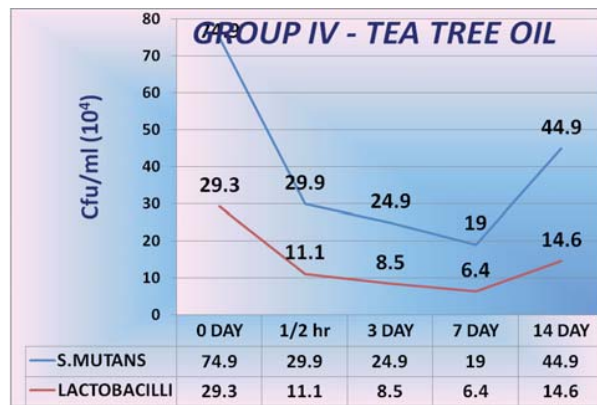


Figure 3. Streptococci mutans and Lactobacilli means (cfu/ml) for tea tree oil group.

The control group did not show statistically significant differences regarding these parameters. Tooth color alteration and systemic adverse effects were noted during the mouthwash regimen. The taste of garlic mouthwash was the worst followed by tea tree oil and curry leaves mouthwash ($\chi^2 = 20.2$, $p = 0.003$). Burning sensation ($\chi^2 = 17.9$, $p = 0.006$) and nausea ($\chi^2 = 29.3$, $p < 0.05$) was more intense in garlic followed by tea tree oil and curry leaves mouthwash. Garlic caused the worst breath followed by curry leaves and tea tree oil mouthwash ($\chi^2 = 27.9$, $p < 0.05$).

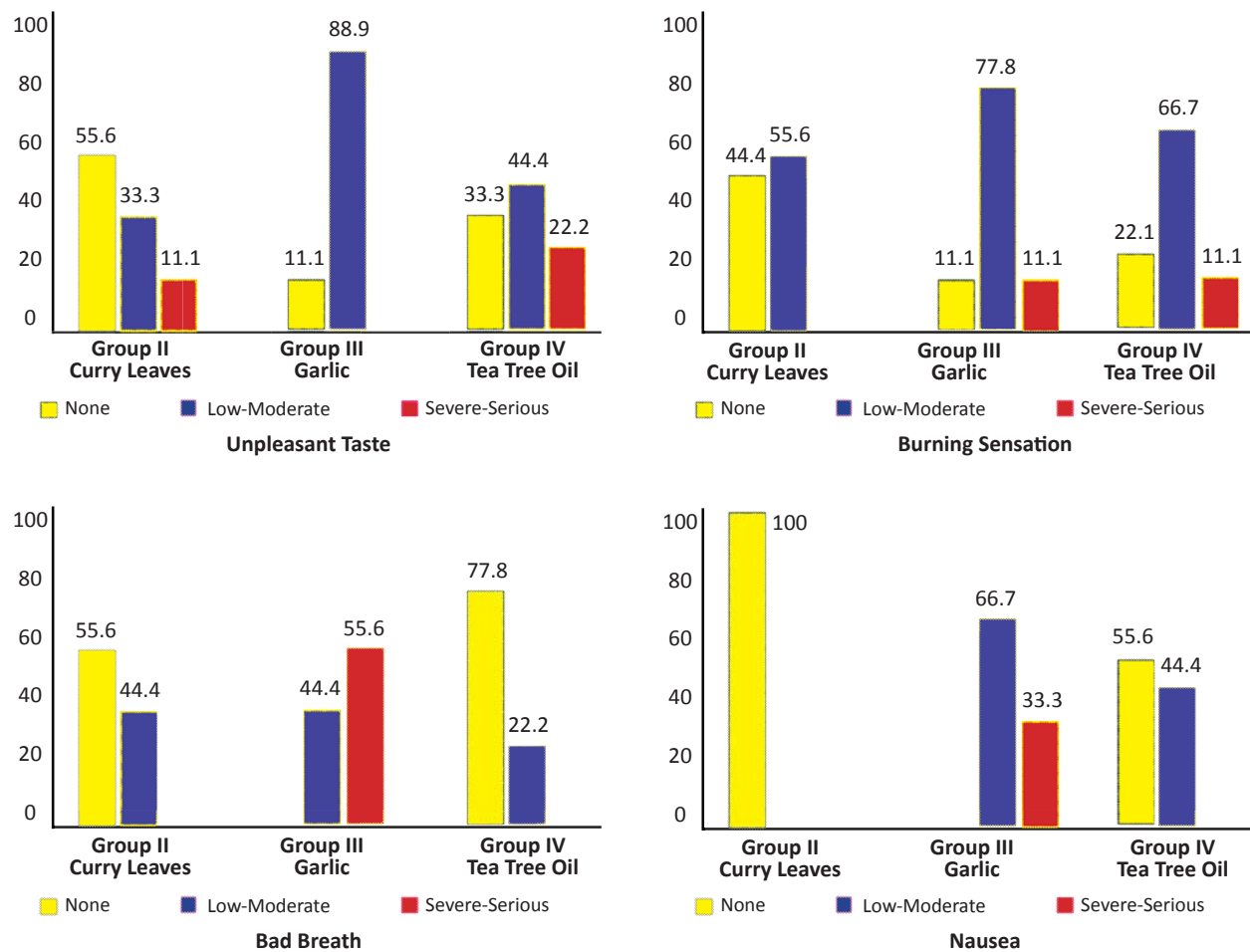


Figure 4. Solution taste, bad breath, burning sensation, tooth color and systemic adverse effects.

DISCUSSION

Salivary mutans streptococcus was used in this study due to its direct correlation with the number of colonized oral sites. So decline in the number of these microorganisms has been considered to be equivalent to a decrease in dental diseases.

The results of our study noticeably express the significant antimicrobial inhibition potential of all experimental mouthwashes. The fresh curry leaves contain 2.65 volatile essential oils and the essential oils in the curry leaves are sufficiently soluble in water. They contain thymol, eucalyptus oil and menthol and the terpenes present have antibacterial and antifungal activity. They have broad antimicrobial effects, are

microbiologically safe and have the ability to penetrate the plaque biofilm killing the microorganisms⁵.

Curry leaves contain 21000 µg total carotene, 7100 µg beta carotene, 93.9 µg total folic acid, 0.21 mg riboflavin, 0.93 mg iron, 830 mg calcium, 57 mg phosphorus and 0.20 mg zinc per 100g¹⁰. The cold extract of curry leaves (10 g of cut fresh curry leaves in 200 ml of distilled water) has a pH of 6.3 to 6.4 and they proposed chlorophyll as an anticariogenic agent¹¹.

In addition to the presence of essential oil, the curry leaves contain chlorophyll, beta carotene and folic acid, riboflavin, calcium and zinc and all these can act on the oral tissues and help in keeping up good oral health. Chewing 2 to 4 fresh curry leaves with 10 to 15 ml of water in the mouth, swishing for 5 to 7 minutes and rinsing the mouth with water can be of help in keeping good oral hygiene and as the curry leaf is a green leafy vegetable it will be safe and economical to use as a mouthwash⁵.

In vitro data revealed that garlic extract could significantly inhibit the growth of *S. mutans* at MIC range of 4-32 mg/ml. It is thought that tooth paste or mouthwash containing optimum concentration of garlic extract might be useful for prevention of dental caries. Concerning the possible side effects of garlic on humans, it has been shown that patients could tolerate intravenous extract of garlic daily for at least 1 month without apparent major toxic damage to liver, kidney or bone marrow. However oral or i.v. administration causes minor side effects such as vomiting, diarrhea and nausea¹².

Tea Tree Oil is effective against a high number of gram-positive and gram-negative bacteria as well as fungi. Its efficacy is due to its ingredients such as terpene hydrocarbons, mainly monoterpenes, sesquiterpenes, and their associated alcohols. Terpenes are volatile, aromatic hydrocarbons and may be considered as polymers of isoprene, which has the formula C₅H₈. These include a terpinen-4-ol chemotype, a terpinolene chemotype, and four 1,8-cineole chemotypes. With biological activity, the antimicrobial activity of TTO is attributed mainly to terpinen-4-ol, a major component of the oil. The antimicrobial activity of TTO is due to hydrocarbons which preferentially partition the biological membranes and disrupt their vital functions. This premise is further supported by data showing that TTO permeabilizes model liposomal systems⁷.

Results of our study illustrate that even at low concentration, 2.5% garlic mouthwash has shown significant adverse effects when compared to 2.5% curry leaves and 0.2% tea tree oil mouthwash. Further research is going on in our department to camouflage the adverse effects related to experimental mouthwashes used in this study.

CONCLUSIONS

- 1) Mouthwashes with 2.5% curry leaves, 2.5% garlic and 0.2% tea tree oil were able to reduce *S. mutans* and *Lactobacilli* count to a significant level.
- 2) 2.5% Garlic mouth wash showed more adverse effects than mouthwashes with 0.2% tea tree oil and 2.5% curry leaves.

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Recebido/Received: 26/12/08
Aprovado/Approved: 11/05/09

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