Original Article

Comparison of the antimicrobial efficacy of chlorhexidine, sodium fluoride, fluoride with essential oils, alum, green tea, and garlic with lime mouth rinses on cariogenic microbes

Ann Thomas, Sneha Thakur, Sanjana Mhambrey

Department of Pedodontics and Preventive Dentistry, AJ Institute of Dental Sciences, Mangalore, Karnataka, India

Corresponding author (email: <snehathakur68@gmail.com>)
Dr. Sneha Thakur, Department of Pedodontics and Preventive Dentistry, AJ Institute of Dental Sciences, Kuntikana, Mangalore - 575 004, Karnataka, India.

Abstract

Background: A number of natural mouth rinse formulations are being proposed as an alternative to the widely used chemical mouth rinses. **Objective:** To evaluate and compare the antimicrobial efficacy of chlorhexidine (0.2%), sodium fluoride (0.05%), fluoride with essential oils (0.05%), alum (0.02 M), green tea, and garlic with lime mouth rinses against *Streptococcus mutans*, lactobacilli, and *Candida albicans*. **Materials and Methods:** The three microbes were isolated from the saliva samples collected from children with severe early childhood caries. The zone of minimum inhibition was assessed using agar diffusion method. The data were statistically analyzed using SPSS software. **Results:** Against *S. mutans* and lactobacilli, chlorhexidine mouth rinse was found to be the most effective as compared to sodium fluoride ($P < 0.001$, $P < 0.001$), fluoride with essential oils ($P < 0.001$, $P < 0.001$), alum ($P < 0.001$, $P < 0.001$), green tea ($P < 0.001$, $P < 0.001$), and garlic with lime ($P < 0.001$, $P < 0.001$) mouth rinses, respectively. But against *C. albicans*, garlic with lime mouth rinse was found to be the most effective as compared to chlorhexidine ($P < 0.001$), sodium fluoride ($P < 0.001$), fluoride with essential oils ($P < 0.001$), alum ($P < 0.001$), and green tea ($P < 0.001$) mouth rinses. Against *S. mutans* and lactobacilli, after chlorhexidine mouth rinse, garlic with lime mouth rinse was found to be significantly more effective than sodium fluoride ($P = 0.053$, $P = 0.001$), fluoride with essential oils ($P < 0.001$, $P < 0.001$), alum ($P < 0.001$, $P < 0.001$), and green tea ($P < 0.001$, $P < 0.001$) mouth rinses. **Conclusion:** As a natural mouth rinse, garlic with lime mouth rinse was found to be the most promising. However, further studies are needed in this field.

Key words: *Candida albicans, caries prevention, herbal mouth wash, home oral hygiene, lactobacilli, severe early childhood caries, Streptococcus mutans*

INTRODUCTION

Mouth rinse as an adjunct to other oral hygiene measures was first propagated by ancient Egyptians and Romans. In the 16th century, a mixture of alum, vinegar myrrh, and wine was reported to have been used for washing the mouth after meals. Hippocrates is also known to have advocated mouth rinsing with a

Access this article online	
Quick Response Code:	Website: www.jispcd.org
	DOI: 10.4103/2231-0762.161759

How to cite this article: Thomas A, Thakur S, Mhambrey S. Comparison of the antimicrobial efficacy of chlorhexidine, sodium fluoride, fluoride with essential oils, alum, green tea, and garlic with lime mouth rinses on cariogenic microbes. J Int Soc Prevent Communit Dent 2015;5:302-8.

mixture of alum, salt, and vinegar in ancient times.[1] Mouth rinses are used generally for their analgesic, anti-inflammatory, antimicrobial, and anticariogenic activities. The most commonly used preventive and therapeutic mouth rises in children are sodium fluoride and chlorhexidine, respectively, for their anticariogenic effect because dental caries is the most prevalent chronic childhood disease.

Chlorhexidine mouth rinse is considered the "gold standard" due to its bacteriostatic and bactericidal properties at low and high concentrations, respectively;[2] but it is not recommended for routine home care use due to its metallic taste and staining.[3] Sodium fluoride mouth rinse remains the most widely recommended routine home care oral hygiene agent in children due to its anticariogenic properties. Yet, its extensive use in very young children is not advised due to the risk of ingestion and fluoride toxicity.[4] Fluoride with essential oil is a newer mouth rinse formulation introduced commercially, may be because of the popularity of essential oil mouth rinse in adults and of fluoride as an anticariogenic agent. Some studies have shown that fluoride with essential oil mouth rinse is effective in promoting enamel re-mineralization and fluoride uptake.[5,6]

In the 21st century, greater awareness about the ill effects of chemical use has led to search for natural remedies; substances like garlic (*Allium sativum)*, lime (*Citrus aurantifolia)*, green tea (*Camellia sinensis)*, alum (potassium aluminum sulfate), etc., have been used since ancient times for their therapeutic properties. Recent literature has brought to light the benefits of various natural substances as mouth rinses.[7] Even as early as 1858, there were reports of antibacterial, antifungal, and antiviral activities of garlic, as observed by Louis Pasteur.[8] Research has shown garlic to have anti-inflammatory and antioxidant properties and the sulfur-containing compounds present in it to have an inhibitory effect on *Streptococcus mutans.*[8-10] Green tea is reported to be very rich in fluoride and catechins, a bioactive component which has anticariogenic effect.[4,11] Maryam hajenorouzali *et al.* reported green tea mouth rinse to be effective in reducing *S. mutans* and lactobacilli and also to be effective as sodium fluoride mouth rinse.[4] Lime, an essential ingredient in most herbal concoctions, is effective against variety of gram-negative and gram-positive microorganisms including *Candida albicans*.[8] Alum, chemically known as potassium aluminum sulfate, has been traditionally used for its antimicrobial and astringent properties.[12]

Various studies have compared the natural against the gold standard mouth rinses and reported varying degrees of efficacy, especially against *S. mutans*,[2-4,12,13] but there is a paucity of reports comparing the various natural mouth rinses. Also, to our knowledge, there are no reports of studies comparing the antimicrobial efficacy against *C. albicans*, a fungus being implicated in the etiopathogenesis of dental caries.

This study was undertaken to evaluate and compare the antimicrobial efficacy of sodium fluoride (0.05%), fluoride with essential oils (0.05%), alum (0.02 M), green tea, and garlic with lime mouth rinses to that of chlorhexidine (0.2%) against *S. mutans*, lactobacilli, and *C. albicans*. The null hypothesis was that there was no statistically significant difference in the anti-microbial activity of each of these mouth rinses. This is an *in vitro* pilot study, which is a part of a larger randomized control trial.

MATERIALS AND METHODS

Ethics

The study protocol was reviewed and approved by the ethical committee of the institution. Written informed consent was taken from parents of the children who were included in the study.

Study design

This *in vitro* study was conducted between August and September 2012. Six different mouth rinses were used which included three commercially available mouth rinses such as chlorhexidine (0.2%) (CLOHEX, Dr. Reddy's), sodium fluoride (0.05%) (PEPSODENT, Hindustan Unilever), and fluoride (0.05%) with essential oils (LISTERINE, Johnson and Johnson) consisting of sodium fluoride and acidulated phosphate fluoride along with eucalyptol and thymol; and three laboratory-prepared mouth rinses: alum, green tea, and garlic with lime. Chlorhexidine mouth rinse was used as a positive control to compare the antimicrobial potential since it has been considered as the gold standard for mouth rinses and is known to have bacteriostatic and bacteriocidal effects.[2]

Preparation of mouth rinses

The laboratory-formulated mouth rinses were prepared under aseptic conditions.

Alum (0.02 M) mouth rinse was prepared by taking weighed quantity of potassium aluminum sulfate which is found in its doctahydrate form [molecular

formula KAl $(SO_4)_2 \cdot 12H_2O$]. It was calculated using its molecular weight: 474.39 (wt of alum) = mol. wt × 0.02 = 9.4878 g. Initially, it was dissolved in 800 ml of distilled water and to it, 1 g of sodium benzoate as a preservative and 0.5 g of sodium saccharine (as a sweetening agent) were added. To 200 ml of distilled water, 0.5 ml of Tween 20 and 0.5 ml of peppermint oil were added and mixed properly. The resultant mixture was mixed with the 800 ml alum solution with the help of a propeller to formulate a clear mouth rinse.

To prepare green tea mouth rinse, dried green tea leaves (obtained by open air drying) were ground to a desirable size using an electrical mill, and then extracted by percolation using distilled water as the solvent. Green tea, which is rich in phenolic compounds (6%), was diluted to obtain a concentration of 0.5% phenolic compound using double distilled water. Authorized additive, peppermint flavor (1 g/l), and sodium saccharine (1 g/l), the sweetening agent, were used to formulate the mouth rinse.[4]

To prepare garlic with lime mouth rinse, 100 g of fresh, washed garlic cloves was macerated in a sterile, ceramic mortar and water was added to obtain a homogenate which was then filtered off with a sterile muslin cloth.[8] The weight of insoluble material was subtracted from the weight of original cloves and the final concentration of the solution was determined to be 1 g/100 ml. About 100 ml of lime juice was extracted from fresh lemons using a juice extractor and added to the garlic extract. Authorized additive, peppermint flavor (1 g/l), sodium saccharine (1 g/l) as the sweetening agent, and sodium bicarbonate (0.5 g) as the preservative were added, and the mixture was mixed properly to prepare a mouth rinse.[8]

Isolation of microbes from saliva

Unstimulated whole saliva samples were collected from three children who fulfilled the inclusion criteria. The inclusion criteria were children aged between 4 and 6 years with severe early childhood caries as defined by American Academy of Pediatric Dentistry[14] and with informed parental consent. Children with positive medical history of antibiotic or steroid therapy 1 week prior to the study were excluded. Two milliliters of unstimulated whole saliva was collected in a sterile container by instructing the children to drool for 3–5 min. Saliva collection was done in the morning between 10.00 and 11.00 a.m. in order to eliminate any bias in the concentration of saliva due to circadian rhythm.[15]

The saliva samples were cultured on selective media such as Mitis Salivarius Agar enriched with bacitracin, Rogosa agar, and CHROM agar to obtain isolates of *S. mutans,* lactobacilli, and *C. albicans*, respectively. Selective agar media were sterilized by autoclaving after the addition of 1% potassium tellurite supplement and then poured on sterile petri plates. After cooling to around 50%, they were allowed to set for 24 h and finally, the saliva samples were taken in serial dilutions. The plates were incubated at around 37°C in the biological incubator for 24–48 h and clear colonies were seen on the plates, indicating the growth.

Susceptibility test

The agar well-diffusion method prescribed by National Committee for Clinical Laboratory Standards (2000) was employed to analyze the antimicrobial efficacy. Suspensions of the microbial isolates were prepared in sterile normal saline and adjusted to 0.5 McFarland's standard, and were uniformly seeded by streaking sterile swab dipped in the suspension onto the Muller-Hinton agar plate surface. Wells, 5 mm in diameter and 4 mm deep, were punched on the agar plates with a sterile borer. Fifty microliters of different mouth rinses and water as a negative control were placed in each of the wells and the plates were incubated at 37°C for 24 h.[8] The diameter of the zone of inhibition of each mouth rinse against each microbe was measured in millimeters and recorded on three culture plates using a Vernier caliper. Each measurement was repeated three times and the mean diameter of the zone of inhibition was determined. A single examiner carried out all the measurements. The examiner was calibrated and the intraexaminer reliability coefficient was found to be 0.84.

The data were subjected to descriptive statistics and individual scores were tested using Kruskal–Wallis test, Wilcoxon rank sum test, and Mann–Whitney test using the SPSS software. The results were considered statistically significant at 0.05 probability level.

RESULTS

The six studied mouth rinses showed considerable inhibitory effect against *S. mutans,* lactobacilli, and *C. albicans.* Figure 1 shows the mean zone of inhibition; which was numerically highest for chlorhexidine (0.2%) mouth rinse against *S. mutans* and lactobacilli, whereas

against *C. albicans*, the mean zone of inhibition was highest for garlic with lime mouth rinse. A very high significant difference was found between the mean zones of inhibition of the different mouth rinses for all the three microbes [Table 1]. Table 2 shows the intercomparison of the antimicrobial efficacy of the different mouth rinses against the three microbes. Against *S. mutans* and lactobacilli, chlorhexidine mouth rinse was the most effective as compared to sodium fluoride ($P < 0.001$, $P < 0.001$), fluoride with essential oils ($P < 0.001$, $P < 0.001$), alum ($P < 0.001$, $P = 0.023$), green tea ($P < 0.001$, $P < 0.001$), and garlic with lime ($P = 0.02$, $P < 0.001$) mouth rinses. Garlic with lime mouth rinse was found to be the second most effective mouth rinse when compared to sodium fluoride ($P = 0.053$, $P = 0.001$), fluoride with essential oils ($P < 0.001$, $P < 0.001$), alum ($P < 0.001$, $P < 0.001$), and green tea ($P < 0.001$, $P < 0.001$) mouth rinses. Sodium fluoride mouth rinse was more effective than alum ($P = 0.02$, $P = 0.001$), green tea ($P = 0.053$, $P = 0.001$), and fluoride with essential oil ($P < 0.001$, $P = 0.001$) mouth rinses against *S. mutans* and lactobacilli, respectively. No significant difference was found between alum and green tea mouth rinses against *S. mutans*; however, they were more effective than fluoride with essential oil mouth rinse ($P = 0.013$, $P = 0.053$). No significant difference was found between the antimicrobial efficacy of alum, green tea, and fluoride with essential oil mouth rinses against lactobacilli.

The intercomparison of the antimicrobial efficacy of the different mouth rinses against *C. albicans* revealed that garlic with lime mouth rinse was significantly the most effective as compared to chlorhexidine ($P < 0.001$), sodium fluoride ($P < 0.001$), fluoride with essential oils ($P < 0.001$), alum ($P < 0.001$), and green tea ($P < 0.001$) mouth rinses, whereas chlorhexidine mouth rinse was the second most effective when

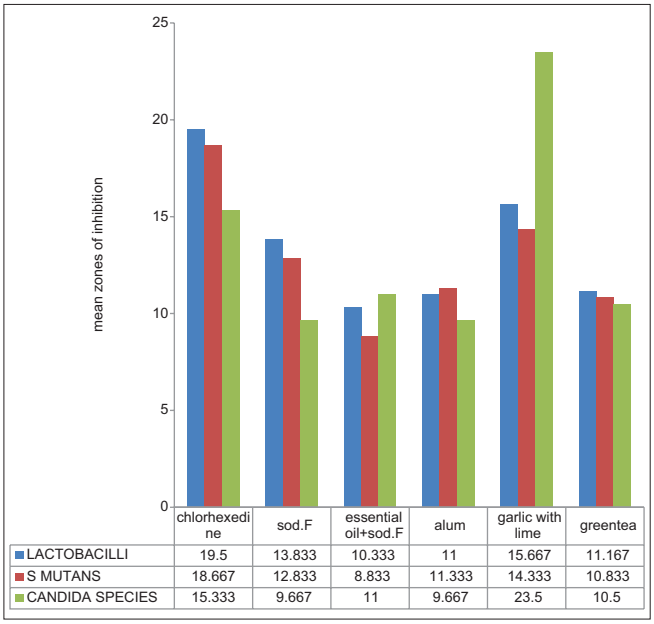

Figure 1: Mean zones of inhibition of the six mouth rinses

Table 1: Comparison of the six mouth rinses on each species separately

	Groups	*n*	Mean	Standard deviation	Statistics/ mean squares	df2 (Welch)/ F (ANOVA)	*P*
Lactobacilli	Chlorhexidine	3	19.5	0.5	37.492	107.976	<0.001
	Sodium fluoride	3	13.833	0.288			
	Sodium fluoride + essential oil	3	10.333	0.577			
	Alum	3	11	0			
	Garlic with lime	3	15.667	1.154			
	Green tea	3	11.167	0.288			
	Total	18	13.583	3.357			
S. mutans	Chlorhexidine	3	18.667	0.577	35.114	64.826	<0.001
	Sodium fluoride	3	12.833	0.288			
	Sodium fluoride + essential oil	3	8.833	1.04			
	Alum	3	11.333	0.577			
	Garlic with lime	3	14.333	0.577			
	Green tea	3	10.833	1.041			
	Total	18	12.806	3.273			
Candida species	Chlorhexidine	3	15.333	0.577	88.622	354.489	<0.001
	Sodium fluoride	3	9.667	0.577			
	Sodium fluoride + essential oil	3	11	0			
	Alum	3	9.667	0.577			
	Garlic with lime	3	23.5	0.5			
	Green tea	3	10.5	0.5			
	Total	18	13.278	5.123			

ANOVA=Analysis of variance; df2/F= Test value

Table 2: Intercomparison of the six mouth rinses

Groups	*S. mutans*			Lactobacilli			*C. albicans*		
	Mean difference	SE	*P*	Mean	SE	*P*	Mean	SE	*P*
Chlorhexidine									
Sodium fluoride	5.833	0.6	<0.001	5.667	0.48	<0.001	5.667	0.408	<0.001
Sodium fluoride + essential oil	9.833	0.6	<0.001	9.167	0.48	<0.001	4.333	0.408	<0.001
Alum	4.333	0.6	<0.001	3.833	0.48	<0.001	−8.167	0.408	<0.001
Green tea	7.333	0.6	<0.001	8.5	0.48	<0.001	5.667	0.408	<0.001
Garlic with lime	7.833	0.6	<0.001	8.333	0.48	<0.001	4.833	0.408	<0.001
Sodium fluoride									
Sodium fluoride + essential oil	4	0.6	<0.001	3.5	0.48	0.001	−1.333	0.408	0.058
Alum	−1.5	0.6	0.02	−1.833	0.48	0.023	−13.83	0.408	<0.001
Green tea	1.5	0.6	0.02	2.833	0.48	0.001	0	0.408	1
Garlic with lime	2	0.6	0.053	2.667	0.48	0.001	−0.833	0.408	0.376
Sodium fluoride + essential oil									
Alum	−2.5	0.6	0.013	−0.667	0.48	0.735	1.333	0.408	0.058
Green tea	−2	0.6	0.053	−0.833	0.48	0.538	0.5	0.408	0.817
Garlic with lime	−5.5	0.6	<0.001	−5.333	0.48	<0.001	−12.5	0.408	<0.001
Alum									
Green tea	0.5	0.6	0.956	−0.167	0.48	0.999	−0.833	0.408	0.376
Garlic with lime	−3	0.6	<0.001	−4.667	0.48	<0.001	−13.83	0.408	<0.001
Green tea									
Garlic with lime	−3.5	0.6	0.001	−4.5	0.48	<0.001	−13	0.408	<0.001

SE=Standard error

compared to sodium fluoride ($P < 0.001$), fluoride with essential oils ($P < 0.001$), alum ($P < 0.001$), and green tea ($P < 0.001$) mouth rinses. Fluoride with essential oil mouth rinse was found to be more significantly effective than alum ($P = 0.058$) and sodium fluoride ($P = 0.058$) mouth rinses, but no significant difference was found in comparison with green tea mouth rinse ($P = 0.817$). Also, no significant difference was found between green tea, alum, and sodium fluoride mouth rinses in their efficacy against *C. albicans*.

DISCUSSION

The most common dental diseases are plaque-related infections. In recent years, there has been a lot of concern about caries prevention, especially in the developing countries and lower socio-economic societies. Hence, there is always a quest for natural mouth rinse which is economical, safe, and easy to prepare, in order to achieve better oral health with widespread use as a home remedy.

In this *in vitro* study, the null hypothesis of no difference in antimicrobial activity of the six studied mouth rinses was rejected. The results of this study confirmed that chlorhexidine, the "gold standard," is the most effective antibacterial mouth rinse when compared to all the other five studied mouth rinses and is also an effective antifungal mouth rinse as compared to the other mouth rinses except garlic with lime mouth rinse. This is in accordance with most of the previous studies comparing its efficacy.[3,16] However, use of chlorhexidine is mostly restricted for therapeutic purpose and not routine home oral care because of its adverse effects.[3,17]

In garlic with lime mouth rinse, a herbal formulation, lime was added to mask the pungent flavor of garlic and it also provided antifungal effect.[8] The characteristic flavor of allicin in garlic induces salivation and salivary clearance, thus providing an additional benefit on caries prevention.[3,16] This mouth rinse was found to be the second most significantly effective antibacterial mouth rinse and the most effective antifungal mouth rinse, when compared to the studied mouth rinses. The *in vitro* antibacterial and antifungal activities of garlic extract have been widely recognized.[8-10,18] Our finding indicates that this mouth rinse can be a very cost-effective formulation which can be easily formulated at home and may lack possible side effects with long-term use, and therefore, it could be considered a good mouth rinse for use as a home oral hygiene measure, especially in low socio-economic groups. However, there is limited data regarding this mouth rinse, which precludes its approval for the clinical prescription at present.

The present study also confirms that sodium fluoride mouth rinse is a potent antibacterial with antifungal

ability and this finding is in agreement with previous reports.[3,17] Fluoride with essential oil mouth rinse is a newer commercially available mouth rinse with the rationale of treating or preventing both dental caries and gingivitis.[5] A number of well-conducted studies have demonstrated the effectiveness of essential oils against gram-negative flora associated with gingivitis and, to some extent, against gram-positive organisms including *S. mutans*.[19,20] The ability of sodium fluoride to significantly reduce or even reverse the initiation and progression of dental caries is also well documented.[3,4,21] The fluoride content in both fluoride with essential oils and sodium fluoride mouth rinses used in this study was 0.05%; yet, sodium fluoride mouth rinse was found to be significantly more efficacious than fluoride with essential oil mouth rinse against the cariogenic bacteria. This finding is contrary to the general hypothesis of the added benefit of essential oil in a fluoride with essential mouth rinse. It could be suggested that the number of available active free fluoride molecules is reduced in fluoride with essential oil mouth rinse. However, fluoride with essential oil had greater antifungal effect against *C. albicans* than sodium fluoride mouth rinse and was also significantly better than alum mouth rinse, while no difference was found with green tea mouth rinse.

Scientific evidence strongly suggests that tea and certain of its components exert a significant anticariogenic effect by its activity against streptococci.[4,11,22,23] However, to our knowledge, only limited literature is available regarding its effect on lactobacilli or *C. albicans*. Our study confirmed its inhibitory effect on *S. mutans* and also found strong inhibitory effect against lactobacilli and *C. albicans*. Green tea mouth rinse was less inhibitory than sodium fluoride mouth rinse against *S. mutans* and lactobacilli, which was contradictory to the previous study.[4]

Studies have indicated alum to be an effective antibacterial agent, especially against the oral bacteria.[12] In our study, alum (0.02 M) mouth rinse showed modest but definite inhibitory effect against the studied microbes, especially *S. mutans* and lactobacilli. Its antifungal effect was similar to sodium fluoride mouth rinse.

CONCLUSION

From the results of this study, it can be concluded that chlorhexidine and garlic with lime are the most effective antibacterial and antifungal mouth rinses, respectively, among the six studied mouth rinses. Garlic with lime mouth rinse has shown promising results, and thus, it can be considered a newer alternative; but further studies on its side effects and long-term use are recommended. Fluoride with essential oil, alum, and green tea mouth rinses had modest effects which were not as comparable to that of sodium fluoride mouth rinse.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Putt MS, Kleber CJ, Smith CE. Evaluation an alum-containing mouthrinse in children for plaque and gingivitis inhibition during 4 weeks of supervised use. Pediatr Dent 1996;18:139-44.
2. Asokan S, Emmadi P, Chamundeswari R. Effect of oil pulling on plaque induced gingivitis: A randomized, controlled, triple-blind study. Indian J Dent Res 2009;20:47-51.
3. Sundas S, Rao A. Comparative evaluation of chlorhexidine and sodium fluoride mouthwashes on streptococcus mutans. J Nepal Dent Assoc 2011;12:17-21.
4. Tehrani MH, Asghari G, Hajiahmadi M. Comparing Streptococcus and Lactobacillus colony count changes following green tea mouth rinse or sodium fluoride mouth rinse use in children (Randomized double-blind controlled clinical Trial). Dent Res J (Isfahan) 2011;8(Suppl 1):S58-63.
5. Zero DT, Zhang JZ, Harper DS, Wu M, Kelly S, Waskow J, *et al*. The remineralizing effect of an essential oil fluoride mouthrinse in an intraoral caries test. J Am Dent Assoc 2004;135:231-7.
6. Benjavongkulchai E, Tamsailom S. Fluoride/zinc/essential oil-containing mouthrinse promotes fluoride uptake and microhardness in enamel *in vitro*. CU Dent J 2013;36:31-6.
7. Rupesh S, Winnier JJ, Nayak UA, Rao AP, Reddy NV. Comparative evaluation of the effects of an alum-containing mouthrinse and a saturated saline rinse on the salivary levels of Streptococcus mutans. J Indian Soc Pedod Prev Dent 2010;28:138-44.
8. Kukreja BJ, Dodwad V. Herbal mouthwashes: A gift of nature. Int J Pharma Bio Sci 2012;3:46-52.
9. Owhe-Ureghe UB, Ehwarieme DA, Eboh DO. Antibacterial activity of garlic and lime on isolates of extracted carious teeth. Afr J Biotechnol 2010;9:3163-6.
10. Fani MM, Kohanteb J, Dayaghi M. Inhibitory activity of garlic (Allium Sativum) extract on multidrug-resistant Streptococcus mutans. J Indian Soc Pedod Prev Dent 2007;25:164-8.
11. Ankri S, Mirelman D. Antimicrobial properties of allicin from garlic. Microbes Infect 1999;1:125-9.
12. Hamilton-Miller JM. Anti-cariogenic properties of tea (Camellia sinensis). J Med Microbiol 2001;50:299-302.
13. Kudva S, Prabhakar S, Pai V, Tegginamani A. Effects of garlic extract on salivary pH: A clinical study. Arch Orofac Sci 2012;7:1-8.
14. American Academy of Pediatric Dentistry; American Academy

of Pediatrics; American Academy of Pediatric Dentistry Council on Clinical Affairs. Policy on early childhood caries (ECC): Classifications, consequences, and preventive strategies. Pediatr Dent 2005-2006;27(Suppl):31-3.
15. Amin M, Kazemi M, Rasaie N. *In vitro* comparison of the effects of garlic juice and chlorhexidine mouthwash on oral pathogens. Jundishapur J Microbiol 2012;5:398-400.
16. Chavan SD, Shetty NL, Kanuri M. Comparative evaluation of garlic extracts mouthwash and chlorhexidine mouthwash on salivary Streptococcus mutans count-an *in vivo* study. Oral Health Prev Dent 2010;8:369-74.
17. Oyanagi T, Tagami J, Matin K. Potentials of mouthwashes in disinfecting cariogenic bacteria and biofilms leading to inhibition of caries. Open Dent J 2012;6:23-30.
18. Iwalokun BA, Ogunledun A, Ogbolu DO, Bamiro SB, Jimi-Omojola J. *In vitro* antimicrobial properties of aqueous garlic extract against multidrug- resistant bacteria and Candida species from Nigeria. J Med Food 2004;7:327-33.
19. Overholser CD, Meiller TF, DePaola LG, Minah GE, Neihaus C. Comparative effects of 2 chemotherapeutic mouthrinses on the development of supragingival dental plaque and gingivitis. J Clin Periodontol 1990;17:575-9.
20. DePaola LG, Overholser CD, Meiller TF, Minah GE, Neihaus C. Chemotherapeutic inhibition of supragingival dental plaque and gingivitis development. J Clin Periodontol 1989;16:311-5.
21. Finn SB, Moller P, Jamison H, Regattieri L, Manson-Hing L. The clinical cariostatic effectiveness of two concentrations of acidulated phosphate-fluoride mouthwash. J Am Dent Assoc 1975;90:398-402.
22. Taylor PW, Hamilton-Miller JM, Stapleton PD. Antimicrobial properties of green tea catechins. Food Sci Technol Bull 2005;2:71-81.
23. Hassani AS, Amirmozafari N, Ordouzadeh N, Hamdi K, Nazari R, Ghaemi A. Volatile components of Camellia sinensis inhibit growth and biofilm formation of oral streptococci *in vitro*. Pak J Biol Sci 2008;11:1336-41.