



## Evaluation of the Antibacterial Effect of Garlic with Lime on Streptococcus Mutans in Children

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### ABSTRACT

**Purpose:** This study was conducted to evaluate the effect of Garlic with lime mouth wash against Chlorhexidine mouthwash on salivary Streptococcus mutans count in a group of Egyptian children. **Materials and Methods:** A total of 40 Egyptian children were included in this study. Children ages ranged from (6 to 12) years old in a good physical condition. Children were randomly distributed into two groups A & B each of 20 children. In children of group A (using Garlic with lime) each participant was instructed to rinse with 5 ml of garlic with lime mouthwash for one minute. In group B (control group using Chlorhexidine mouthwash 0.1%) each participant was instructed to rinse with 5 ml of CHX for one minute. **Results:** There was a significant reduction in Streptococcus mutans count in both groups. **Conclusion:** garlic with lime mouthwash were successful as an antimicrobial agent. It significantly reduced the total bacterial count in the saliva of children when compared to a potent antiseptic like Chlorhexidine.

### INTRODUCTION

Dental caries is a public health problem because it is one of the most common chronic and widespread conditions and multifactorial diseases affecting the human population <sup>(1)</sup>. The caries lesion appearance is determined by three main factors: The host (the saliva and teeth), the micro flora, and the substrate or diet <sup>(2)</sup>.

The etiology of dental caries and variations in its prevalence are associated with differences in dietary habits (the consumption of sugar)

### KEYWORDS

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changes in the oral hygiene pattern, the virulence of microflora in oral and dental plaque and alteration in the oral protective mechanisms<sup>(3)</sup>. Cariogenic microorganisms like *Streptococcus mutans* has a central role in dental caries as it can adhere to salivary pellicle and other bacteria and produce strong acid which eventually leads to cavity formation<sup>(4)</sup>.

Mechanical plaque control methods like brushing and flossing aid to limit the growth of oral microbes but removal of plaque remains a challenge from inaccessible areas of the oral cavity. Therefore, the use of the antimicrobial agent is important to limit the growth of cariogenic microorganisms and prevent dental caries<sup>(5)</sup>.

Chlorhexidine mouth wash is considered the best choice for its bactericidal and bacteriostatic properties but due to its metallic taste and brownish discoloration of the teeth and restorations it is not recommended for routine home care use<sup>(6)</sup>. Recently, the use of medicinal plants with therapeutic properties and natural compounds have demonstrated anticariogenic effect, anti-inflammatory, antibacterial and antioxidant properties as well as biocompatibility which explains the people's growing interest in plants<sup>(7-8)</sup>. because the use of synthetic drugs have adverse effects and drug resistance which led to the needs for safe alternatives<sup>(4)</sup>.

Garlic (*Allium sativum*) has been known to have antibacterial, antifungal and antiviral properties also scientific reports show that garlic have anti-inflammatory and antioxidant property and it have an inhibitory effect on S.M due to sulfur-containing compounds present in it. Mouth wash containing garlic prevent the change in salivary pH, therefore promote remineralization. Allicin present in garlic have a specific flavor which stimulate saliva secretion, and enhance its anticariogenic effect by salivary clearance<sup>(9)</sup>.

Lime (*Citrus aurantifolia*) is used in the production of most herbal fabrication due to its antimicrobial activities against gram-negative and gram-positive bacteria, as well as *Candida albicans*. Thus, this study aimed to evaluate the antimicrobial action

of Garlic with lime extract as mouthwash, against chlorhexidine mouthwash on salivary S.M count in oral cavity of children.<sup>(10)</sup>

## MATERIAL AND METHODS

**Materials used:** Garlic and lime were collected from a local market, Cairo, Egypt, Chlorhexidine mouthwash (0.1%): (Hexitol, Arab Drug Company, Cairo, Egypt) and Mitis Salivarius Bacitracin (MSB): Base enriched with sucrose to selectively isolate oral streptococci and inhibit other microorganisms.

**Case Selection:** A total number of 40 children were selected from a number of orphanages across Cairo, Nasr City. Inclusion criteria: Cooperative children of ages ranging from (6-12) years, and in a good physical condition. No history of recent antibiotic administration or using antimicrobial mouth rinse (within the previous 2 weeks). Exclusion criteria: Children with any systemic diseases, Children with any untreated active carious lesions, children with topical fluoride treatment, Children with history of allergy to any mouth rinse or drug.

**Methods:** Children were randomly distributed into two groups A and B; each of 20. (Group A) consisted of 20 children using garlic and lime mouth wash. (Group B) consisted of 20 children using Chlorhexidine mouthwash (0.1%). Each subject was directed to rinse with 5 ml of the mouthwash; for one minute; once a day for 2weeks, Saliva sampling from each subject was carried out before intervention and after 30 minutes and after 2 weeks of intervention at least 1 hr after a meal.

**Preparation of garlic with lime:** To prepare garlic with lime mouth wash, 100 gm of fresh, washed garlic cloves were macerated in a sterile, ceramic mortar and distilled water were added to obtain a homogenate, which was then filtered off with a sterile muslin cloth. The weight of insoluble material was subtracted from the weight of original cloves and the final concentration of the solution were determined to be 1 gm/100 mL. About 100 mL of lime juice were extracted from fresh lemons using

a juice extractor and added to the garlic extract. Authorized additive, peppermint flavor (1 gm/L), sodium saccharine (1 gm/L), as sweetening agent, and sodium bicarbonate (0.5 gm) as preservative were added, and the mixture were mixed properly to prepare a mouth wash. (Fig1)



Figure (1) Preparation of garlic

**Collection of saliva sample**

A suitable amount of non-stimulated saliva samples were collected from each child by asking him/her to spit in a sterile plastic container till, at least 1 hr. after meal. **Baseline sample (S1):** The initial sample was taken before using the mouthwash. **Second sample (S2):** The second sample was taken after 30 minutes of using the mouthwash.

**Third sample(S3):** The second sample was taken after 2 weeks of using the mouthwash.

**Statistical Analysis**

Statistical analysis was performed with SPSS 18.0 (Statistical Package for Scientific Studies, SPSS, Inc., Chicago, IL, USA) for Windows

Values were presented as mean, standard deviation (SD) values. Data were explored for normality using Kolmogorov-Smirnov test of normality. The results of Kolmogorov-Smirnov test denoted that data were normally distributed (parametric data), thereby, comparison between both groups used by independent t test, while comparison for intragroup

used by ANOVA and Tukey’s post hoc tests (effect of time within the same group).

The percent change was calculated by the formula

$$\frac{\text{Value after}-\text{value before}}{\text{Value before}} \times 100$$

The significance level was set at  $p \leq 0.05$ .

**RESULTS**

The results of our study showed (Table 1) & (Fig. 1) that after treatment, a similar percent decrease in chlorohexidine (-73.68±11.83), in comparison to garlic and lime group (-73.69±5.71). Independent t test detected that the difference between groups was not statistically significant ( $p=1$ ), (Table 1, Fig 2)

**Table (1): Comparison of mean percent change in colony forming unit of Streptococcus mutans (%) in garlic &lime and chlorhexidine groups after treatment**

Groups	Mean	SD	Min	Max	T	P
<b>Garlic and lime extract</b>	-73.69	5.71	-84.74	-62.31		
<b>Chlorohexidine</b>	-73.68	11.83	-84.74	5.71	0.01	1ns

Significance level  $p < 0.05$ , ns=non-significant

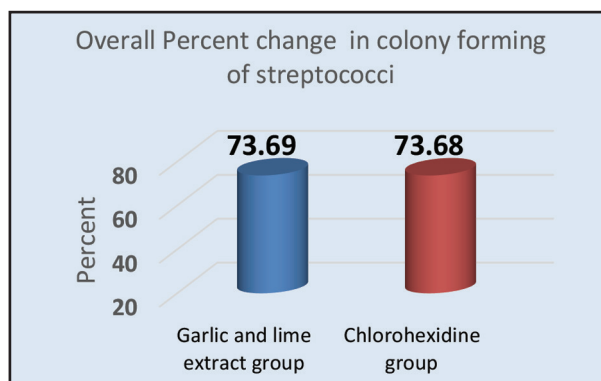


Figure (2) Bar chart showing mean percent change in colony forming unit of Streptococcus mutans (%) in garlic &lime and chlorhexidine groups after treatment.

## DISCUSSION

Oral health has a great impact on individuals physically and psychologically, also affects the rate of growth, chewing, tasting of food, speech, enjoyment of life and social communication. Children with severe caries suffer from pain, acute and chronic infections, discomfort, sleeping, and eating disorders, which affect the children quality of life. A key role for dental caries prevention is obtained by maintaining a good oral hygiene measures through regular removal of dental plaque and food deposits<sup>(11)</sup>.

Streptococcus Mutans are the main cause of dental caries. Dental caries occurs through adhesion of the streptococcus mutans to the enamel pellicle and breaking down of carbohydrates, the microbial metabolism of carbohydrates will lead to acidic environment, which will result in demineralization of hard tooth structure<sup>(12-13)</sup>.

Mouthwash is used in addition to regular mechanical cleaning, brushing and flossing on a daily use for prevention of oral disease. It is suggested to reduce inflammation, halitosis, treat infection, relieve pain, and/ or to deliver fluoride locally to prevent caries<sup>(14)</sup>. Plaque induced caries is a local disease therefore this study was done on mouth wash due to its local effect which is more effective than the systemic one<sup>(15)</sup>.

Chlorhexidine remains the gold standard to which other antiplaque and gingivitis agents are compared for its clinical efficacy. It has an antimicrobial effect on gram-negative and gram-positive bacteria as well as on fungi and some viruses<sup>(16)</sup>. Its effectiveness can be attributed to its bactericidal and bacteriostatic effects and its substantivity within the oral cavity. It has the ability to be adsorbed onto hard and soft tissues in the mouth, which lingers its action period after application of a formulation<sup>(17)</sup>.

As a result of the drawbacks with chemicals in mouth rinses, alternative antimicrobial treatments, such as antibacterial compounds extract from plant,

can be proposed to control this disease<sup>(18)</sup>. Herbal medicine is a comprehensive system, which treat disorders and maintain good health by the use of different parts of plants and their extracts<sup>(19)</sup>.

Garlic is stated for its antibacterial properties by Louis Pasteur and also has reported for its antifungal and antiviral activities. It has also shown that garlic have anti-inflammatory and antioxidant property; and inhibitory effect on S.M due to sulfur-containing compounds present in it.<sup>(9-10-11-20)</sup>

Sulphur-containing compounds have been found by the chemical analyses of garlic cloves. These sulphur based compounds are responsible for the antimicrobial effect of garlic. One of these compounds being allicin and its byproducts namely diallyl sulphide (DAS) and diallyl disulphides (DADS). The reactive allicin molecules have a very short half-life, as they react with many of the surrounding proteins, including the allinase enzyme, making it into a quasi-suicidal enzyme.

The biological activity of allicin extracted from fresh garlic is attributed to (a) its antioxidant function, (b) its ability to inhibit the sulphhydryl enzymes by attach to the sulphur groups in enzymes, proteins and modify their activities, (c) its ability to break through the cell membrane into cells. The sulphur compounds could be utilized in the dental caries prevention as it has an inhibitory effect on the adherence of S.M to in the dental plaque, which cause demineralization of the teeth and dental caries. As a result, the extracts of garlic may be used to stop the chain of caries etiology, by affecting the host factors and the microbial flora<sup>(21)</sup>.

Lime (*Citrus aurantifolia*) is used in the production of most herbal fabrication. It has antimicrobial activities against gram-negative and gram-positive bacteria, as well as *Candida albicans*<sup>(10)</sup>. In garlic with lime mouth rinse, a herbal formulation, lime was added to mask the strong flavor of garlic and it is provided antifungal effect as well. In general, a combination of garlic and lime have a synergistic effect<sup>(9-10)</sup>.

The current study is in agreement with the results of the study which assessed and compared the antimicrobial effect of garlic extract and chlorhexidine (CHX) mouthwash solution on oral salivary microorganisms. Both the garlic extract and CHX mouthwash were effective against salivary microbial population. Garlic has antimicrobial activity with no side effects such as dental staining and other cytotoxic effects when compared to CHX mouthwash. Further clinical investigations are needed for standardization and preparation of garlic containing toothpastes and mouthwashes. It was concluded that mouthwash containing garlic extract can be used as an alternative to CHX mouthwash <sup>(22)</sup>.

The results of the present study seem to support the results achieved by other previous study that assessed and compared the efficacy of garlic extract, tea tree oil, chlorhexidine, cetylpyridinium chloride, and ultraviolet (UV) toothbrush sanitizing device as toothbrush disinfectants against S.M. On comparing S.M colony counts, a highly significant difference was observed in all the groups. Garlic group showed the highest reduction in S.M colonies. It was concluded that garlic was the most effective among the antimicrobial agents <sup>(23)</sup>.

The results of this study also supported with another study which evaluate and compare the antimicrobial efficacy of chlorhexidine, sodium fluoride, fluoride with essential oils, alum, green tea, and garlic with lime mouth rinses against S.M, lactobacilli, and *Candida albicans*. It was concluded that chlorhexidine and garlic with lime are the most effective antibacterial and antifungal mouth rinses, respectively, among the six studied mouth wash. Garlic with lime mouth rinse has shown promising results thus, it can be considered a newer alternative. The finding of this study 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 especially in low socio-economic groups <sup>(9)</sup>.

This result agreed with other study compared the efficacy of neem, garlic, green tea and chlorhexidine mouthwash as toothbrush disinfectants. Then subjected to microbial analysis to check the presence of S.M. All test solutions showed a statistically significant reduction of S.M count. When chlorhexidine mouthwash was compared with neem, garlic, and green tea there was no statistical difference between them. Finally, it was concluded that neem, garlic, and green tea are equally efficacious as chlorhexidine and these herbal products can be used as potent alternatives to chlorhexidine as disinfectant for toothbrushes <sup>(24)</sup>.

The results of the current study also supported the result of this study seem to support the results achieved with an invitro study determined and compared the antibacterial activity of soft neck and hard neck species of garlic against cariogenic bacteria. Hard neck garlic extract has shown an inhibitory effect on the cariogenic organisms S.M and *Lactobacillus acidophilus* then comes the soft neck garlic extract and chlorhexidine mouth rinse. As a result of this garlic have shown a therapeutic effect on dental caries and other oral infections <sup>(25)</sup>.

## CONCLUSIONS

1. Garlic with lime mouthwash was successful as an antimicrobial agent it significantly reduced the salivary level of S.M which are the main etiologic factor in dental caries when compared with chlorhexidine 0.1% mouthwash
2. Garlic with lime extract is natural, easy to prepare and can be used as home care product.
3. In long term use of garlic with lime mouthwash was safe and effective alternative to chlorhexidine with no side effect.

## CONFLICT OF INTERES

None declared.



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