

Original Article

Efficacy of Different Concentrations of Garlic Extract in Reduction of Oral Salivary Microorganisms

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Abstract

Background: Garlic is a plant with antimicrobial effects and different concentrations of garlic extract can decrease oral microorganisms. This study evaluates the effectiveness of different concentrations of garlic extract in an oral salivary microbial population.

Methods: A total of 40 patients were selected based on information obtained through a questionnaire. Saliva samples were collected from all 40 subjects in dry plastic vials by spitting. Each sample was divided into 3 groups (2 cases and 1 control). One milliliter of sample was added to 9 mL of sterile physiologic serum and mixed. Then, this mixture was serially diluted to prepare a 10^{-3} suspension. One mL of garlic hydro-alcoholic extract was added to 1 mL each of the case specimens and transferred to a Trypticase Soy Agar (TSA) culture medium. Case specimens were exposed to 40% and 70% concentrations of garlic extract after 30 and 60 seconds. Control specimens were prepared by the same method, but without exposure to garlic extract. All samples were incubated at 37°C for 48 hours. Assessments were made based on colony counts to determine inhibitory activity of garlic extracts on oral salivary microorganisms.

Results: There was a significant reduction in colony forming unit (CFU) at 30 and 60 seconds for 40% concentrations of garlic extracts in comparison to the control group. In other words, 78% and 83.5% reduction in CFU, respectively. On the other hand, for the 70% concentrations, an 86.5% and 90.8% reduction occurred in CFU at 30 and 60 seconds, correspondingly. The mean colony counts of salivary microbial population at the 70% concentration were 771.72 ± 703.86 at 30 seconds and 524.8 ± 497.4 at 60 seconds.

Conclusion: According to our findings, garlic extract is effective in the reduction of an oral microbial population. It may be useful as an alternative product and new treatment modality with fewer side effects.

Keywords: Garlic extract, oral microorganism, saliva

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Introduction

One of the most important universal health problems is oral disease.¹ There is a well-known association between oral infections and microbial activities. More than 750 species of bacteria dwell in the oral cavity and a number of these are associated with oral diseases.² Resistance by pathogenic bacteria to currently used antibiotics and chemotherapeutics has increased the global requirement for alternative safe, efficacious and cost-effective treatment options and products for such infections, particularly in developing countries.^{3,4}

Traditional plants and natural products can treat bacterial infections. Even though natural products are not inevitably safer than synthetic antibiotics, health care professionals should recognize the value of herbal antibiotics.^{5,6}

Garlic (*Allium sativum*) is one of the most extensively investigated medicinal plants in use since ancient times due to its antibacterial, antifungal, and antiviral properties.⁷ Allicin is produced by the enzymatic activity of alliinase (a cysteine sulfoxide lyase) after crushing a garlic clove. There is extensive support that allicin and other thiosulfinates are responsible for the range of remedial effects described for garlic.⁸ Garlic extract has been shown to have a wide spectrum inhibitory effect on the growth of various gram-positive

and gram-negative bacteria such as: *Micrococcus*, *Enterobacter*, *Escherichia*, *Klebsiella*, *Lactobacilli*, *Pseudomonas*, *Salmonella*, *Shigella*, *Proteus*, *Helicobacter pylori*, *Staphylococcus*, and *Streptococcus* species.^{6,8-10} Moreover, it is also active against multi-drug resistant (MDR) organisms such as *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Mycobacterium tuberculosis*.⁶ Garlic extract mouth wash is effective at reducing total salivary bacterial counts.^{9,10}

A number of antibacterial agents, such as cetylpyridinium chloride, chlorhexidine, amine fluorides, or products that include these agents have been used in the prevention and management of oral diseases. These agents have been shown to be toxic, stain teeth, or possibly cause oral cancer, as with the case of ethanol which is frequently found in mouthwashes.^{11,12} Consequently, the search for alternative products such as natural phytochemicals isolated from plants used in traditional medicine would be reasonable.¹³ Therefore, the purpose of the present study is to compare the efficacy of different concentrations of garlic extract on reduction of oral salivary bacteria.

Materials and Methods

A total of 40 patients were selected from those referred to the Department of Oral Medicine, Faculty of Dentistry, Qazvin University of Medical Sciences to participate in this *in vitro* experimental study. The protocol was approved by the Institutional Review Board of Qazvin University Medical Sciences and all subjects gave written informed consent. A specially prepared questionnaire provided information on personal details such as past medical history, which included any recent antibiotic exposure. Participants who consumed

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drugs in the past month before study onset, were pregnant or had any kind of localized or systemic diseases were excluded.

Saliva collection

Four milliliter unstimulated whole salivary samples were obtained by spitting, in the absence of chewing movement, into dry plastic vials. During this procedure, the test subject was instructed to sit in a relaxed position. The collected saliva samples were centrifuged (2000×g for 10 min) and supernatants refrigerated until further analysis. Samples were collected at the same time of day (10 am to 12 am) and at least 2 hours after the last intake of food or drink. Each sample was divided into 3 groups (2 cases and 1 control). The vials were labeled (A: control; B₁ and B₂: case). Each vial was mixed completely on a shaker, after which 1 mL of saliva was added to 9 mL of sterile physiologic serum by a sterile pipette, and mixed. Then, this mixture was serially diluted to prepare suspensions of 10⁻³ concentration.

Garlic extract preparation

In order to prepare the garlic extract, garlic cloves were purchased from a local market in Hamedan, washed, peeled, and cut into pieces to exclude raw garlic extract. Garlic cloves were dried to protect them from changes in chemical composition. By subtracting the weight of the insoluble material from the weight of the original cloves, the final concentration of the garlic extract in solution was determined to be 40 and 70% (w/v). The hydro-alcoholic short extraction method was used to produce garlic extract.¹⁴ In this method, for production of fluid or dry extracts, the ratio of the garlic material used to the volume or weight of the final product is as following example. For instance, a 1:1 fluid extract would include the components of one gram garlic: one milliliter of final product. The raw extract was placed into a sterile container and refrigerated until used.

Procedure

One mL of garlic hydro-alcoholic extract was added to 1 mL of case specimen and transferred to a culture medium of trypticase Soy Agar (TSA). The *t*-test dependent control samples were prepared by the same method, but without exposure to garlic extract. Hence, salivary specimens consisted of two case and one control specimens. Cases were exposed to 40 and 70 mg/mL of garlic extract after 30 and 60 seconds. Agar plates were incubated at 37°C for 48 hours to allow for microbial growth. Microbial colonies were counted by an independent interpreter to evaluate the results.

Statistical analysis

Frothy salivary samples obtained from patients were used in this study. Each experiment was divided into two cases and one control group. Data were evaluated by parametric statistical analysis. Unpaired student's *t*-test compared different concentrations of garlic extract and time between the two groups. *P* values of less than 0.05 were statistically significant.

Results

In this study, the growth inhibitory activities of 40% and 70% concentrations of garlic extract were investigated. There was a significant reduction in colony forming unit (CFU) at 30 and 60 seconds for the 40% concentrations of garlic extracts in comparison to the control group. The 40% concentration at 30 seconds showed a 78% reduction and at 60 seconds there was an 83.5% reduction in CFU. The 70% concentrations had an 86.5% reduction at 30 seconds and 90.8% reduction in CFU at 60 seconds. The mean colony counts

of the salivary microbial population at the 70% concentration were 771.72 ± 703.86 at 30 seconds and 524.8 ± 497.4 at 60 seconds. Statistical analysis demonstrated no significant differences in oral microbial population between the two different times (*P* = 0.074). The mean colony count of salivary microbial population at 40% concentration was 1235.7 ± 765.06 for 30 seconds exposure and 941.52 ± 651.15 for 60 seconds exposure. Statistical analysis demonstrated no significant differences in oral microbial population in the two different times for the 40% concentration (*P* = 0.068). However, there were significant differences between mean colony counts with exposure to 40% concentrations of garlic extract for 30 seconds (1235.7 ± 765.05) when compared with the mean colony counts exposed to 70% concentration of garlic extract for 60 seconds (524.8 ± 497.4; *P* = 0.0001). In summary, although there was not a significant reduction of CFU in the control group (5731 ± 132 at 30 and 60 seconds), however, both case groups showed significant reductions in CFU.

Discussion

In general, quality of life is influenced by chronic conditions and systemic diseases linked to oral health. The relationship between oral diseases and oral microbiota is well recognized. The incidence of oral disease, improved bacterial resistance to antibiotics, side effects of some present bacterial agents and financial concerns produce a need for alternative preventative, safe, efficient and reasonably priced treatment options. Consequently, investigations into alternative products and new treatment modalities with fewer side effects would be logical. Natural plants that have been used as traditional medicines are regarded as good choices.¹⁵ From prehistoric times, garlic (*Allium sativum*; Liliaceae) has been used as a medicine due to its antimicrobial effects.¹⁶

Garlic is recognized to have antibacterial, antifungal, and anti-proteolytic activity.¹⁷ It has been previously used for treatment of various oral microorganisms, but to our knowledge this is the first description that evaluates the effectiveness of garlic extract activity against a total collection of oral salivary microorganisms. Our present *in vitro* study has exhibited that garlic extract can successfully inhibit the growth of oral microorganisms.

A comparison between two concentrations of garlic extract (40% and 70%) at different intervals (30 and 60 seconds) demonstrated no significant statistical differences between the two intervals in reduction of microorganisms, although the reduction in oral salivary microorganisms was greater at 60 seconds. In this study, there was a definitive reduction in oral salivary microbial population in the 40% concentration of garlic extract exposed for 60 seconds. The effectiveness of the 40% concentration of garlic extract at 60 seconds was even greater than the 70% concentration of garlic extract at 30 seconds.

The antimicrobial properties of garlic extract have been identified. A variety of garlic properties demonstrated broad spectrum activity in opposition to oral gram-positive and negative bacteria.^{6,8-10,18} The active component of garlic extract is allicin whose mechanism of action partially inhibits DNA and protein synthesis, and entirely inhibits RNA synthesis as the most important aim. Correspondingly, DNA transcription and other DNA activities are influenced by allicin.¹⁹ The fact that antimicrobial action of garlic is primarily attributable to allicin has been initially confirmed by Cavallito and Bailey.⁷

Garlic is also effective against antibiotic resistant organisms.²⁰ These properties of garlic improve its effectiveness against even highly resistant bacterial strains.¹⁹ On the other hand, some investi-

gators have demonstrated that certain mucoid bacterial strains were discovered to be resistant to allicin. For unknown reasons, it is presumed that penetration of allicin into the bacteria was restrained by hydrophilic capsular or mucoid layers.¹⁷

Antimicrobial agent of raw garlic extract is more effective than presently used antibiotics. The inhibition of DNA or RNA synthesis has a similar mechanism in both garlic and antibiotics.¹⁹ Moreover, the 50% toxic dose concentrations of allicin from garlic were greater than the dose needed for some of the newer antibiotics.

Strong antifungal activities of garlic extract inhibit the formation of mycotaxine, which is similar to the aflatoxin of *Aspergillus parasiticus*. Responsible for inhibition of fungal growth, pure allicin has a high anticandidal activity with minimum inhibitory concentrations of 7 µg/mL.^{21,22} In addition, it is also effective against diverse clinically important yeasts and can reduce both germination of spores and growth of hyphae.^{17,23}

Antiviral properties of fresh garlic extracts have been illustrated by *in vitro* and *in vivo* experimental studies against human cytomegalovirus, influenza virus type 3, vaccine virus, vesicular stomatitis virus and human rhinovirus type 2.¹⁷

Low concentrations of garlic extracts have shown beneficial results for oral health. Groppo et al. have found that after 2 weeks, mouthwash which contained 2%–5% of garlic extract significantly decreased salivary levels of streptococci.²⁴

Chen et al. have stressed that consumption of garlic extract with glucose containing foods could be harmful to the teeth because of the stimulation of acid production. On the other hand, they illustrated the efficacy of garlic extract and diallyl sulfide against *S. mutans* in garlic stimulated consequence of salivation that diminishes demineralization and raises the potential for remineralization.²⁵

In this study it has been revealed that antimicrobial effects of garlic extract could significantly inhibit the growth of oral microorganisms after 30 seconds. Thus, it seems that antimicrobial properties of garlic extracts are produced immediately after chewing and initial absorption of garlic. It is assumed that consumption of toothpaste or mouth wash containing an optimum concentration of garlic extract might be practical for the prevention and treatment of oral microbial diseases. There is no evidence in humans for toxic damage to the liver, kidneys, or bone marrow by the use of daily garlic extract for at least one month.²⁶ Considering that garlic is essentially a collection of numerous properties that are all beneficial in the management of oral microbial diseases, it may be considered an alternative or supplementary medicine for patients with this disease. In addition, since there is no evidence for severe adverse effects by the use of 40% and 70% concentrations of garlic extracts, it seems they may be less harmful for patients compared to other antimicrobial drugs with similar effects. These findings advocate that based on capability, garlic extracts could provide benefit as an anti-infective therapy. Nevertheless, lack of sufficient *in vivo* studies prohibits its clinical practice recommendation at the present time. Further clinical investigations for standardization and preparation of toothpastes and mouthwashes containing this antimicrobial agent for the prevention of oral microbial diseases is proposed in order to confirm the efficacy of garlic for their treatment.

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