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Antimicrobial activity of garlic against oral streptococci

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Dates:
Accepted 8 January 2007

To cite this article:
Int J Dent Hygiene **5**, 2007; 109–115
Groppo FC, Ramacciato JC, Motta RHL, Ferraresi PM, Sartoratto A. Antimicrobial activity of garlic against oral streptococci.

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Abstract: The antimicrobial activity of two garlic clones' (1: purple and 2: white) crude extracts against oral microbiota was evaluated *in vitro* (study 1) and *in vivo* (study 2). Study 1 consisted of the evaluation of minimum inhibitory (MIC) and bactericidal (MBC) concentrations against nine streptococci strains. In study 2, a 2.5% garlic (clone 2) solution was used as a mouthwash in a 5-week study by 30 subjects. Blood agar and Mitis Salivarius Bacitracin agar were inoculated with subjects' saliva to quantify oral microorganisms and *mutans* streptococci. Study 1 showed MIC ranging from 0.5 to 32.0 mg ml^{-1} for clone 2 and from 8 to 64.0 mg ml^{-1} for clone 1. MBC ranged from 1.0 to 128.0 mg ml^{-1} and from 8.0 to 128.0 mg ml^{-1} regarding clones 2 and 1 respectively. Study 2 showed that 2.5% garlic mouthwash solution had good antimicrobial activity against *mutans* streptococci and oral microorganisms. Maintenance of reduced salivary levels of streptococci was observed after 2 weeks at the end of mouthwash use. Unpleasant taste (100%), halitosis (90%) and nausea (30%) were reported by subjects after the end of the study. It was concluded that the garlic clones have antimicrobial properties *in vitro* against streptococci and anticariogenic properties against oral microorganism in spite of its adverse effects.

Key words: *allium sativum*; antimicrobial activity; garlic; oral streptococci

Introduction

Garlic antimicrobial activities have been recognized for centuries being many of its therapeutic properties first mentioned in 1500 BC in an Egyptian recipe named Papirus Ebers. Currently, it is used in folk medicine for the treatment of many diseases (1) and for the preservation of food products due to its antiseptic and disinfectant properties (2).

Allium sativum L. Liliaceae or simply garlic contains various biologically active constituents, like alliin, alliinase, allicin, S-allycystein, diallylsulphide and allymethyltrisulphide. Alliin is an amino acid, which is converted into allicin by alline-lyase catalyses when the bulbs are crushed. Allicin is the precursor of sulphur-containing compounds, which are responsible for the flavour, odour and pharmacological properties. Once exposed to air, allicin is further converted into diallyldisulphide, which has antibacterial effects, and the reduction by cysteine will disrupt the disulphide bond in microbial proteins (3). Bacterial growth inhibition and bactericidal properties are mainly attributed to allicin and thiosulphonates found in garlic (4, 5) but other sulphur-containing compounds, such as ajoene, also decrease bacterial growth (3, 6, 7).

Pure garlic extract had more efficient antimicrobial properties than tetracycline against human-caecum bacteria (8). Garlic also showed to be a strong fungicide agent against *Candida albicans*, which is a fungi usually present in the oral surfaces (9).

The growth of 19 *Helicobacter pylori* strains was inhibited using garlic concentrations ranging from 2 to 5 mg ml^{-1}, being the concentration necessary to kill the strains (minimum bactericidal concentrations, MBC) twice as much as the concentration to inhibit them – minimum inhibitory concentrations (MIC) (10). Elnima *et al.* (11) reported that 25% garlic extract showed good antimicrobial activity against human oral microbiota.

Dental disease prevention is commonly associated with a reduction of some oral-bacterial strains (13), such as *S. mutans*, *S. sanguis* and *S. sobrinus*, which are associated with dental caries and commonly found on the surface of hard dental tissues (12, 13) and *S. salivarius* on the surface of oral mucosa (14).

There is a variety of garlic clones in some Latin-American countries and the white clone is one of the most used in culinary. These clones have different morphological, anatomic and molecular characteristics and the most used clones in Brazil are the white and purple ones.

Thus, the aim of this study was to observe the antimicrobial activity *in vitro* of two garlic clones against oral streptococci and to test the most effective clone against human salivary microbiota in an *in vivo* study.

Materials and methods

In vitro study

Preparation of garlic extracts

Two garlic clones, white and purple, were obtained in the Research Center for Chemistry, Biology and Agriculture, State University of Campinas, Campinas, Brazil. The dry peel involving the bulbs was removed.

Aqueous garlic extract was prepared by using 100 g of fresh rootless bulbs and 100 ml of distilled/deionized water, which were ground in a blender for 10 min. The resulting extract was filtered in a paper filter and sterilized through 0.2 μm membrane filter by using a vacuum pump. All residues were weighed and the concentration of the final solution was considered to be 25% (w/v) or 250 mg ml^{-1}.

Analysis of garlic extract compounds

The extracts were analysed using a gas chromatograph (Hewlett-Packard – HP-5890), a mass selective detector (Hewlett-Packard – HP-5971), equipped with DB-1 capillary column (25 m × 0.2 mm × 0.33 μm). Carrier gas was helium at a flow rate of 1.0 ml min^{-1}. The injection temperature was 240°C and the detector was 300°C. The column temperature programme was 60°C (2 min) to 300°C at 4°C min^{-1}. The injection volume was 1.0 μl. The detector was quadropole system with ionization energy of 70 eV. Both extracts were assayed under the same conditions, and the NIST98 electronic library of GC/MS was used.

Bacteria

Streptococcus sobrinus ATCC 27607, *Streptococcus mutans* OMZ 175, *Streptococcus salivarius* AE112, *Streptococcus sanguis* ATCC 10556 and *Streptococcus cricetus* HS-6 were used as standard strains.

Four strains of streptococci were collected from volunteers in a previous study. These strains were identified as streptococci by using colony morphology, Gram staining, and selective culture medium. Biochemical tests were carried out and *S. sobrinus*, *S. mutans*, *S. salivarius*, and *S. sanguis* were identified (14, 15).

Minimal inhibitory and bactericidal concentrations

Eleven culture tubes containing 5 ml of Mueller–Hinton broth were used for each strain. Progressive concentrations of extract of purple (group 1) or white (group 2) garlic were added in the first 10 tubes. The last tube had microorganisms only (positive control). An extra tube was used as negative control (without microorganisms or extract). The concentrations of the garlic extract were 0.25, 0.5, 1, 2, 4, 8, 16, 32, 64 and 128 mg ml^{-1}.

All tubes received 250 μl of 10^6 ufc ml^{-1} and were incubated in 10% CO_2 at 37°C, for 18 h. After this time, the culture

medium was observed. By using a comparative visual scale, opacity of medium was indicative of bacterial growth. The first tube, in which bacterial growth was not observed, was considered as MIC.

Amounts of 5 μl from the tubes that did not present bacterial growth were spread on Petri dishes containing blood agar (5% sheep's blood). The concentration, which did not cause any bacterial growth in the blood agar, was considered as MBC.

In vivo study

This study was approved by Ethical committee of the Dental School of Piracicaba (protocol # 35/99). Thirty healthy subjects (15 men and 15 women, 18–35 years) having all teeth except third molars and presenting no allergy were selected. They did not use any antimicrobial agent during at least 1 month before the study began. All subjects formally agreed with all study aspects and signed the consent form. Sodium fluoride dentifrice (Tandy® – Kolynos, 1100 ppmF) was used as the standard dentifrice after the first week (basal measurements).

Saliva samples were collected using a previously described technique (12). All samples were sonicated (Vibra Cell 400 W, Sonics & Materials Inc. – 5% amplitude, 9.9 s cycle, six pulses) and diluted in a saline solution (100 and 1000 times).

The diluted samples (5 μl) were spread on blood agar (agar base; Difco Co., Detroit, MI, USA – and 5% sheep's blood) and MSB (Difco Co. – Mitis Salivarius agar, bacitracin 200 U l^{-1}, 15% sucrose, and 1% potassium tellurite). The Petri dishes were placed in an incubator (Jovan IG 150) with 10% CO_2 at 37°C for 48 h. Subsequently, the dishes with blood agar were placed in an aerobic incubator at 37°C, for an additional 24 h. Total oral microorganism counts were performed in blood agar and *mutans* streptococci counts in MSB. Figure 1 shows the procedure for saliva sample handling.

The subjects did not receive any treatment or standard dentifrice during the first week. Saliva samples were collected in the morning of the third (sample 1) and sixth (sample 2) days. The mean of sample 1 and sample 2 was considered as BASELINE for both oral microorganisms and *mutans* streptococci.

In the second week, all subjects were submitted to 1-min mouthwashes using 10 ml of vehicle solution (distilled/deionized water, 5% spearmint essence and 2% sorbitol) and standard dentifrice. Mouthwashes were carried out 30 min after the last tooth brushing of the day (16). Saliva samples were collected in the morning of the third (period 1) and sixth (period 2) days. The reduction (in %) of oral microorganisms and *mutans* streptococci counts in relation to baseline counts was used to express the effect of vehicle alone.

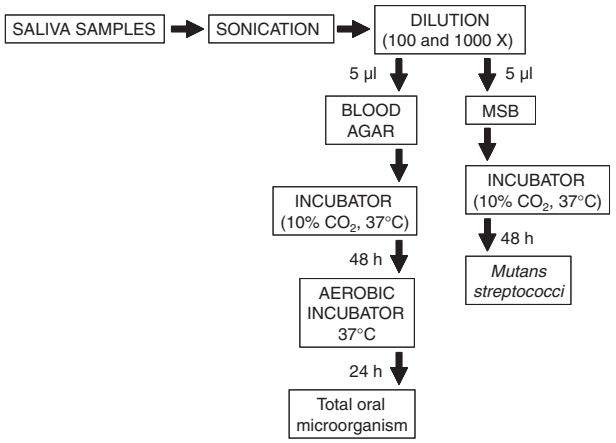


Fig. 1. Flow chart of the procedure for saliva samples handling.

In the third week, the subjects began to use a 1-min mouthwashes of 2.5% garlic solution. They used 10 ml of mouthwash as previously described for 7 days. This solution was prepared using the white clone and by using the same technique described in the *in vitro* study. The solution was diluted to 2.5% using the vehicle solution. Saliva samples were collected in the morning of the third (period 3) and sixth (period 4) days. The reduction (in %) of oral microorganisms and *mutans* streptococci counts in relation to baseline counts was used to express the effect of 2.5% garlic mouthwash.

In the two consecutive weeks, all subjects stopped the treatment. Saliva samples were collected once a week to determine oral microorganisms and *mutans* streptococci counts (period 5 = 4th week and period 6 = 5th week). The reduction (in %) of oral microorganisms and *mutans* streptococci counts in relation to baseline counts was used to express the residual effect of 2.5% garlic mouthwash. Figure 2 shows the distribution of groups and the sample collections.

In each sample-collecting day, all subjects filled a form inquiring about the solution's taste, halitosis, burning sensation, alteration in teeth colour and adverse effects during the experimental period. They classified these parameters as: without change, moderate or severe alteration.

All oral microorganisms and *mutans* streptococci counts observed were statistically analysed by Kruskal–Wallis test (at 5% significance level) by using a computer software (Bioestat 1.0 for Windows). The results from the test were expressed as a percentage.

Results

The chromatograms of both purple and white garlic clones showed chemical similarity due to the same retention time

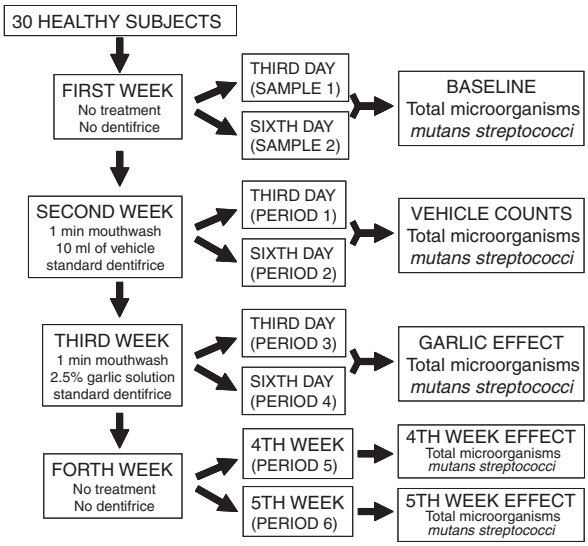


Fig. 2. Flow chart of the distribution of groups and the sample collections.

peaks of their components. Different peak areas could mean differences in concentration of each extract component. However, determination of the composition was not possible due to the insufficient extract concentration of either purple or white garlic clone. The chromatograms of two garlic extract clones are shown in Fig. 3.

Minimum inhibitory concentrations range considering the white clone was 0.5–32 mg ml^{-1}, while the range considering purple clone was 8–64 mg ml^{-1}. MBC range considering the white clone was 1–128 mg ml^{-1}, while the range considering purple clone was 8–128 mg ml^{-1} values. Streptococci strains collected from volunteers and standard microorganisms had different antimicrobial susceptibility profile considering the two garlic clones. MIC and MBC of the extracts against all microorganisms are shown in Table 1.

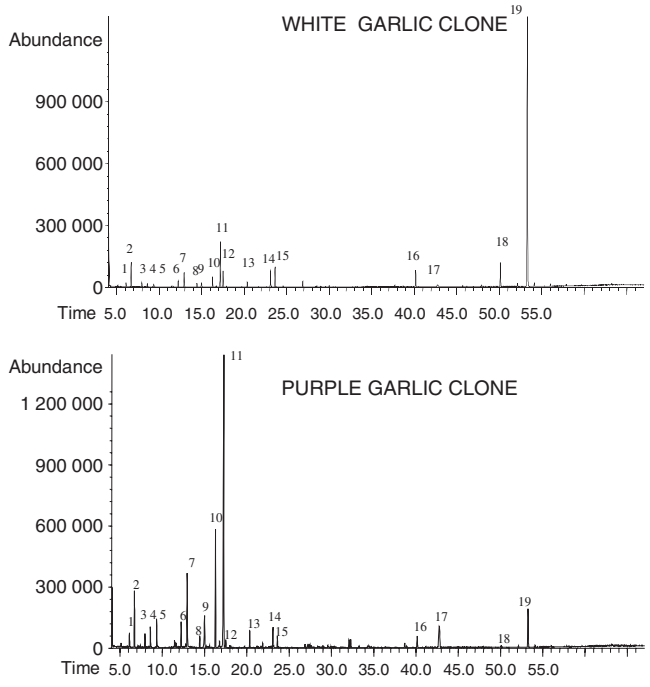


Fig. 3. Gas chromatograms of extracts of white and purple garlic clones.

Figure 4 shows the reduction of total oral microorganism and *mutans* streptococci (in %) in relation to baseline counts. Periods 1 and 2 are the reductions induced by vehicle use. Periods 3 and 4 are the reductions induced by the treatment (2.5% garlic solution). Periods 5 and 6 are, respectively, the first and second week after the end of treatment, i.e. the residual effect of the treatment.

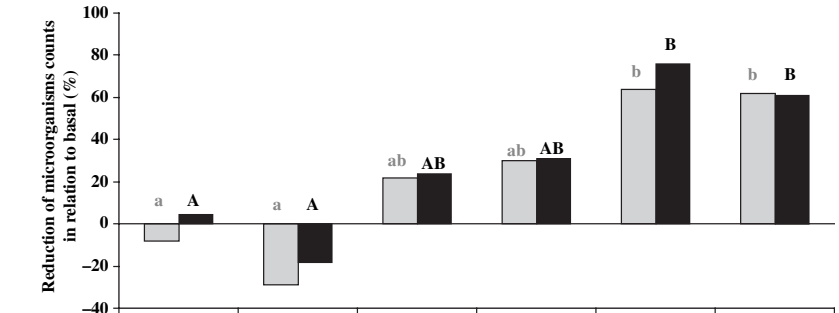
The effect of treatment (periods 3 and 4) and vehicle (periods 1 and 2) did not show statistically significant differences ($P > 0.05$). Although the effect of treatment (periods 3 and 4) and the residual effect (periods 5 and 6) did not show statistically significant differences ($P > 0.05$), the residual effect exhibited significant difference ($P < 0.05$) from vehicle periods (1 and 2). These observations are valid for both *mutans* streptococci and total oral microorganisms.

Solution's taste, halitosis, burning sensation, alteration in the colour of teeth and adverse effects are shown in Fig. 5. The vehicle solution did not affect anyone of these parameters. The use of the treatment (2.5% garlic solution) induced at least a moderate unpleasant taste and burning sensation in all subjects. Halitosis was pointed out by 90% of subjects. Nausea was the unique systemic effect related (30%). Alterations in the colour of teeth were not verified. The most severe parameters pointed out were unpleasant taste and halitosis.

Table 1. **MIC and MBC of garlic extracts against all microorganism strains**

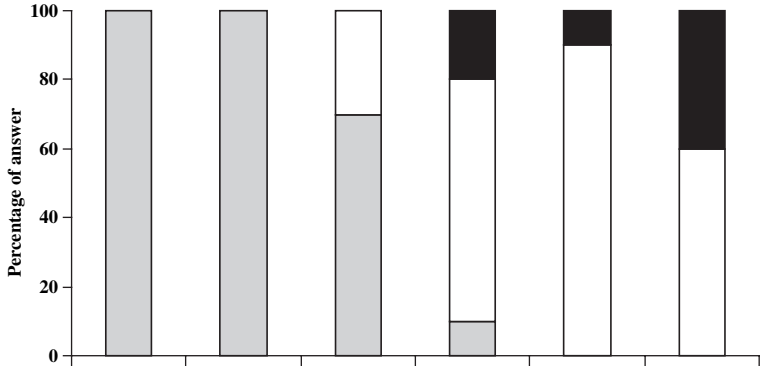
	MIC (mg ml^{-1})		MBC (mg ml^{-1})	
Streptococci	White	Purple	White	Purple
Streptococci sobrinus ATCC 27607	0.5	32.0	1.0	128.0
*S. sobrinus**	2.0	16.0	8.0	32.0
Streptococci sanguis ATCC 10556	2.0	16.0	8.0	128.0
*S. sanguis**	8.0	16.0	32.0	64.0
Streptococci mutans OMZ 175	8.0	64.0	8.0	64.0
*S. mutans**	32.0	16.0	128.0	128.0
Streptococci salivarius AE112	8.0	8.0	128.0	8.0
*S. salivarius**	8.0	32.0	32.0	32.0
Streptococci cricetus HS-6	8.0	32.0	8.0	128.0

MIC, minimum inhibitory concentration; MBC, minimum bactericidal concentration.
*Streptococci strains isolated from volunteers.



	PERIOD 1	PERIOD 2	PERIOD 3	PERIOD 4	PERIOD 5	PERIOD 6
streptococci	–8.1	–28.9	21.7	29.8	63.8	61.7
oral	4.4	–18.3	23.6	30.7	76.1	61.1

Fig. 4. Percentage of reduction in microorganism counts (oral and *mutans* streptococci) in relation to baseline counts in each period. Periods 1 and 2 are the difference between baseline and vehicle. Periods 3 and 4 are the difference between baseline and treatment. Periods 5 and 6 are the difference between baseline and the first and second week after the end of treatment respectively. Different upper case letters mean statistically significant differences ($P < 0.05$) among periods regarding total oral microorganisms and lower case letters regarding streptococci.



	Vehicle	Teeth color	Adverse effects	Halitosis	Burning	Taste
Severe	0	0	0	20	10	40
Moderate	0	0	30	70	90	60
Without change	100	100	70	10	0	0

Fig. 5. Percentage of positive answers about adverse effects, burning sensation, halitosis, and taste of solution.

Discussion

Shashikanth *et al.* (8) observed significant reduction of faecal streptococci by using raw garlic extract, which was orally administered in rats. While the use of raw extract could increase the antibacterial activity, it was not used due to the impossibility of observation on bacterial growth in the *in vitro* study. Moreover, without any filtration it may contaminate the culture broth.

One previous study showed the antimicrobial properties of garlic against oral streptococci. In this previous study, 25% garlic extract was able to inhibit oral streptococci (*S. mutans*, *S. milleri* and *S. sanguis)* ranging from 1/64 to 1/128 dilutions (11). These results were similar to the results of the present study.

There are a greater number of studies showing antimicrobial activity of garlic against other microorganisms than against oral bacteria including yeast, virus and human intestinal protozoan parasites. De *et al.* (2) observed a potent antimicrobial activity *in vitro* against *Bacillus subtilis*, *Escherichia coli* and *Saccharomyces cerevisiae*. Arora and Kaur (17) observed a significant bactericidal effect of garlic extract against *Staphylococcus epidermidis*, *Salmonella typhi* and various yeasts. Even bacteria resistant to antimicrobial agents were sensitive to extracts of garlic.

Cellini *et al.* (10) observed 5 mg ml^{-1} as MIC_{90} by using garlic extract against *H. pylori*. *Staphylococcus aureus* showed 50 mg ml^{-1} as MIC_{90} (5). The MIC_{90} was 10 mg ml^{-1} against dermatophytes (18). In the present study, we found similar values, showing that garlic in small concentrations could inhibit a number of different microorganisms.

Avato *et al.* (19) studied six different mixtures of garlic-distilled oils containing diallyl disulphide (DDS) and diallyl trisulphide (DTS) against *C. albicans*, *C. tropicalis*, *B. capitatus*,

S. aureus, *B. subtilis*, *P. aeruginosa* and *E. coli*. The results supported a specific antifungal activity more than an antibacterial activity and implicated DDS as the active constituent. However, DDS in garlic oils showed a significant dose-dependent inhibitory activity.

Allicin is another important active constituent of freshly crushed garlic homogenates. Pure allicin exhibits antibacterial activity against a wide range of Gram-negative and Gram-positive bacteria, including multidrug-resistant and enterotoxicogenic strains of *E. coli*, antifungal activity (particularly against *C. albicans*), antiparasitic activity (including against *Entamoeba histolytica* and *Giardia lamblia*) and antiviral activity. The main antimicrobial effect of allicin is due to its chemical reaction with thiol groups of various enzymes, e.g. alcohol dehydrogenase, thioredoxin reductase and RNA polymerase (20).

Living bacteria in dental plaque are generally more resistant to antimicrobial agents than bacteria in batch culture (standard bacteria), which is normally used for the *in vitro* susceptibility test (21). In the present study, the streptococci collected from the volunteers showed higher resistance when compared with the corresponding standard strains. However, microorganisms are considered to be tolerant to a specific antimicrobial agent when MBC is 32 times higher than MIC (22). Thus, including the clinical isolates, all microorganisms used in the present study were susceptible to the garlic extracts. The efficacy in reducing the streptococci in the *in vivo* assay provided evidence to the susceptibility of oral streptococci.

Salivary *mutans* streptococci counting was used in this study due to its direct correlation with the number of colonized oral sites (12). A decline in the number of these microorganisms has been considered as a decrease in dental diseases (13, 23). Garlic caused a significant reduction of salivary streptococci counting in the *in vivo* study. Thus, 2.5% garlic has anticariogenic potential.

Very few studies evaluated clinically the effects of garlic or its constituents. Ledezma *et al.* (24) studied the safety and effectiveness of ajoene (0.6%, gel) for the treatment of *Tinea corporis* and *Tinea cruris*. They evaluated 60 soldiers with clinical and mycological diagnosis of dermatophytosis. The results confirmed that ajoene can be used in the topic treatment of superficial mycoses. Similarly, our results indicated the possibility to use garlic as oral antiseptic. Further studies are necessary to establish which garlic constituent can be more useful as mouthwash.

A significant reduction in oral microorganisms was observed in a previous study (11) after using 10% garlic solution. However, a terrible taste and odour were verified at this concentration. The lower concentration used in the present study showed satisfactory antimicrobial activity; however, in spite of its low concentration, the 2.5% garlic showed a high percentage of adverse effects, including nausea. Further studies are necessary to reduce these adverse effects.

Conclusion

1 Garlic extract inhibited and killed all oral streptococci strains tested;

2 The white garlic clone was more effective than the purple one, and its chemical composition needs further definition;

3 The garlic solution mouthwash exhibited antimicrobial properties against oral microorganisms and streptococci *in vivo*, in spite of its adverse effects.

Acknowledgements

The authors thank the financial support of FAPESP/CNPq and Mr Jorge Valerio for his assistance in manuscript preparation.

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