

Antibacterial activity of garlic extract on cariogenic bacteria: An *in vitro* study

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Abstract

Background: Garlic (*Allium Sativum*) is ubiquitous, small and commonly used spice for processing food. There are many types of garlic and differ in shape, size, color, taste, number of cloves per bulb and storability. **Objectives:** To determine and compare the antibacterial activity of soft neck and hard neck species of garlic against cariogenic bacteria (*Streptococcus mutans* and *Lactobacillus acidophilus*). **Materials and Methods:** The well diffusion method was used to evaluate the antibacterial activity of garlic against *Streptococcus mutans* and *Lactobacillus acidophilus*. After incubation in an appropriate culture medium, diameter of zone of inhibition was measured to assess the antibacterial efficacy of garlic extract. Chlorhexidine mouthwash (ICPA HEALTH PRODUCTS LTD.) was kept as control group. Results were statistically analyzed using Kruskal Wallis test and independent 't' test. Thus, zone of inhibition (in mm) was analyzed using mean of all the readings obtained and the level of significance at <0.05 was considered statistically significant at 5% of level of significance. **Results:** Maximum zone of inhibition was found with hard neck garlic extract (24mm) followed by soft neck garlic extract (18mm) and Chlorhexidine (17mm) against *Streptococcus mutans* and *Lactobacillus acidophilus*. **Conclusion:** Action of garlic against *Streptococcus mutans* and *Lactobacillus acidophilus* raises the possibility that it can be used for dental caries and other oral infections possibly.

Keywords: Antibacterial, Chlorhexidine, garlic, *Lactobacillus acidophilus*, *Streptococcus mutans*

Introduction

Spices are the flavor of life. Throughout the world, cultures and countries utilize variety of spices for many reasons. These purposes may include enhancing flavor and preserving perishable food. Spices are defined as any dried, fragrant or aromatic vegetable or plant substance that contributes flavor in a whole, broken or ground form.^[1] Many of the spices used daily have been documented to be antimicrobial and have medicinal value as well.^[2]

Garlic is one of the useful aromatic spices. This pungent wonder of nature is botanically known as *Allium sativum* L. It is prophylactic as well as therapeutic medicinal plant. Some of the literature suggests that this medicinal plant was found in Avesta, a collection of Zoroastrian holy writings that was probably compiled during the sixth century BC. It has also played an important medicine to Sumerian and the ancient Egyptians. There is evidence that during the earliest Olympics in Greece, garlic was fed to the athletes for increasing stamina.

The word garlic is derived from the old English word garleac i.e spear leek. The 'gar' means spear (referring to spear shaped leaves) and 'leac' means leek.^[3] The origin of garlic dates back from 5000-6000 years. It is a native to central Asia, but it is difficult to trace the country of its origin.^[3] It is widely recognized for having an array of medical benefits, with antiviral, anti-fungal and antibacterial properties.^[4-6]

Garlic is made up of portions known as cloves, which may be separated for cooking and eating.^[1] Historically, garlic has been used for centuries worldwide by various societies to combat infectious diseases. Garlic can be provided in the form of capsules and powders, as dietary supplements and thus differ from conventional food or food ingredients. Garlic

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is also effective against antibiotic resistant organisms. Garlic extract has antimicrobial activity against oral bacterial species, particularly gram-negative species.

In India, garlic has been used to prevent wound infection and food spoilage. As per Ayurvedic classics it possesses wide range of pharmacological action including antimicrobial and anti-cariogenic. It is common ingredient of many medicinal and dietary medicinal formulations of Ayurveda. Most bacteria are sensitive to the extracts of plants such as clove, garlic, mustard, onion, oregano, turmeric etc. Spices such as garlic, turmeric and cinnamon have been used as antimicrobial agents in their raw form for the treatment of wounds and injuries and joint pains etc. The natural products are found to be more effective with least side effects as compared to commercial antibiotics so that reason they are used an alternated remedy for treatment of various infections. Emergence of drug resistance; molecular changes in organisms, which might result in increased virulence; and development of drug hypersensitivity are the most commonly reported undesirable effects of antibiotic usage.^[7] So, now there is need for alternative solutions. Dental caries is one of the most prevalent diseases and affects quality of life also. *Streptococcus mutans* and *Lactobacillus acidophilus* are the organisms which are responsible for initiation and progression of it. Natural phytochemicals can be an effective alternative to antibiotics and represent a promising approach in the prevention of dental caries and other oral infection. It has the ability to kill all oral streptococci strain.

Different types or subspecies of garlic are available like porcelain, rocambole, spanish, artichoke, smoked garlic etc., but most notably available species are soft neck garlic and hard neck garlic. Garlic is separated into these two categories based on tendency of each variety to develop flower stalks, its hardness and pattern of its clove formation. Other differences between soft neck and hard neck garlic are mentioned in Table 1. Literature review showed less published online data regarding dental health benefits of garlic. The objective of this *in vitro* study was to assess the antibacterial effect of soft neck and hard neck species of garlic extract against dental caries pathogens.

Materials and Methods

The present *in-vitro* study was conducted at the Department of Microbiology, Annasaheb Chudaman Patil Medical College, Dhule. (Maharashtra) and ethical clearance was obtained from the Institutional Ethical Review Committee. For the present study blood agar was used as a culture media, while nutrient broth was used for bacterial isolates preservation.^[2,7] Freeze dried strains of *Streptococcus mutans* (MTCC 497) and *Lactobacillus acidophilus* (MTCC 10307) were obtained from IMTECH (Institute of Microbial Technology), Chandigarh. Bactericidal activity was determined by agar well diffusion method.^[8] The above mentioned two different strains of organisms were first transferred to BHI broth and incubated for 24 hours to check their viability. These suspensions were

Table 1: Difference between hard neck and soft neck garlic

Hard neck garlic	Soft neck garlic
Produce a flower stalk (scape). Flowers usually abort and form 'bulbils'.	Do not produce a seed stalk. A partial flower stalk may be produced and bulbils will form directly above the bulb.
Less productive.	More productive.
Closely related to wild garlic.	Considered to be the most domesticated varieties.
Have small aerial 4 to 12 cloves surrounding the flower stalk.	Have 10-40 cloves arranged in multiple layers.
Difficult to braid.	Easy to braid.
Cold-tolerant, grown in cooler climate.	Less cold-tolerant, grown closer to equator.
Can not be stored well. May either start to form roots or start to dry out within a few months after harvest.	Can be stored for 6 to 8 months without significant deterioration. Generally, has a much larger shelf life.
Example: Rocambole, purple stripe, glazed purple stripe, marbled purple stripe.	Example: Artichoke, silver skin.

then smeared on blood agar plate. After 24 hours of incubation at 37°C, 9-10 colonies were transferred to 10ml BHI broth for its further use. These solutions were aseptically introduced and evenly spread on the blood agar plate (Lawn/Carpet culture).^[9] After removing excess solution, the inoculum was allowed to dry for few minutes at room temperature with the lid closed to avoid contamination. With the aid of a sterile 5mm metal borer, 3 equally spaced wells were bored in the agar plate aseptically.

Extract preparation

Extract preparation was carried out in the Department of Pharmacology, Annasaheb Chudaman Patil Medical College, Dhule (Maharashtra). Total 200 gms. of fresh soft neck and hard neck species of garlic were purchased from a retail food store of Dhule city, out of which 50gms of rootless garlic bulbs were cleaned, peeled off and first washed with water and then with 250ml of distilled water thoroughly. These garlics were then chopped into fine pieces, crushed and homogenized in sterile mortar and pestle^[10] under aseptic conditions. Obtained homogenized mixtures were then filtered with Whatman filter paper no 1.^[11] Almost 30-35ml extracts were obtained of 100% concentration from each species and immediately used for testing on microorganisms. (*S. mutans* and *L. acidophilus*) Chlorhexidine mouthwash was used as a control because it is the most common ingredient in mouthwash so it can be used as a standard to compare the effects of new products.

50 µl extract of each species of garlic and chlorhexidine were poured in the respective prepared wells with the help of sterile and disposable dropper. After 24 hours of incubation in an incubator at 37°C, agar plates were observed for zone of inhibition (areas without growth of test organisms). It was measured as the maximum width from the edge of the well to the periphery of the inhibition zone with the help of Vernier caliper.^[12] Maximum zone of inhibition would determine the inhibition of bacterial growth on an agar plate, so maximum

zone was measured. Similarly experiment was repeated in triplicate to assess the antibacterial efficacy for each species of garlic and a total of 6 plates were prepared. The principal investigator who is the primary individual responsible for the preparation, conduct and administration of a research was responsible to measure the zone of inhibition in the Dept. of Microbiology and measured the maximum zone of inhibitions on all the agar plates [Figure 1].

Statistical analysis

The statistical package for the Social Sciences (version 20, IBM, Armonk, NY, United States of America) was used for data analysis. Data was presented as mean \pm standard deviation of mean zone of inhibition. Levene test was used to test homogeneity of variances of data. Data obtained was organized in an excel sheet and subjected to statistical analysis. Comparison of zone of inhibition of *Streptococcus mutans* and *Lactobacillus Acidophilus* in between three groups was done using Kruskal Wallis test. Comparison of mean zone of inhibition in between either of 2 groups was done using independent *t* test. The data exhibited a normal and homogeneous distribution; thus, zone of inhibition (in mm) was analyzed using mean of all the readings obtained and the level of significance at <0.05 was considered statistically significant.

Results

There was statistically significant ($P < 0.05$) difference in zone of inhibition of *Streptococcus mutans* and *Lactobacillus acidophilus* in between the three groups [Table 2].

There was statistically significant difference in zone of inhibition of *Streptococcus mutans* and *Lactobacillus acidophilus* in between the either of pair of three groups [Table 3].

It was seen that garlic species selected for the present study were effective against cariogenic organisms. Hard neck garlic extract showed maximum zone of inhibition followed by soft neck garlic extract and Chlorhexidine mouthwash.

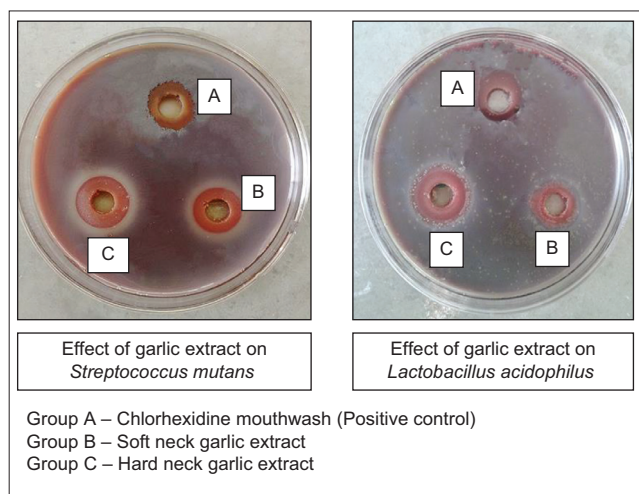


Figure 1. Effect of garlic extract on *Streptococcus mutans* and *Lactobacillus acidophilus* in group A,B and C

Discussion

Chlorhexidine till date has proven to be the most effective anti-plaque agent and it is the most common ingredient in mouthwash so was used as standard to compare the effects of new products^[11] but it can discolor the teeth and tongue and can cause loss of taste, burning sensation of the oral mucosa and subjective dryness of the oral cavity.^[13] A large number of bacterial species have become resistant to antibacterial drugs. This means that there is a need to develop alternative strategies. Garlic is known to act as an antibiotic and no resistance to it has been reported. Garlic is a strong antimicrobial agent and acts as an inhibitor on both gram-positive and gram-negative bacteria. There are many available types or subspecies of garlic, most notably soft-neck and hard neck garlic.^[14] Very less data is available on comparison of different species of *Allium sativum*. On the basis of availability of different species of *Allium sativum* in market, hard neck and soft neck species were selected for the present study.

Allicin (allyl 2-propenethiosulfinate or diallyl thiosulfinate) is the principal bioactive compound present in the aqueous extract of garlic or raw garlic homogenate. When garlic is chopped or crushed, allinase enzyme is activated and produce allicin from alliin (present in intact garlic). It is also seen that different extracts were different in their antimicrobial effectiveness depending upon the extractive solvent used. Oloke and Kolawole showed that bioactive components of any medicinal plant may differ in their solubility depending on the extractive solvents used.^[15]

The antibacterial activity of garlic is widely attributed to allicin. It is known that allicin has sulfhydryl modifying activity and is capable of inhibiting sulfhydryl enzymes. Allicin is not present in raw garlic. It is formed rapidly by the action of the enzyme, allinase.^[16] It reacts very rapidly with free thiol groups, via thiol-disulphide exchange and therefore, it is thought that its main mechanism of antimicrobial action is through interaction with thiol-containing enzymes, including cysteine proteases and alcohol dehydrogenases. Because these enzymes tend to be essential for bacterial nutrition and metabolism, it has been suggested that development of resistance to allicin arises 1000-fold less easily than it does to certain antibiotics.

With regard to the results of the present study, garlic juice was more effective against oral pathogens when compared with Chlorhexidine mouthwash and can be recommended as a new type of mouthwash. This is in accordance with the study

Table 2: Comparison of zone of inhibition of *Streptococcus mutans* and *Lactobacillus acidophilus* in between three groups (Kruskal Wallis Test)

Micro-organisms	<i>Streptococcus mutans</i>	<i>Lactobacillus acidophilus</i>
Chi-Square	7.057	7.386
Degree of freedom	2	2
<i>P</i>	0.029	0.025

Table 3: Comparison of mean zone of inhibition of *Streptococcus mutans* and *Lactobacillus acidophilus* in between three groups (Independent 't' test)

Micro-organisms Comparison groups	<i>Streptococcus mutans</i>			<i>Lactobacillus acidophilus</i>		
	Group A vs. B	Group A vs. C	Group B vs. C	Group A vs. B	Group A vs. C	Group B vs. C
Mean diff.	-1.33	-7.0	-5.7	-2.0	-8.0	-6.0
<i>t</i>	-2.828	-14.849	-12.021	-4.243	-16.971	-12.73
<i>P</i>	0.047	<0.001	<0.001	0.013	<0.001	<0.001

Group A: Chlorhexidine mouthwash (as a control), Group B: Soft neck garlic extract, Group C: Hard neck garlic extract

conducted by Mansour Amin *et al.* who reported the efficacy of garlic juice was higher than Chlorhexidine against tested organisms. Fatemeh Ahmadi Motamayel *et al.* compared alcoholic extracts of eucalyptus and garlic against cariogenic pathogens i.e., *Streptococcus mutans* and *Lactobacillus acidophilus* and showed that both bacteria, *S. mutans* and *Lactobacillus acidophilus*, were resistant to eucalyptus extract but sensitive to garlic extract and the inhibition zone diameter increased as concentration of garlic extract increased.

Present study showed more inhibitory effect of garlic on *Lactobacillus acidophilus* compared to *Streptococcus mutans*. This might be a result of genetic differences among the organisms. The structural differences of the bacterial strain may also play a role in the bacterial susceptibility to garlic constituents. So, further research should be done to know the reasons behind the same. Contents of garlic may differ due to geographic location of the farm, soil, climate, temperature, type/or species of garlic. So this might be the reason why hard neck garlic was more effective than soft neck garlic in the present study. Although species of garlic selected for the present study were purchased from the same market but there is no assurance of its from same geographical area or climatic conditions. So further research is necessary to overcome this limitation.

Conclusion

Hard neck garlic extract showed maximum zone of inhibition or antibacterial activity against cariogenic organisms *Streptococcus mutans* and *Lactobacillus acidophilus*, followed by soft neck garlic extract and Chlorhexidine mouthwash. Its action against these organisms raises the possibility that garlic may have therapeutic use for dental caries and possibly other oral infections.

Recommendation

The present study has its own limitations. This study was performed on standard strains of microorganisms obtained from IMTECH, Chandigarh. Further studies on garlic and cariogenic bacteria present in oral cavity (mouth derived) are necessary.

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Conflicts of interest

There are no conflicts of interest.

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