Original Research Article

# Antibacterial efficacy of hydroalcoholic extract of garlic: An Invitro study

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## Abstract

**Background:** Streptococcus mutans is the predominant bacteria seen in oral diseases. A wide variety of oral hygiene products are used as disinfectants for proper maintenance of oral hygiene and to eradicate the micro-organisms, such as Cetylpyridinium chloride, Chlorhexidine, Triclosan, or antibiotics are of choice. Because of its disadvantages, there is a need for alternate, safe agent to remove the *Streptococcus mutans* infection. **Aim:** The aim of this study is to evaluate the antimicrobial activity of Hydroalcoholic extract of garlic against *Streptococcus mutans*. **Materials and Methods:** The *Streptococcus mutans* (ATCC35668) samples were then treated with different concentrations of Hydroalcoholic extract of garlic for different time periods. The effect of each test agent was determined by calculating the colony forming units and percentage killings of the *Streptococcus mutans* bacteria. The data obtained is statistically analyzed using student t test. **Results:** The present study shows that the Hydroalcoholic extract of garlic shows larger diameter zone of inhibition and greater inhibitory potential by MIC and MBC assays against *Streptococcus mutans*. **Conclusion:** From the study, it can be concluded that, Hydroalcoholic extract of garlic has a significant antimicrobial effect against *Streptococcus mutans* and can be used as oral hygiene agent for proper maintenance of oral hygiene.

**Key Words:** *Streptococcus mutans*, Antimicrobial activity, Colony forming unit, Hydroalcoholic extract of garlic.

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## INTRODUCTION

Maintenance of good oral hygiene is the key to the prevention of oral diseases. The importance of oral microflora being responsible for mouth odor and most oral diseases is well documented.[1] Dental plaque is the major cause of dental caries and periodontal disease. The disease process may involve enamel, dentin and cement, causing decalcification of these tissues and disintegration of the organic substances. Plaque is a habitat for different microorganisms.[2] Streptococcus mutans is one of the main opportunistic pathogens of dental caries which plays a central role in fermentation of carbohydrates resulting in acid production, and leading to the demineralization of the tooth enamel.[3] Worldwide, approximately 36% of the population has dental caries in their permanent teeth. In baby teeth it affects about 9% of the population.[4] Risk of caries includes physical, biological, environmental, behavioral and lifestyle-related factors. Several studies have previously demonstrated the antibacterial potency of various plant extracts against oral pathogens.[5] Moreover, with the continuous use of synthetic drugs and chemical formulations, the microbes developed a wide resistance. Therefore, nowadays researchers are focusing towards the naturally available products, in order to overcome the shortcomings caused by the usage of modern medicaments. Nature is considered as an infinite resource for discovery and development and supply of drugs. Nature is always a foremost drug house with a wide variety of plants, animals and microorganisms. In India, since ancient periods, the use of natural products as a medicine (Ayurvedam), is in practice. Innumerable plant extracts, animal products are used to treat a large variety of clinical conditions. Garlic is considered as the most

effective medicinal agent with a lot of therapeutic uses. Garlic also acts as a bioenhancer for various antimicrobial, anti fungal and anti cancer drugs thus making them very effective (6). Therefore, the present study details the invitro antibacterial potential of Hydroalcoholic extract of garlic against *Streptococcus mutans*.

## MATERIALS AND METHODOLOGY

**Preparation of Garlic Extract:** Hydroalcoholic extract of garlic was prepared according to the method described by Suffness and Douros.[7] The fresh garlic was thoroughly cleaned, shade dried and extraction procedure was carried out. Briefly, one hundred grams of garlic powder was extracted with 500 ml of 50% ethanol at 50 to 60°C in a Soxhlet apparatus for 72 hours. The hydroalcoholic extract of Garlic was then filtered and evaporated to dryness at 50°C. The residue obtained was stored at 4°C until further use.

**Disc Diffusion Method:** The antimicrobial disc were made by using Whatman, no.3, filter paper discs measuring 7 mm were cut and impregnated with 10 mL of Garlic Hydroalcoholic extract. The discs were allowed to remain at room temperature until complete diluent evaporation and kept under refrigeration until ready to be used. In this method, with the standardized inoculum of Streptococcus mutans (ATCC35668), inoculated onto the entire surface of a Mueller-Hinton agar (MHA) plate with a sterile cotton-tipped swab to form an even lawn. The discs loaded with Garlic Hydroalcoholic extract were placed onto the surface of the inoculated agar plate. The disks used for the disk diffusion assay contains the standardized known amount of the Garlic Hydroalcoholic extract, which diffuses into the agar when in contact with the agar surface. The plate is then incubated under standardized conditions following Clinical and Laboratory Standards Institute (CLSI) guidelines. During incubation, the Garlic Hydroalcoholic extract diffuses into the agar medium and inhibits the growth of the Streptococcus mutans bacteria, producing a "Zone of inhibition" around the disk. The diameter of this zone is measured and the results are interpreted as resistant, intermediate, or susceptible using the standard guidelines. The bigger the diameter of the inhibition zone, the more susceptible is the microorganism to the antimicrobial. Tests were performed in duplicate.

**Determination of minimum inhibitory concentration (MIC) and minimal bactericidal concentrations (MBC):** MIC was determined by using the broth microdilution method in Mueller Hinton Broth. Briefly, serial doubling dilutions of the extract were prepared in a 96-well microtiter plate. Further, each well was added with 10 µl of indicator solution and 10 µl of Mueller Hinton Broth. Finally, 10 µl of bacterial suspension ($10^6$ CFU/ml) was added to each well to achieve a concentration of $10^4$ CFU/ml. The plates were prepared in triplicates, and then they were placed in an incubator at 37°C for 18–24 hours. The color change was then assessed visually. The lowest concentration at which the color change occurred was taken as the MIC value. The average of 3 values was calculated providing the MIC values for the tested extract. The MIC is defined as the lowest concentration of the extract at which the microorganism does not demonstrate the visible growth. The microorganism growth was indicated by turbidity. Bacterial cells from the MIC test plate were sub-cultured on solid nutrient agar by making streaks on the surface of the agar. The plates were incubated overnight at 37°C and the MBCs were determined after 24 h. Plates that did not show growth were considered to be the MBC for the extract or drug used. The experiment was carried out in duplicate.

**Statistical Analysis:** The data obtained were statistically analyzed using Student t test to compare the results between the groups. All the data were expressed as Mean ± SEM and the difference of $p<0.05$ or more was considered significant.

## RESULTS

**Table 1:** Growth inhibition diameters of *Streptococcus mutans* obtained by disc diffusion method

| Microbial strain | Zone of inhibition (mm) Mean ± SEM | |
|---|---|---|
| | 24 hrs | 48 hrs |
| *Streptococcus mutans* | 14±0.56 | 16.2±0.71 |

**Table 2:** MIC and MBC assays showing the antibacterial efficacy of hydroalcoholic extract of Garlic against *Streptococcus mutans*.

| Group | MIC µg/ml | MBC µg/ml |
|---|---|---|
| Untreated | Not Detected | Not Detected |
| Ciprofloxacin | 10 | 50 |
| Hydroalcholic extract | 25 | 100 |

**Disc Diffusion assay:** Antimicrobial activity of Garlic Hydroalcoholic extract was evaluated based on the diameters of clear inhibition zone surrounding the paper discs. Table 1 shows the results of antimicrobial activity of Garlic Hydroalcoholic extract against Streptococcus mutans by disc diffusion after 24 and 48 hrs incubation. The present study shows that, incubation of Streptococcus mutans with Garlic Hydroalcoholic extract coated disc for 48 hrs incubation shown larger diameter zone of inhibition (15.25±0.31) than that of 24hrs (11.80±0.27), indicating effective inhibition of growth of Streptococcus mutans by Garlic Hydroalcoholic extract.

**MIC and MBC assay:** In the present study, MIC was calculated for the Hydroalcoholic extract of Garlic against Streptococcus mutans. Ciprofloxacin used as positive control exhibited potent antibacterial with MIC values ranging from 0.06-0.10 µg/ml. Whereas, Hydroalcoholic extract of Garlic extracts showed MIC against the test bacteria with MIC values ranging from 10-25 µg/ml indicating the antibacterial efficacy of garlic extract when compared with untreated group. The MBC was calculated against the S.mutans, the garlic extract group values were ranging from 50-100 µg/ml, where as the ciprofloxacin showed MBC values ranging from 25-50 µg/ml (Table II).

## DISCUSSION

In the past four decades, microbial ecologists including microbiologists, taxonomists, molecular biologists, biochemists, epidemiologists, and dental scientists have accumulated information which has led to the identification of the presumed pathogens of human dental caries and periodontal diseases[8]. So in order to prevent dental diseases it is important to decrease the bacterial load of plaque. Dental plaque consists of various pathogenic microorganisms of which *streptococcus mutans* is the most pathogenic[9]. A wide variety of oral hygiene products are used as disinfectants for proper maintenance of oral hygiene and to eradicate the micro-organisms, such as Cetylpyridinium chloride, Chlorhexidine, Triclosan, or antibiotics are of choice. Because of its disadvantages, there is a need for alternate, safe agent to remove the Streptococcus mutans infection. Recently much attention has been paid to extracts and biologically active compounds that are isolated from natural species and used in herbal medicine[10]. The aim of this study is to evaluate the antimicrobial activity of Hydroalcoholic extract of garlic against *Streptococcus mutans*. Several studies showed the effect of garlic extract on various bacterial strains[11]. Highly standardized methods are essential for all types of susceptibility testing. These are highly sensitive to variations like media formulation, pH, inoculum density and incubation conditions. In addition, the agar diffusion methods are strongly influenced by agar depth, growth rate of the bacteria, diffusion rate of the antimicrobial agent. Diffusion tests are low cost compared to most methods, but colony forming unit method can be regarded as the standard method for testing the microbial susceptibility. Results from present study showed the ability of antimicrobial activity of Hydroalcoholic extract of garlic against S.mutans bacteria by disc diffusion by forming larger diameter zone of inhibition after 24 and 48hrs of aerobic incubation. Our present results are in accordance with earlier studies, Reuter and co-workers[12] assessed the inhibitory effects of Allium sativum extract on drug resistant pathogenic bacteria using the disc diffusion method and reported that all the tested bacterial strains in their study were susceptible to garlic extract, which confirms our results. Present study observations showed Hydroalcoholic extract of garlic exhibited higher inhibitory potential against S.mutans. The antimicrobial activity of Hydroalcoholic extract of garlic is attributed may be due to the presence of allicin active component of the garlic. The enzyme alliinase present in garlic converts Alliin to Alliicin, participates in the metabolism of cysteine in proteins, causing the disruption to the epidermal junction of the cells leading coagulative necrosis of the tissues[13]. In the present study, MIC and MBC was also calculated for the Hydroalcoholic extract of Garlic against Streptococcus mutans. Ciprofloxacin used as positive control exhibited potent antibacterial with MIC values ranging from 0.06-0.10 µg/ml. Whereas, Hydroalcoholic extract of Garlic extracts showed MIC against the test bacteria with MIC values ranging from 10-25 µg/ml indicating the antibacterial efficacy of garlic extract when compared with untreated group. The MBC was calculated against the S.mutans, the garlic extract group values were ranging from 50-100 µg/ml, where as the ciprofloxacin showed MBC values ranging from 25-50 µg/ml. Hydroalcoholic extract of Garlic also showed effective inhibitory potential over the Streptococcus mutans with MIC values ranging from 10-25 µg/ml indicating the antibacterial efficacy of garlic extract when compared with untreated group. The MBC was calculated against the S.mutans, the garlic extract group values were ranging from 50-100 µg/ml. So, from the results it can be derived that the antimicrobial activity of Hydroalcoholic extract of Garlic against Streptococcus mutans.

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