

Evaluation of Antimicrobial Activity of *Allium sativum* (Garlic) *in vitro*: A Pilot Study

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ABSTRACT

Background: Dental calculus and its microbial activity are considered as major causative factors for progression of inflammatory disease of the periodontium. To prevent the microbial activity of oral cavity, various chemical agents have been practiced, the long-term usage of which results in adverse reactions. To minimize its adverse side effects, treatment modalities have been changing toward herbal medications. Therefore, with minimal side effects, effectiveness of *Allium sativum* (garlic) against dental calculus formation is considered as vital. The aim of the study is to analyze the antimicrobial activity of garlic using calculus samples. **Materials and Methods:** Dental calculus samples were cultured using brain–heart infusion reagent. For antibiotic assay, Kirby–Bauer antimicrobial susceptibility procedure was carried. Freshly prepared aqueous garlic extract (AGE) and chlorhexidine were kept in cultured agar plate, and after 24 h, incubation inhibition zones were calculated. **Results:** Greater inhibition zones were seen around AGE compared to chlorhexidine. **Conclusion:** Study shows definite antimicrobial activity of *A. sativum*.

KEYWORDS: *Allium sativum*, antimicrobial, chlorhexidine, inhibition zone

INTRODUCTION

Dental calculus is the major causative factor for progression of periodontal disease. It consists of intricate microbial community of more than thousands bacteria per milligram. Recently, specific pathogens are identified in calculus which progress to chronic periodontal diseases.^[1] Progression of inflammatory periodontal disease is due to impaired host response to the periodontal pathogens. Oral health can be achieved by removal of residual microorganisms.^[1]

From ancient times, the curative value of garlic (*Allium sativum*) is well documented. The properties of garlic against coronary thrombosis,^[2] inhibition of platelet aggregation,^[3] atherosclerosis^[4] and mainly its antibacterial, antifungal, and antiprotozoal properties are widely known.^[5]

Allicin as diallyl thiosulfinate is responsible for the antimicrobial activity of garlic.^[6] The main purpose of the present study is to analyze the antimicrobial activity of garlic against dental calculus.

Aims and objectives

1. To analyze the antimicrobial activity of garlic in oral cavity using calculus samples
2. To compare its efficacy with chlorhexidine.

MATERIALS AND METHODS

Sample collection

After obtaining institutional ethical clearance, 15 patients were randomly selected from the outpatient Department of Periodontics. Subgingival calculus samples were collected using curettes from molar region in culture bottles.

Preparation of culture

Culture was prepared using brain–heart infusion broth reagent agar plate, where the sample was inoculated using streaking method. This culture was incubated at

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How to cite this article: Thirupathi P, Elanchezhiyan S, Nethravathy RR, Vennila K, Esther PV, Meenalochani TB. Evaluation of antimicrobial activity of *Allium sativum* (Garlic) *in vitro*: A pilot study. J Indian Acad Dent Spec Res 2018;5:5-7.

Access this article online	
Quick Response Code: 	Website: www.jiadsr.org
	DOI: 10.4103/jiadsr.jiadsr_28_17

37°C for 48 h. After 48 h, colonies of microbial growth were noted.

Preparations of aqueous garlic extract

Bulbs of garlic (*A. sativum*) were purchased from a local store. Fresh cloves of garlic (100 g > were peeled, minced, and ground in a blender. One hundred grams of grounded garlic pulp was agitated with 200 ml of double distilled water, occasionally stirred and refrigerated for 4 days then filtered using microspore filter paper of size 0.2 mm cloth to remove the larger particles, and centrifuged at 2500 rpm for 30 min. The supernatant and pellets are collected separately.

Antibiotic assay

Kirby–Bauer antimicrobial susceptibility test procedure

The main purpose of performing Kirby–Bauer test^[7] is for the evaluation of antimicrobial activity of chemotherapeutic agents. For this procedure, Mueller–Hinton agar, the medium of choice with a pH range of 7.2–7.4, is poured into plates to a uniform depth of 5 mm and refrigerated on solidification. Before use, the plates are transferred to an incubator at 37°C for 10–20 min to dry the moisture that develops on the agar surface.

Following incubation, the plates are examined for the presence of growth inhibition, which is indicated by a clear zone surrounding each disk. The susceptibility of an organism to a drug is determined by the size of the zone, which itself is dependent on variables such as:

1. Ability and rate of diffusion of the antibiotic into the medium and its interaction with the test organism
2. The number of organisms inoculated
3. The growth rate of the organism, and
4. The degree of susceptibility of the organism to the antibiotic.

The susceptibility of an organism to a drug is determined by the size of the zone. A measurement of diameter of the zone of inhibition in millimeters is made and its size is compared. Based on this comparison, the test organism is determined to be resistant, intermediate, or susceptible to the antibiotic. Inhibition zone with range of 17–20 mm is considered as susceptible to antibiotics.

Agar plates were placed right-side up in an incubator heated to 37°C for 10–20 min. A sterile cotton swab was dipped into peptone water and then into a well-mixed test culture of one patient, and excess inoculum was removed by pressing the saturated swab against the inner wall of culture tube. Onto the agar surface, the swab was streaked horizontally, vertically, and around the outer edge of the plate to ensure a heavy growth over the entire surface. All the agar plates were allowed to dry for about 5 min.

In cultured agar plate, four wells were prepared for each sample. Using a dispenser on agar surface, first well

from left side is left as empty, second well is applied with supernatant garlic extract, followed by pellet of garlic extract in third well, and standard chlorhexidine were applied on the fourth well. [Figure 1].

All plates were incubated in an inverted position for 24–48 h at 37°C. Results were evaluated and compared. All 15 samples had clear inhibition zone with a diameter range of 20–30 mm for prepared aqueous garlic extract (AGE).

RESULTS

After 48 h, inhibition zones were formed on the agar plates impregnated with extracted supernatant and pellet garlic. The inhibition zone signifies the reduction of microorganisms on the agar plate [Figures 2]. These inhibition zones on the agar plates looked circular in shape, and the zones were measured with the help of a millimeter scale. Mean and standard deviation for zones of inhibition implies that when compared with the control group chlorhexidine, both supernatant and pellet garlic extract show statistically significant inhibition zones [Table 1].

DISCUSSION

Periodontal disease which encompasses several pathological conditions affects the tooth-supporting

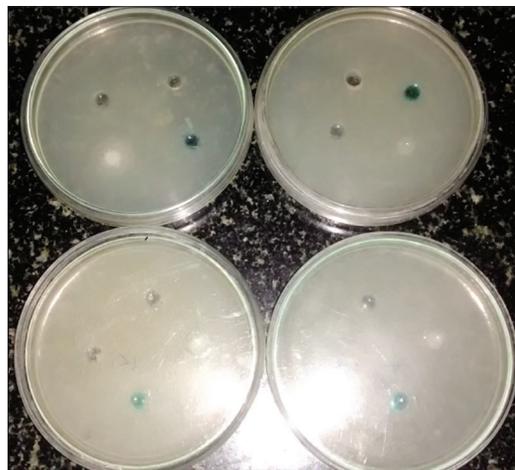


Figure 1: Prepared well for antimicrobial susceptibility

Table 1: Mean±standard deviation zones of inhibition of extracts

	Supernatant garlic	Pellet garlic	Chlorhexidine
Mean value	23.38±0.67	21.38±0.67	13.04±0.13
Standard deviation	1.06±0.62	0.84±0.08	0.63±0.24
When compared with control	<i>t</i> : 29.47 (<i>P</i> <0.001)*	<i>t</i> : 30.17 (<i>P</i> <0.001)*	

**P*<0.001 – statistically significant 95% CI. CI=Confidence interval

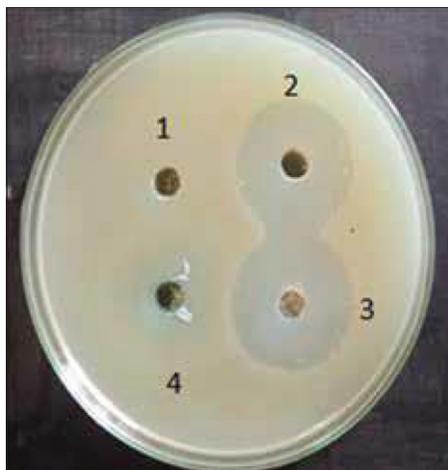


Figure 2: Formation of inhibition zone

structure. The clinical signs of periodontitis are changes in the morphology of gingival tissues, bleeding on probing, as well as periodontal pocket formation. This periodontal pocket provides an ideal environment for the growth and proliferation of anaerobic pathogenic bacteria.^[1] Dental plaque is formed by sequential colonization of microorganisms on the tooth surface. It is a structured, resilient, and yellow-grayish substance that adheres tenaciously to intraoral hard substance. One gram of plaque contains nearly 100,000,000,000 bacteria.

The accumulation of plaque on the tooth surface is prevented by anti-plaque agents. The efforts of dental researchers have resulted in the availability of a wide range of anti-plaque agents, but available antimicrobial agents produce resistant strains. Chlorhexidine mouthwash may stain the teeth.

With renewed interest in the anti-infective properties of medicinal plants and foods, garlic in particular is widely utilized as a folk remedy for its antimicrobial and other beneficial effects. The inhibitory activity of AGE against many microorganisms has been mentioned in clinical reports for nearly 100 years, and its use can be particularly important when traditional therapies are found to be too toxic.^[8]

Allicin present in garlic, react with various enzymes of thiol groups^[8] which inhibits trypsin and protease thus produces bactericidal effect on oral pathogens.^[5] It is active against *Porphyromonas gingivalis*, *Klebsiella*, *Staphylococcus*, *Helicobacter pylori*, and *Serratia* species and also active against *Streptococcus mutans* and *S. faecalis*.^[9]

Chlorhexidine is taken as control group,^[10] due to its bactericidal and bacteriostatic activity^[11,12] and its inhibition zone is around 13.4 mm. The wide inhibition zone was comparatively present around garlic extract due to the presence of allicin.^[4]

In this study, reduction of microorganisms is confirmed by the formation of inhibition zones on Mueller–Hinton agar plates. The formation of inhibition zones takes place after 24 h with mean value of 23.04 mm diameter around supernatant well of garlic extract and 21.38 mm diameter around pellet well of garlic extract. Results indicate a considerable antibacterial activity of garlic. Henceforth, garlic extract can be used to control periodontal diseases and limit the progression of periodontitis.

CONCLUSION

This *in vitro* study concludes that the commonly used natural herbs among the rural people, such as garlic, have beneficial antimicrobial activity. Hence, further studies are required in formulating the anti-plaque agents based on herbs such as *A. sativum*.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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