Antibacterial Activity of Garlic Extract against *Streptococcus mutans* and *Lactobacillus acidophilus*: An *In Vitro* Study

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**Abstract**

**Background:** Pulpotomy is a therapeutic conservative procedure involving the amputation of the infected coronal pulp and dressing the remaining noninfected pulp tissue with a suitable material. An antimicrobial pulp dressing material is key to avoid failure of the treatment due to any residual microflora. *Allium sativum*, commonly known as “Garlic” is one of the most widely researched natural entities due to its antimicrobial, antioxidant, and antifungal properties. The aim of the present study was to evaluate the antimicrobial efficacy of garlic extract (*A. sativum*) in the concentrations of 100%, 50%, and 25% against *Streptococcus mutans* and *Lactobacillus acidophilus* using the agar well diffusion method.

**Methodology:** The microorganisms *S. mutans* and *L. acidophilus* were isolated from the patient’s saliva and were identified using the biochemical test. Five wells were prepared using a sterile template in agar plates. The garlic extract in the concentrations of 100%, 50%, and 25%, formocresol (positive control), and ethanol (negative control) was added to the prepared wells. The agar plates were incubated at 37°C for 24 hours and 48 hours in a candle extinction jar for *S. mutans* and an anaerobic jar for *L. acidophilus*, respectively.

**Results:** All the tested concentrations of the garlic extract demonstrated antimicrobial activity against *S. mutans* and *L. acidophilus*. Formocresol in 1:5 concentration (positive control) showed excellent antimicrobial activity and no antimicrobial activity was observed with ethanol (negative control) at 24 hours and 48 hours. Antimicrobial activity is reduced by reducing the concentration of garlic extract. It was found that *L. acidophilus* was more sensitive to different concentrations of garlic extract than *S. mutans*.

**Conclusion:** All the tested concentrations of garlic extract exhibited varying antimicrobial activities against *S. mutans* and *L. acidophilus*. Considering the antimicrobial activity, *A. sativum* can be considered as a suitable alternative to formocresol as a pulpotomy medicament.

**Keywords:** *Allium sativum*, Antimicrobial activity, Formocresol, *L. acidophilus*, *S. mutans*.

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**Introduction**

The main objective of pulp therapy in the deciduous dentition is to maintain the healthy status and integrity of the primary teeth and their supporting tissues in children.1 Pulpotomy is a vital pulp therapy procedure involving amputation of the coronal portion of the affected or inflamed pulp tissue, thereby retaining the vitality and function of all or part of the remaining radicular portion of the pulp.2 Various medicaments have been used for dressing the radicular pulp after the amputation of coronal pulp tissue such as formocresol,3,4 glutaraldehyde,5 ferric sulfate,6 collagen material,7 mineral trioxide aggregate,8,9 and Biodentine10; however, some of these medicaments are reported to cause adverse effects in the human body.11 The ideal material for capping pulp must possess superior biological and physical properties such as sealing of the remaining pulpal tissue, should be biocompatible,12 and should possess antimicrobial activity.13

With this concern, a search for natural medicaments as alternatives for agents such as formocresol as a pulp dressing material has become vitally important. *Allium sativum* is one of the most widely researched medicinal plants14 due to its antibacterial,15 antioxidant,16 and antifungal17 properties. Allicin and other components such as thiosulfonates present in the garlic are considered to be responsible for the therapeutic benefits reported.18 As the eradication of microorganisms is essential for the success of pulpotomy procedures, the assessment of antimicrobial efficacy of pulpotomy medicaments becomes imperative.19

The microflora of cariously exposed vital pulps included a subset of microbial species associated with advanced carious lesion biofilms and are commonly predominated by *Streptococcus mutans*, *Lactobacillus acidophilus*.18,19 Thus, this study aimed to evaluate the antibacterial activity of garlic extract in the concentrations of 100%, 50%, and 25% against *S. mutans* and *L. acidophilus* using the agar diffusion method.

**Methodology**

**Isolation of the Organisms**

From the sample of patient’s saliva, microorganisms such as *S. mutans* and *L. acidophilus* were isolated and cultured on milts salivarius bacitracin agar (HMEDIA, Mumbai) and *Lactobacillus* selection agar, respectively. The agar plates were incubated in anaerobic and aerobic conditions for *L. acidophilus*.
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Statistical Analysis
The obtained data were tabulated and subjected to statistical analysis using Statistical Package for Social Sciences version 24 software (SPSS Inc, Chicago, IL, USA). Descriptive and inferential statistics including the mean zones of inhibition was calculated. Analysis of Variance (ANOVA) test was used to compare the mean values at different concentrations and pairwise comparison was carried out using Post hoc Tukey test. p-value less than 0.05 was considered statistically significant.

RESULTS
The mean zones of inhibition (Fig. 2) were recorded for different A. sativum concentrations and control groups against L. acidophilus and S. mutans at 24 hours and 48 hours are shown in Table 1. All the concentrations of A. sativum showed significant antimicrobial activity against the experimental bacteria at 24 hours and 48 hours.

Antimicrobial Activity of A. sativum against S. mutans (Fig. 3)
All experimental concentrations of A. sativum displayed significant antimicrobial activity against S. mutans both at 24 and 48 hours. The mean zone of inhibition produced was highest for 100% A. sativum (13 ± 0.50 mm) and gradually reduced with decreased concentrations. However, the recorded antimicrobial activity of A. sativum was less when compared to formocresol which was used as a positive control. Though it was not statistically significant, a minimal increase in mean inhibition zones was observed from 24–48 hours.

Antimicrobial Activity of A. sativum against L. acidophilus (Fig. 4)
Formocresol was observed to exhibit the highest antimicrobial activity against L. acidophilus (36 ± 0.50 mm), followed by 100% (20 ± 0.50 mm), 50% (16 ± 0.50 mm) A. sativum extract, and least was found with 25% concentration (12 ± 0.50 mm). Negative control ethanol did not produce any inhibition zone. A minimal increase in antimicrobial activity was found at 48 hours when compared to 24 hours. However, this was not statistically significant.

Preparation of Ethanolic Extract of Garlic
An amount of 30 mL of ethanol was added to 30 gm of freshly peeled and weighed garlic and were homogenized in a sterile mortar and pestle to make a paste. The mix was then rigorously stirred using a magnetic stirrer for 1 hour at 1000 rpm. The extract was then filtered using Whatman’s filter paper-2. The filtered ethanolic extract of garlic was transferred to sterile petri dishes for 6 hours to allow for evaporation. The final extract thus obtained was of 100% concentration and dilutions of 50% and 25% were made with sterile distilled water by adding appropriate volumes to the concentrated extract.

Antibacterial Assay
After the addition of different concentrations of garlic extract to the experimental and control wells, the plates were then incubated at 37°C in candle extinction jars for S. mutans and in anaerobic jars for L. acidophilus for 48 hours. Using Vernier calipers, the microbial inhibition zones were measured in millimeters at 24 and 48 hours incubation periods. The test was performed in triplicates for each microorganism for the homogeneity of the results.
Garlic Extract against S. mutans and L. acidophilus

Agar well diffusion test is widely used for evaluating the antimicrobial activity among in vitro studies. This method facilitates direct comparisons of materials against tested microorganisms and can be used to demonstrate the activity of freshly prepared materials. The results of the agar diffusion test are semiquantitative that is derived by measuring the mean diameter of complete inhibition zones of bacterial growth around the disks after a specific period of incubation. However, the selection of the microorganisms and agar medium, control and standardization of inoculation density, reading points of the inhibition zones are several factors that affect the results of the diffusion tests in an agar medium. Nevertheless, consistent results can be produced if these variables are controlled carefully.

In this study, A. sativum extract in all the tested concentrations exhibited varying antimicrobial activity. Formocresol in 1:5 concentration produced a maximum zone of inhibition followed by 100%, 50%, and 25% garlic extract in decreasing manner. No antimicrobial property was exhibited by ethanol, which served as a negative control. It could also be inferred that L. acidophilus was more sensitive and susceptible to A. sativum extract when compared to S. mutans.

### Table 1: Mean zones of inhibition (mm) of different concentrations of garlic extract (GE) against the tested microorganisms at 24 and 48 hours

|               | S. mutans | L. acidophilus |
|---------------|-----------|---------------|
|               | Mean      | SD           | p-value | Mean | SD | p-value |
| 24 hrs        |           |             |         |      |    |         |
| 100% GE       | 13.0      | 0.500       | <0.001  | 100% GE | 20.0 | 0.500 | <0.001 |
| 50% GE        | 11.0      | 0.500       |         | 50% GE | 16.0 | 0.500 |
| 25% GE        | 9.0       | 0.500       |         | 25% GE | 12.0 | 1.000 |
| Positive control | 30.0   | 1.000       |         | Positive control | 36.0 | 0.500 |
| Negative control | 0.0     | 0.000       |         | Negative control | 0.0  | 0.000 |
| 48 hrs        |           |             |         |      |    |         |
| 100% GE       | 13.5      | 0.500       | <0.001  | 100% GE | 21.0 | 1.000 | <0.001 |
| 50% GE        | 11.5      | 0.500       |         | 50% GE | 16.5 | 0.500 |
| 25% GE        | 10.0      | 0.500       |         | 25% GE | 12.5 | 0.500 |
| Positive control | 30.0   | 0.500       |         | Positive control | 36.0 | 0.500 |
| Negative control | 0.0     | 0.000       |         | Negative control | 0.0  | 0.000 |

### Discussion

Pulpotomy is the procedure involving complete or partial amputation of the inflamed or affected coronal part of the pulp tissue and covering the residual pulp tissue with the medicaments to conserve the vitality of the radicular portion of the dental pulp and to prolong the integrity and function of the deciduous dentition. Formocresol has been considered as a standard pulpotomy medicament for primary teeth. However, several drawbacks such as cytotoxicity, mutagenicity, carcinogenicity, immunologic, biochemical, and teratogenicity in the host have been reported. This led to the search for natural medicaments to replace formocresol for dressing the dental pulp.

One such medicine in that category is A. sativum, known commonly as “Garlic.” A. sativum is a medicinal plant that was researched extensively. Garlic has antimicrobial and antioxidant properties, regulates the immune system and can contribute to wound healing. Its activity depends on component allicin which is produced on the enzymatic activity of alliinase as well as thiosulfinates. The use of A. sativum in dentistry involves its use as a root canal irrigant, mouth wash, as nonvital and vital pulp therapy medicament in primary teeth. In our study, we aimed to evaluate the antibacterial activity of 100%, 50%, and 25% garlic extract against L. acidophilus and S. mutans using the agar diffusion method.

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Garlic Extract against S. mutans and L. acidophilus

The antibacterial activity of A. sativum is due to the contents such as ajoene, a sulfur-containing compound, and allicin (allyl 2-propenethiosulfinate or diallyl thiosulfinate). It has been reported that allicin has the ability to modify sulfhydryl groups and can inhibit sulfhydryl enzymes. Raw garlic does not contain allicin. It is produced rapidly by the action of the enzyme, alliinase. Alliinase enzyme is usually activated when garlic cloves are crushed or chopped, thereby producing allicin from allin (present in intact garlic). Antimicrobial activity of allicin has been attributed to its reaction with free thiol groups of bacterial enzymes such as alcohol dehydrogenase, RNA polymerase, cysteine proteases which are essential for bacterial metabolism and nutrition.

The results of this study demonstrating the antimicrobial property of garlic extract against S. mutans and L. acidophilus are in accordance with the other investigations conducted by Mohammad SG et al., Kshirsagar MM et al., Fatemeh AM et al., Mansour A et al. and Groppo FC et al. Fatemeh AM et al. investigated the antimicrobial activity of alcoholic extracts of garlic and eucalyptus against caries forming bacterial strains S. mutans and L. acidophilus. They reported that both the tested strains were sensitive to garlic extract and resistant to eucalyptus extract. They also noted that the antimicrobial property was reduced with decreased concentration of A. sativum extracts. Houshmand B et al. tested various concentrations of garlic extract against bacteria isolated from dental plaque and observed that even at 5% concentration, garlic extract showed significant antimicrobial activity. In our study, the least concentration used (25%) also exhibited significant antimicrobial activity.

In our study, formocresol showed greater antimicrobial activity than garlic extract. Mohammad SG et al. in their study tested the microbiological efficacy of A. sativum and formocresol in primary teeth when used for mortal pulpotomy. They observed a marked reduction in the counts of S. mutans, L. acidophilus after application of both the medicaments. They also observed better results with A. sativum oil when compared to formocresol. However, our study demonstrated better antimicrobial activity with formocresol. This variation in results can be attributed to the obvious differences in the study methodology applied.

The inhibitory activity of garlic extract on L. acidophilus was shown to be more effective than S. mutans and this is in agreement with the findings reported by Kshirsagar MM et al. and Houshmand B et al.
Limitations of the Study

- It is imperative to consider that in vitro tests do not reproduce the actual conditions of the oral cavity and clinical inferences should be drawn with caution.
- Although significant care was taken to standardize the experimental conditions, the determination of inhibition zone values depends on technical details that are different with different laboratories. The size of the inhibition zones is dependent on the diffusibility of the test material in agar, which in turn is influenced by the molecular weight, negative charge, composition of samples, pH, and the thickness of the agar culture medium.

CONCLUSION

Within the limitations of this study, it can be concluded that:

- All the tested concentrations of garlic extract exhibited varying antimicrobial activities against S. mutans and L. acidophilus.
- The highest antimicrobial activity against the tested microorganisms was demonstrated by formocresol followed by various concentrations of garlic extract in decreasing order.
- Considering the antimicrobial activity, A. sativum can be considered as a suitable alternative for formocresol as a pulpotomy medicament in deciduous teeth. However, further clinical studies are recommended.

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