

Original Research Article

EXTRACTION OF GARLIC AND ENHANCING ANTIBIOTIC ACTIVITY OF ALLICIN

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ABSTRACT:

Historically garlic has been used for centuries worldwide by various societies to combat infectious disease. Garlic can be provided in the form of capsules and powders as dietary supplements and thus differ from conventional foods or food ingredients. Louis Pasteur was the first to describe the antibacterial effect of onion and garlic juices. Alliums vegetables particularly garlic exhibit a broad antibiotic activity against both Gram-positive and Gram-negative bacteria. In India, garlic has been used to prevent wound infection and food spoilage. In present work the extraction of garlic was done and antimicrobial activity was tested by cup plate agar diffusion method and also the antibiotic enhancing effect was compared with Azithromycin which was used as Antibacterial standard. The zone of inhibition was calculated by measuring diameter of the zone of no microbial growth.

Keywords: Garlic, Extraction, Broad Spectrum Antibiotic.

INTRODUCTION

Garlic (*Allium sativum*) is one of the most extensively researched medicinal plants and its typical odour and antibacterial activity depends on allicin produced by enzymatic activity of allinase (a cysteine sulfoxide lyase) on allin after crushing or cutting garlic clove.^{1, 2} Allicin and other thiosulphinates are believed to be responsible for the range of therapeutic effects reported for garlic. Its close relatives include the onion, shallot, leek, chive³ and rakkyo⁴. There are extensive literatures on antibacterial effects of fresh garlic extract. Garlic extract has been reported to inhibit growth of various gram-positive and gram-negative bacteria including: *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, and *Pseudomonas aeruginosa*. The antifungal⁸ and antiviral⁹ activity of garlic extract have also been reported. The objective of the present investigation was to determine the inhibitory activity of garlic extract on bore plate method. Historically, garlic has been used for centuries worldwide by various societies to combat infectious disease. Garlic can be provided in the form of capsules and powders, as dietary supplements, and thus differ from conventional foods or food ingredients. Louis Pasteur was the first to describe the antibacterial effect of onion and garlic juices. Alliums vegetables, particularly garlic exhibit a broad antibiotic activity against both Gram-positive and Gram-negative bacteria. In India, garlic has been used to prevent wound

infection and food spoilage. Recent reports also demonstrated the inhibitory activity of garlic aqueous extracts on numerous bacterial and fungal species. Therapeutic effect of garlic is possible because of its oil- and water-soluble organ sulfur compounds, which are responsible for the typical odor and flavor of garlic. Thiosulfinate play an important role in the antibiotic activity of garlic. Due to the occurrence of unpleasant side effects and increasing resistance to the synthetic pharmaceuticals, there has been increasing interest in the quest for natural alternatives. In this paper, we evaluated the antimicrobial activity of crude extracts of garlic against four bacterial pathogens i.e. against two Gram positive and two Gram negative pathogens. *Allium sativum* belongs to the family Alliaceae⁵, commonly known as garlic. The therapeutic effects of Garlic on the cardiovascular system, antibacterial, antiviral, antifungal, antiprotozoal, anticancer, antioxidant, immuno-modulatory, anti-inflammatory hypoglycemic and hormone like effects. This study will focus only on antibacterial activity of *A. sativum* cloves.

Structure and occurrence:

Allicin features the Thiosulfinate functional group, R-S-(O)-S-R. The compound is not present in garlic unless tissue damage occurs and is formed by action of the enzyme allinase on allin. When Garlic is crushed or otherwise damaged; the allin reacts with the enzyme allinase also found naturally in garlic. Allinase acts as a catalyst and results in the transformation of allin into Allicin (diallyl thiosulphinate).

MATERIALS AND METHODS:**MATERIALS:****Chemicals:**

Isopropanol was obtained from Ashonuj chem. Pvt.Ltd.n-Hexane and Iodine crystals was procured from S.D.Lab.chemical centre. Ethanol was obtained from Changshu yangyuan chemical china.

METHODS:

Active ingredient in Garlic: Many drugs used by consumers for medicinal purposes are derived from plants. Plants can be called the first chemist because they can synthesize large molecules for biological activity. The compounds are used by plants as defense against plant eating insects and other are by-products of growth processes of plants. A number of these plant compounds have a property or chemical activity useful to humans.

Part-I

Allicin is produced due to the interaction of the garlic enzyme allinase with the substrate allin. Allinase was isolated chemically stabilized and coupled to a solid matrix thus enabling the efficient conversion of synthetic nature –identical allin to allicin. Aqueous solutions of allicin can be stored in the cold at 4°C for month.

Part-II: TLC Method^{6, 7, 8}

The cloves of garlic were crushed by using mortar and pestle and the crushed garlic and obtained oil was placed in 50 ml beaker. Then the garlic and oil was soaked in 20 ml ethanol and the beaker was closed with glass plate and allows it to stand for half an hour. Then 15 ml of n-hexane and 5 ml of isopropanol was added in a beaker to produce a 3:1 hexane isopropanol solvent. The solvent mixture was poured into a TLC chamber. No

more than $\frac{1}{4}$ of an inch of solvent should be added to the chamber. The saturation of TLC chamber was done by dipping a rectangle piece of filter paper that fits into the TLC chamber. By using a pencil drawn a line approximately 3 cm from the bottom of the TLC plate. Using a capillary spotter placed one drop of the garlic/ethanol mixture on the penciled line on the TLC plate. Then the TLC plate was placed in TLC chamber till the mobile phase run $\frac{3}{4}$ th height of plate. Then the plate was withdrawn and dried at room temperature and then placed in a fumed Iodine chamber. Then the R_f was calculated.



Figure 1: TLC plate of Allicin

2. Experimental Methods -

Fresh Garlic and leaves of *Allium sativum* collected from agricultural local market of Satara, adequate quantity (100gm) of samples was kept in fresh polythene bags. Then, the garlic bulbs were peeled, weighed (100 g), and cleaned garlic were taken and surface sterilized using ethanol. The ethanol was allowed to evaporate in a sterile laminar flow chamber, and the garlic was homogenized aseptically using a sterile mortar and pestle. The homogenized mixture was filtered through sterile cheesecloth. This extract was considered as the 100% concentration of the extract. The crude extracts of Garlic *A. Sativum* used to purify, identify the Allicin by TLC method. The solution prepared for each concentration of *A. Sativum* Garlic by micropipette. contained different quantity (100 μ g/ml, 200 μ g/ml, 300 μ g/ml) of allicin of *A. Sativum* this solution were aseptically transferred on the medium surface to determine the antibacterial activity of all concentrations of *A. sativum* Garlic by Agar well diffusion assay. The selected strains of bacteria were inoculated into 10 mL of sterile nutrient broth, and incubated at 37°C for 8 hours. The cultures were swabbed on the surface of sterile nutrient agar plates using a sterile cotton swab. Agar wells were prepared with the help of sterilized cork borer with 10 mm diameter. Using a micropipette, different concentrations of garlic extracts (100 μ g/ml, 200 μ g/ml, and 300 μ g/ml) were added to the wells in the plate. The plates were incubated in an upright position at 37°C for 24 hours. The diameter of inhibition zones was measured in mm and the results were recorded.



Figure 2:300µg/ml Azithromycin-**Figure 3:**Azithromycin standard
Allicin against *Staphylococcus aureus*. against *Staphylococcus aureus*.

Antibiotic sensitivity testing:

The cultures were enriched in sterile nutrient broth for 6-8 hours at 37°C. Using sterile cotton swabs, the cultures were aseptically swabbed on the surface of sterile nutrient agar plates. Using an ethanol dipped and flamed forceps, different concentrations of Allicin and combination of Allicin and Azithromycin (standard Antibacterial) were aseptically placed over the seeded agar plates sufficiently separated from each other to avoid overlapping of inhibition zones. The plates were incubated at 37 °C for 24 hours and the diameter of the inhibition zones was measured in mm. All the media used in the present investigation were obtained from Hi-media Laboratories Ltd., Mumbai, India.

Antimicrobial activity:

Antimicrobial activity was carried out by cup plate agar diffusion method. Extract at concentration of 100µg/ml, 200µg/ml, 300µg/ml were tested against bacteria. Antimicrobial activity was tested on *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *E. coli*, *B. subtilis*. The plates were incubated at 37 °C for 24 hours. The antimicrobial enhancing effect was compared with Azithromycin which was used as Antibacterial standard. The zone of inhibition was calculated by measuring diameter of the zone of no microbial growth.

RESULTS AND DISCUSSION:**Table no. 1** Antibiotic activity testing by Allicin extract

Concentration/cultures	<i>Staphylococcus aureus</i> Diameter (mm)	<i>Bacillus subtilus</i> (mm)	<i>Eschrichia coli</i> (mm)	<i>Pseudomonas auerogenosa</i> (mm)
100 µg/ml Area of inhibition of Allicin	-	5	-	7
200 µg/ml Area of inhibition of Allicin of Allicin	10	9	11	9
300 µg/ml Area of inhibition of Allicin of Allicin	15	10	12	13
Azithromycin + Allicin(100 µg/ml)	14	13	12	13
Azithromycin + Allicin(200µg/ml)	17	15	14	16
Azithromycin + Allicin(300 µg/ml)	20	17	18	18
Azithromycin (standard)	12	17	14	13

AE: Aqueous extract of Allicin, -:zone not appeared

Determination of antimicrobial activity from time immemorial man has been using various parts of the plants against common ailments with varying degree of success. The knowledge of drug has developed together with the evolution of scientific and social progress. Drugs derived from plants are effective, easily available, and less expensive and rarely have side effects. The practitioners of traditional and indigenous medicine mainly use medicinal plants and herbs for preparation of therapeutic substance. Initial screening for the potential antibacterial and antifungal compounds from plants may be performed by using the crude extracts. The two most commonly used methods to determine antimicrobial susceptibility are the dilution assay and the disc or agar well diffusion assay. In the present investigation different concentrations of the aqueous garlic extracts (AGE) was tested for their inhibitory activity on 2 Gram-negative and 2 Gram positive bacteria. Antibacterial activity of different concentrations of AGE by agar well method was tested.

Diameter of Inhibition zone (mm) of different concentration Number: Test organisms at 100µg/ml, 200µg/ml, 300µg/ml - All test organisms were inhibited by up to 25% concentration and the activity was a linear function of concentration. At 300 µg/ml Azithromycin + Allicin the maximum zone of inhibition was observed against *Staphylococcus aureus*, a Gram positive organism and the minimum was against *Pseudomonas aeruginosa*, a Gram-negative organism. This indicates that Allicin has the potential of a broad spectrum of activity against both Gram-positive and Gram-negative

bacteria. But we can see the variation in the size of the inhibition zone among the different group of bacteria. This may be due to the lipid content of the membranes of the different groups of the microorganisms and the permeability of allicin and other garlic constituents. There are a greater number of studies showing antimicrobial activity of garlic against bacteria, fungi, virus and human intestinal protozoan parasites. The antibacterial activity of garlic is widely attributed to allicin. Allicin interferes with RNA production and lipid synthesis. If RNA cannot be produced, or produced in less amount then protein synthesis will be severely affected. It would be stopped at every stage due to the absence of messenger RNA, ribosomal RNA and transfer RNA. If amino acids and proteins cannot be produced then growth and development of the organism will not occur as they are essential for all parts of cell structure. Also, as lipid synthesis is affected, other parts of the cell are interfered with. The main effect was that the Phospholipids Bilayer of the cell wall cannot form correctly in both Gram positive and Gram negative bacteria. All these things contribute to the bacteria cannot grow in the presence of allicin. The 300 µg/ml Allicin showed a higher inhibition zone, when compared to the activity with the commercially used antibiotic i.e. Azithromycin. Antibiotics were used for therapy but many of the pathogenic bacteria are resistant. The Gram positive organism *Staphylococcus aureus* was more sensitive than other bacteria tested. Our results are aqueous extracts of garlic to possess potent bacteriostatic principle against many bacteria at varying concentrations.

DISCUSSION:

Garlic has been used through history for both culinary and medicinal purposes. The Garlic plant bulb is the most commonly used part of plant. The extract was found to contain the compound allicin which is confirmed by TLC method. The *Allium sativum* contains allicin, allin shows antibiotic enhancing activity. The *Allium sativum* contains Allicin, shows antibiotic enhancing activity by using cup plate agar diffusion method using different concentrations like 100 µg/ml, 200 µg/ml, 300 µg/ml against Gram positive and Gram negative organisms like *B. subtilis*, *Staphylococcus aureus* and *E. coli*, *Pseudomonas aeruginosa*. Combination of antibiotic and aqueous extract of Allicin indicates that *Allium sativum* has tremendous potential to enhancing effect of antibiotic.

CONCLUSION:

From this study and the earlier reports it is clear that garlic appears to satisfy all of the criteria for antibacterial agents, being cheap and safe. Since the introduction of antibiotics there has been tremendous increase in the resistance of many bacterial pathogens. Scientists advance in their search for new bacterial targets to attack bacteria evolve and as a result a large number of bacterial species have become resistant to antibacterial drugs. Hence, search for new antimicrobials is very important in recent times. Because garlic is known to act synergistically with antibiotics and resistance has not been reported for garlic more dose-response preclinical studies and eventually clinical studies should be done to assess the use of an antibiotic-garlic combination for bacteria that are difficult to eradicate. In view of the strong antibiotic properties and the complete absence of development of resistance further investigation upon the principles of the antimicrobial activity of juices from *Allium* species merits consideration. From this study it was

concluded that allicin used as new source for antimicrobial drug. It is necessary to do further research on molecular level to improve formulation of this combination therapy which will definitely give promising results.

ACKNOWLEDGEMENTS:

The author would like to convey his sincere gratitude to his colleague Mr. sunil hindole for his kind support and necessary help to make the original research paper. He is thankful to his Principal Dr. R. B. Jadhav for his advice and valuable suggestions. Finally He would like to thank all laboratory staffs of Arvind Gavali College of Pharmacy, Jaitapur, Satara for their continuous help regarding this work.

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