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Pharmacological Study

Antimicrobial activity of fresh garlic juice: An *in vitro* study

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Abstract

Introduction: Antimicrobial resistance has been a global concern. Currently, interest has been focused on exploring antimicrobial properties of plants and herbs. One such botanical is *Allium sativum* (garlic). **Aim:** To evaluate the antimicrobial activity of fresh juice of garlic. **Materials and Methods:** Varying concentrations of fresh garlic juice (FGJ) were tested for their antimicrobial activity against common pathogenic organisms isolated at SSG Hospital, Vadodara, using well diffusion method. Moreover, minimum inhibitory concentration (MIC) and minimum lethal concentration (MLC) of FGJ were tested using broth dilution method. Sensitivity pattern of the conventional antimicrobials against common pathogenic bacteria was tested using disc diffusion method. **Results:** FGJ produced dose-dependent increase in the zone of inhibition at a concentration of 10% and higher. MIC of FGJ against the pathogens ranged from 4% to 16% v/v whereas MLC value ranged from 4% to 32% v/v with *Escherichia coli* and *Staphylococcus aureus* spp. showed highest sensitivity. **Conclusion:** FGJ has definite antimicrobial activity against common pathogenic organisms isolated at SSG Hospital, Vadodara. Further studies are needed to find out the efficacy, safety, and kinetic data of its active ingredients.

Key words: *Allium sativum*, antimicrobial, fresh juice of garlic, minimum inhibitory concentration, minimum lethal concentration, zone of inhibition

Introduction

Plants as a source of medicinal compound have continued to play a vital role in the maintenance of human health since ancient times. One such botanical is garlic. Garlic (*Allium sativum* L.) belongs to the *Liliaceae* family and falls within the group of onion. The bulb and cloves of the garlic are the most commonly used parts of the plants for medicinal purpose. It has a typical pungent odor, and antibacterial activity depends on the sulfur-containing compounds, the major one being allicin, which is produced by enzymatic (alliin lyase) hydrolysis of allicin after cutting and crushing of the cloves.[1,2] Throughout history, many different cultures have recognized the potential use of garlic for prevention and treatment of different diseases. Recent researches have focused on four main areas: Heart disease, cancer, infectious disease, and antioxidant effects. Indeed, garlic is found to have antihypertensive,[3,4] antioxidant,[5] antiplatelet,[6] antitumor,[7,8] and lipid-lowering actions.[9,10] Moreover, a recent study also points out towards the antimicrobial action of garlic.[11-13]

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The objectives of the present study were to evaluate the antibacterial activity of fresh garlic juice (FGJ) using *in vitro* techniques, against common pathogenic bacteria (*Escherichia coli*, *Proteus mirabilis*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*).

Materials and Methods

Preparation of fresh garlic juice

Fresh garlic was purchased from local market. Garlic bulbs were peeled, weighed 100 g, and cleaned. Clean cloves were crushed in sterile mortar and pestle and then the mixture was filtered through a sterile cheese cloth. This filtrate was considered 100% fresh garlic extract,[14] was stored at −20°C, and was thawed before use. Every week, FGJ was prepared.

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Ten grams of raw garlic yielded 4 ml of juice (i.e., 2.5 g/ml). Each time 100% garlic juice (undiluted) was inoculated on nutrient agar media and incubated at 37°C overnight and was found to be sterile.

The concentrated (100%) juice was further diluted to 10–25%, 50% by mixing with appropriate sterile distilled water.[15]

Sample collection

Twenty-five samples each of *E. coli*, *K. pneumoniae*, *P. mirabilis*, *P. aeruginosa*, and *S. aureus* were collected from the clinical specimens of urine, stool, pus, and cerebrospinal fluid sent to the microbiology laboratory for culture and sensitivity testing.

Isolation of pure culture

A nichrome inoculating loop was used to inoculate the infective material on nutrient agar. The inoculum was spread into four quadrants of the nutrient agar plate with the help of a nichrome inoculation loop. The loop was sterilized between each successive quadrant streak. The purpose of this technique was to dilute the inoculum sufficiently on the surface of agar medium so that well-defined colonies of bacteria can be obtained. The isolated colonies were sub-cultured individually to obtain pure culture isolates. The pure colonies thus obtained were picked up for confirmatory biochemical tests. Standard biochemical tests were performed and isolated organisms were confirmed.

Preparation of standard inoculum

Pure colonies were picked up with the help of a straight wire and emulsified in a test tube containing saline, and the turbidity was matched with 0.5% McFarland standard containing 1×10^7 CFU/ml approximately.

Well diffusion method

Mueller Hinton Agar (MHA) was poured in plates of 9–10 cm; depth of agar was 3–4 mm. With sterile cotton swab, the test culture was spread evenly over the plate successively in three directions to obtain an even inoculum. The plate was allowed to dry for 3–5 min. Wells of 5 mm diameter were cut on the surface of the agar. Thirty microliter of 5%, 10%, 25%, 50%, and 100% solutions (v/v) of FGJ was added to different wells and in one well, normal saline was added. The plates were incubated at 37°C for 24 h. The zone of inhibition was measured by a scale to the nearest mm including disc diameter.[16]

Broth dilution method

Serial dilutions of FGJ were prepared in Mueller-Hinton broth so that each tube contains 1 ml of diluted garlic juice. One milliliter of standard inoculum of the microorganism was added to each concentration of garlic juice and to a tube of the growth medium without antimicrobial agent, which serves as a growth control. An uninoculated tube of the medium was incubated to serve as a negative growth control. After 24 h of incubation, the tubes were examined for turbidity, indicating growth of the microorganisms. The lowest concentration of the FGJ that inhibits growth of the organism was designated the minimum inhibitory concentration (MIC).[17,18] A loopful of inoculum from each of the tubes of broth was subcultured to solid agar plates that were divided into six sections. The lowest concentration of FGJ that allowed <0.1% of the original inoculum to survive was considered to be the minimum lethal concentration (MLC).[16,17]

Disc diffusion method

The surface of the MHA plate was inoculated with the test organisms. Inoculum was standardized by matching the turbidity with 0.5% McFarland standard and then with a sterile cotton swab, the test culture was spread evenly over the plate successively in three directions to obtain an even inoculum. The plate was allowed to dry for 3–5 min. Commercially available readymade antibiotic discs (Himedia Laboratories, Mumbai Maharashtra) were placed on the surface. The plate was incubated overnight at 37°C and the zone of inhibition was measured.[18]

Statistical analysis

One-way ANOVA followed by Tukey's multiple comparison tests was applied for comparison between two mean values as a measure of test of significance. MLC and MIC values are expressed as mean ± standard error of the mean. $P < 0.05$ was considered as statistically significant.

Results

FGJ was tested against common bacterial pathogens using agar well diffusion method, and results of the zone of inhibition at different concentrations of FGJ are presented in Table 1.

All pathogenic bacteria showed statistically significant dose-dependent increase in the zone of inhibition at FGJ concentration of 10% and higher compared to control. Ten percentage of FGJ showed ≥10 mm zone of inhibition in *E. coli* and *S. aureus*, while 25% concentration of garlic juice showed >15 mm of the zone of inhibition in *S. aureus* [Figure 1], *P. mirabalis* [Figure 2] *and E. coli*. Garlic juice at 100% concentration produced >20 mm of the zone of inhibition for all the bacteria excluding *P. aeruginosa*.

Table 1: Zone of inhibition at different concentration of fresh garlic juice for different pathogenic bacteria using well diffusion method

Bacteria (*n*=25)	Zone of inhibition (mm)						
	Control	5%	10%	25%	50%	100%	*F*
Escherichia coli	5.0±0	6.28±0.73	10.96±0.57*	15.52±0.60*	20.93±0.70*	26.44±0.72*	191.20
Klebsiella pneumoniae	5.0±0	5.0±0	6.04±0.21	9.88±0.43*	14.44±0.60*	20.68±0.79*	197.14
Proteus mirabilis	5.0±0	5.0±0	8.80±0.21*	16.12±0.43*	21.72±0.62*	28.08±0.64*	529.38
Pseudomonas aeruginosa	5.0±0	5.0±0	5.64±0.36	5.68±0.23	8.96±0.28*	12.48±0.36*	139.41
Staphylococcus aureus	5.0±0	6.72±0.55	9.80±0.47*	15.56±0.53*	21.16±0.53*	26.44±0.57*	306.37

Data: Mean±SEM, *P<0.001 versus control value using one-way ANOVA and Turkey's multiple comparison tests df=5; 144. SEM: Standard error of mean

MIC and MLC of the FGJ for the organisms were determined using broth dilution method [Table 2]. MIC value of FGJ for all the bacteria excluding *P. auregenosa* ranged from 4% to 8% v/v whereas MIC value of FGJ for *P. auregenosa* ranged from 4% to 16% v/v.

MLC value for *S. aureus* and *E. coli* [Figure 3] ranged from 4% to 8% v/v while that of *P. mirabilis* and *K. pneumoniae* [Figure 4] ranged from 8% to 16% v/v whereas MLC value for *P. aeruginosa* ranged from 16% to 32% v/v.

The results suggest that FGJ exhibits higher activity against *S. aureus* and *E. coli*. Since *S. aureus* is reported to be the major pathogen resistant toward most antibiotics, hence may receive special attention from the researchers.

Discussion

Since 1940s, the development of effective and safe drugs to deal with bacterial infections has revolutionized medical treatment, and the morbidity and mortality from microbial disease have been dramatically reduced. Unfortunately, the development of effective antibacterial drugs has been accompanied by the emergence of drug-resistant organisms. The phenomenon of resistance imposes serious constraints on the options available for the medical treatment of many bacterial infections.

In the present study, almost all the 25 different strains of all the pathogenic bacteria were resistance to nalidixic acid and ceftazidime, while they were mildly sensitive to cefotaxime, moderately sensitive to nitrofurantoin and norfloxacin, and were highly sensitive to netilmicin, ciprofloxacin, and ofloxacin. Thus, only three antimicrobial agents out of seven studied

Table 2: Minimum inhibitory concentration and minimum lethal concentration of fresh garlic juice for different pathogenic bacteria

Bacteria (*n*=25)	MIC % (v/v)	MLC % (v/v)
Escherichia coli	4-8	4-8
Staphylococcus aureus	4-8	4-8
Klebsiella pneumonia	4-8	8-16
Proteus mirabilis	4-8	8-16
Pseudomonas aeruginosa	4-16	16-32

MIC: Minimum inhibitory concentration, MLC: Minimum lethal concentration

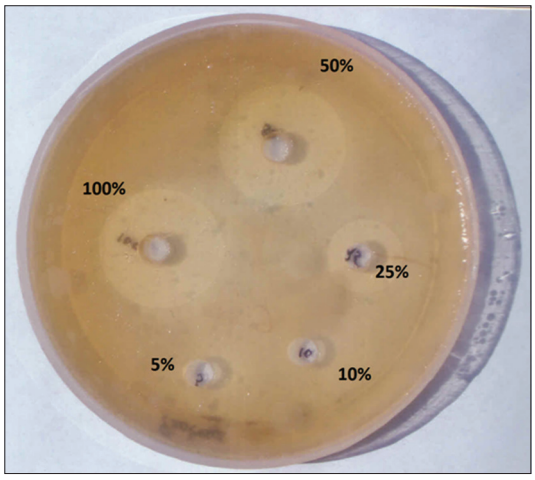

Figure 1: Zone of inhibition at different concentrations of fresh garlic juice for *Staphylococcus aureus* using well diffusion method

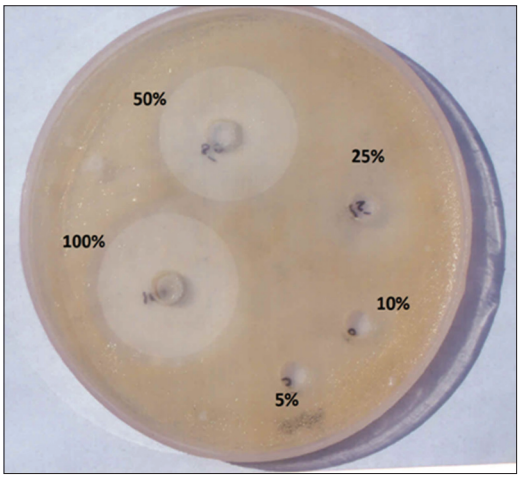

Figure 2: Zone of inhibition at different concentrations of fresh garlic juice for *Proteus* using well diffusion method

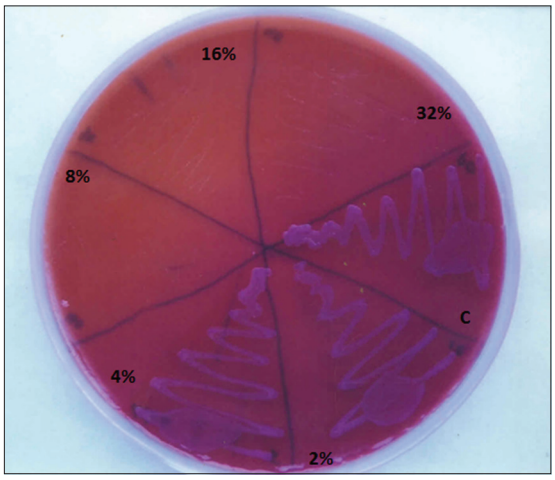

Figure 3: Minimum lethal concentration of fresh garlic juice for *Escherichia coli*

Figure 4: Minimum lethal concentration of fresh garlic juice for *Klebsiella*

showed high antimicrobial sensitivity against the most common pathogenic bacteria.

The present study has demonstrated that FGJ effectively inhibited the growth of all the five bacterial pathogens tested, namely *E. coli*, *K. pneumoniae*, *P. mirabilis*, *P. aeruginosa*, and *S. aureus*, though their sensitivity to the FGJ varied. The MIC values were found to be in the range of 4–16% v/v [Table 2].

In previous studies,[19] allicin exhibits its antimicrobial activity mainly by immediate and total inhibition of RNA synthesis, although DNA and protein syntheses are also partially inhibited, suggesting that RNA is the primary target of allicin action. The structural differences of the bacterial strains may also play a role in the bacterial susceptibility to garlic constituents. Apart from antimicrobial action, garlic is found to have antifungal activity.[20]

It is clear that FGJ may be useful as an antimicrobial agent against the above-mentioned pathogens. The present study suggests that garlic is active against organisms that are found to be resistant to conventional antibiotics, which is in agreement to that reported in previous studies.[21,22] Moreover, studies also indicate that combination of garlic extracts with conventional antimicrobials leads to partial or total synergism.[23,24]

Conclusion

This preliminary screening study suggested that garlic used in traditional medicine have potentials as antibacterial agents for a variety of Gram-positive as well as Gram-negative organisms. Further detailed studies are needed to evaluate the possibility of the use of garlic as an antibacterial agent alone or in combination with conventional antibacterial.

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Conflicts of interest
There are no conflicts of interest.

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हिन्दी सारांश

लहसुन स्वरस का रोगाणुरोधी गतिविधि का मूल्यांकन

सीमा यादव, नियति ए. त्रिवेदी, जगत डी. भट्ट

रोगाणुरोधी प्रतिरोध एक वैश्विक चिंता का विषय रहा है। वर्तमान में, पौधों और जड़ी बूटियों के रोगाणुरोधी क्षमता की खोज पर ध्यान केंद्रित किया गया है। ऐसा ही एक वनस्पति लहसुन है। लहसुन के ताजे रस का रोगाणुरोधी गतिविधि का मूल्यांकन करने के लिए ताजे रस के अलग अलग सांद्रता में आम रोगजनक जीवाणुओं के खिलाफ उनके रोगाणुरोधी गतिविधि के लिए एस.एस.जी. अस्पताल, वडोदरा में वेल डिफ्युजन विधि के द्वारा परीक्षण किया गया। इसके अलावा, लहसुन स्वरस की न्यूनतम निरोधात्मक सान्द्रता (एम.आई.सी.) और न्यूनतम घातक सान्द्रता (एम.एल.सी.) का ब्रोथ डायल्युशन विधि का उपयोग कर परीक्षण किया गया। आम रोगजनक जीवाणुओं के खिलाफ पारंपरिक रोगाणुरोधी की संवेदनशीलता पैटर्न का डिस्क प्रसार विधि का उपयोग कर परीक्षण किया गया। लहसुन स्वरस के १०% और अधिक सांद्रता में वृद्धि के साथ निषेध के क्षेत्र में वृद्धि देखी गई। रोगजनकों के खिलाफ लहसुन स्वरस की एम.आई.सी. ४–१६%व्ही/व्ही पायी गयी, जबकि एम.एल.सी. मूल्य ४–३२% व्ही/व्ही पायी गयी। ई. कोलाई और एस. ऑरियस के साथ सबसे अधिक संवेदनशीलता पायी गयी। लहसुन का ताजा रस आम रोगजनक जीवाणुओं के खिलाफ निश्चित रोगाणुरोधी है। भविष्य के अध्ययनों मे इसके सक्रिय संघटक की क्षमता, सुरक्षा और बलगतिकी का पता लगाने की जरूरत है।