

Research Article

Efficacy of antibacterial activity of garlic cloves from Tamil Nadu and Jowai region

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ABSTRACT

Background: The development of antibiotic resistance has become a global health challenge which is causing ineffectiveness of the available antibacterial agents leading to increase in diseases and death rate. Therefore this study intends to investigate the antibacterial action of Aqueous Garlic Extract (AGE) against 9 multidrug-resistant gram-positive and gram-negative bacterial isolates, including *Staphylococcus aureus*, *Enterococcus* species, β hemolytic streptococcus, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Klebsiella pneumoniae*, *Escherichia coli*, *Vibrio cholerae* and *Serratia marcescens*.

Methods: Antibacterial activity of different concentrations of AGE by well-diffusion method was recorded by measuring the diameter of zone of inhibition. The Tamil Nadu garlic cloves as well as Jowai region garlic cloves showed antibacterial activity against both GPC and GNR.

Results: The maximum zone of inhibition was observed in Tamil Nadu garlic than that of Jowai region, but the only bacteria which showed a better zone of inhibition with Jowai region than Tamil Nadu garlic was *Pseudomonas aeruginosa*.

Conclusion: Thus our study reveals that garlic not only makes the food more spicy & edible with its flavour but can also be used as an effective antibacterial agents against MDR gram positive & gram negative bacteria.

Keywords: Bacterial isolates, Antibacterial activity, Tamil Nadu garlic and Jowai region garlic extracts

INTRODUCTION

The microbial infections are the major cause of morbidity and mortality in the developed and developing country, although a number of antimicrobial agents are available for the treatment and management of infectious diseases. In addition, misuse of the antibiotics which can lead to the development of antibiotic resistance is also a major health concern.¹ Therefore, there is a perpetual need to exploit new bioactive principles with high safety index.

Historically, medicinal plants have been a source of novel drug compounds. Plants derived products have made

large contributions to human health and well-being. Green pharmacy may become the base for the development of medicines by providing a pharmacophore which could be used for the development of new drug with novel mechanisms of action.² A number of medicinal plants have been screened for the antimicrobial activity in recent years³ and efforts has been taken to identify their active constituents.⁴

Garlic is one of the edible plants which have generated a lot of interest throughout human history as a medicinal panacea. A wide range of microorganisms including bacteria, fungi, protozoa and viruses has been shown to

be sensitive to crushed garlic preparations. Moreover, garlic has been reported to reduce blood lipids and has anti-cancer effects. Chemical analyses of garlic cloves have revealed an unusual concentration of sulphur-containing compounds (1–3%).^{5,6} A daily dose of 1 ml/kg body weight of garlic extract for six months can result in significant reduction in oxidant (free radical) stress in the blood of patients with atherosclerosis and cholesterol circulating in the bloodstream.

Garlic (*Allium sativum*) is a common spice used for flavoring and has been traditionally popular with strong folkloric awareness. It is the edible bulb of lily family, *Liliaceae*. It contains aromatic sulphur based compounds, which contribute to the characteristic odour and taste. Antimicrobial activity of garlic is attributed to its key component allicin, which is a volatile & active ingredient; it acts by partial inhibition of DNA and protein synthesis and also total inhibition of RNA synthesis as a primary target.⁷

The main advantage of using the herbal antimicrobial drug is that the side effects are reduced: The side effects mainly include damage to the normal intestinal flora, bone marrow depression, dysentery, local inflammation and damage to liver.⁸

The present study was aimed at determining the *in vitro* antibacterial activity between TN and Jowai region garlic clove extracts on the isolates of gram-positive and gram-negative organisms with the view to find an alternative means of treating infections caused by them.

METHODS

Microorganism

9 different strains of gram positive & gram negative organisms: *Staphylococcus aureus*, *Enterococcus* species, β hemolytic streptococcus, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Klebsiella pneumoniae*, *Escherichia coli*, *Vibrio cholerae* and *Serratia marscesnes* cultures were collected from our hospital Shri Sathya Sai Medical College And Research Institute, Thiruporur. The cultures were streaked onto individual agar plates and incubated at 37°C for 24 h.

Preparation of aqueous garlic extract (AGE)

The Tamil Nadu garlic cloves were obtained from the local market & Jowai region garlic cloves were obtained from the North East region. The spices were cleaned and washed using sterile distilled water. 1 gram of both types of garlic was homogenized using a sterile blender and the extract was sieved using sterile cheese cloth. The concentrations of 100 μ L, 50 μ L and 25 μ L were prepared by mixing with appropriate volumes of sterile distilled water.⁹

Antibacterial activity

The antibacterial properties of both Tamil Nadu and Jowai garlic cloves were studied by agar well diffusion method and disk diffusion methods.¹⁰

Agar well diffusion method

The selected bacterial strains were inoculated into 10 ml of sterile nutrient broth and incubated at 37°C for 16-18 h. Using a sterile cotton swab, the nutrient broth cultures was swabbed on the surface of sterile agar plates. Agar wells were prepared with the help of sterilized cork borer with 6 mm diameter. Using a micropipette, concentrations of 100 μ L, 50 μ L, and 25 μ L solution of garlic were added to different wells in the plates. The plates were incubated in an upright position at 37°C for 24 h. The diameters of inhibition zone (in mm) were measured.

Antibiotic sensitivity testing

The microorganisms were also tested for their sensitivity against the antibiotics ciprofloxacin, vancomycin, gentamicin, linezolid, amoxiclav by disc diffusion method. The cultures of test microorganism were enriched in sterile nutrient broth for 16 h at 37°C. Sterile cotton swabs were used to transfer the test cultures aseptically on the surface of sterile nutrient agar plates. Flamed forceps was used to place the antibiotic disc aseptically over the seeded agar plates. The plates were incubated at 37°C for 24 h and the diameters of inhibition zones were measured. Antimicrobial susceptibility was tested by the disk diffusion method according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI, 2007).¹¹

RESULTS

The antibacterial activity of Tamil Nadu garlic was compared with the antibacterial activity of Jowai region garlic with 3 GPC and 6 GNR which are listed in Table 1.

Table 1: Comparison of the antibacterial activity of Tamil Nadu garlic with Jowai region garlic with 3 GPC and 6 GNR.

GNR	GPC
<i>Pseudomonas aeruginosa</i>	<i>Staphylococcus aureus</i>
<i>Proteus mirabilis</i>	<i>Enterococcus species</i>
<i>Klebsiella pneumoniae</i>	β hemolytic streptococcus
<i>Escherichia coli</i>	
<i>Vibrio cholerae</i>	
<i>Serratia marscesnes</i>	

The Tamil Nadu garlic as well as Jowai region garlic showed antibacterial activity against both GPC and GNR. All the 3 GPC exhibited high sensitivity zone with 100 μ L of Tamil Nadu garlic (Table 3) along with *Vibrio cholerae* which is GNR (Table 2).

Table 2: Antibacterial activity of Tamil Nadu and Jowai region garlic - gram negative bacilli.

Organisms isolated	Tamil Nadu garlic zone of inhibition (mm)				Jowai region garlic zone of inhibition (mm)			
	Negative control	100µL	50 µL	25 µL	Negative control	100µL	50 µL	25 µL
<i>Pseudomonas aeruginosa</i>	NZ	20mm	18mm	16mm	NZ	25mm	20mm	19mm
<i>Proteus mirabilis</i>	NZ	28mm	25mm	20mm	NZ	20mm	17mm	15mm
<i>Klebsiella pneumoniae</i>	NZ	30mm	22mm	20mm	NZ	16mm	15mm	14mm
<i>Escherichia coli</i>	NZ	28mm	26mm	25mm	NZ	21mm	20mm	19mm
<i>Vibrio cholerae</i>	NZ	32mm	30mm	29mm	NZ	23mm	16mm	15mm
<i>Serratia marscesnes</i>	NZ	26mm	24mm	20mm	NZ	18mm	16mm	15mm

NZ - No zone

Table 3: Antibacterial activity of Tamil Nadu and Jowai region garlic - gram positive cocci.

Organisms isolated	Tamil Nadu garlic zone of inhibition (mm)				Jowai region garlic zone of inhibition (mm)			
	Negative control	100µL	50 µL	25 µL	Negative control	100µL	50 µL	25 µL
<i>Staphylococcus aureus</i>	NZ	32mm	30mm	20mm	NZ	25mm	23mm	20mm
<i>Enterococcus species</i>	NZ	30mm	23mm	20mm	NZ	25mm	20mm	15mm
<i>β hemolytic streptococcus</i>	NZ	27mm	26mm	25mm	NZ	24mm	18mm	16mm

NZ - No zone

The 50 µL extract of Tamil Nadu garlic zone showed a better zone in both GPC and GNB isolates but maximum diameter was obtained in *Staphylococcus aureus* and *V. cholerae*-30mm, followed by *β hemolytic streptococcus* and *Escherichia coli*-26mm.

The 25 µL concentration of the garlic gave best results for *V. cholerae*-29mm, followed by *β hemolytic streptococcus* and *Escherichia coli*-25mm.

These zones are less than the results obtained in the previous study: In vitro antibacterial activity and sensitivity of garlic extract at different pH and temperature¹² for *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. But the 50 µL and 25 µL concentration showed a better result in our study, when compared to the previous study which are listed below in Table 4.

Table 4: Antibacterial activity of previous study.

Test organisms	Diameter of inhibition zone (mm) of different concentrations			
	100%	50%	25%	10%
<i>Escherichia coli</i>	33	16	10	0
<i>Klebsiella pneumoniae</i>	39	21	10	0
<i>Pseudomonas aeruginosa</i>	25	12	7	0
<i>Staphylococcus aureus</i>	33	16	8	0

The Jowai region garlic zone showed a strong positive zone of inhibition of 25mm in GPC namely *Staphylococcus aureus*, *Enterococcus species* where as GNB like *Escherichia coli* showed 21mm, followed by 24mm in *β hemolytic streptococcus* and *V. cholerae*, exhibiting 23mm while *Pseudomonas aeruginosa* had 25mm with 100 µL extract of Jowai region garlic.

Jowai region garlic showed 20mm zone of diameter with other Gram negative bacteria like *Proteus mirabilis*, *Serratia marscesnes* had 18mm and 16mm with *Klebsiella pneumoniae* in 100 µL (Figure 4). The lowest diameter of 14mm was seen in *Klebsiella pneumoniae* for 25 µL concentration of Jowai region garlic.

The results obtained from our study, clearly showed that all the bacterial strains which are resistant to antibiotics (Ciprofloxacin, vancomycin, gentamicin, linezolid, amoxiclav) were highly susceptible to garlic clove extracts. It is evident that the Tamil Nadu and Jowai region garlic clove extracts showed better and broader spectrum activity when compared to the antibiotics (Figure 2).

**Figure 1: Showing Tamil Nadu and Jowai region garlic.**



Figure 2: Kirby Bauer's disk diffusion technique with conventional antibiotic disc, showing with resistance to GNB & GPC.

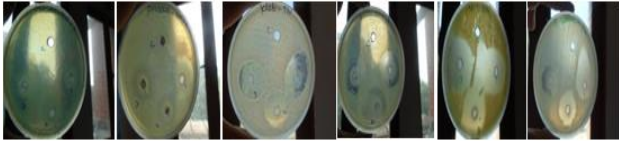


Figure 3: Antibacterial activity of Tamil Nadu garlic by well diffusion method for GNB.



Figure 4: Antibacterial activity of Jowai region garlic by well diffusion method for GNB.

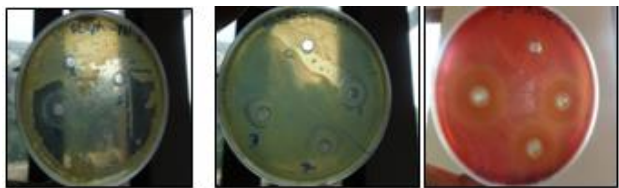


Figure 5: Antibacterial activity of Tamil Nadu garlic by well diffusion method for GPC.

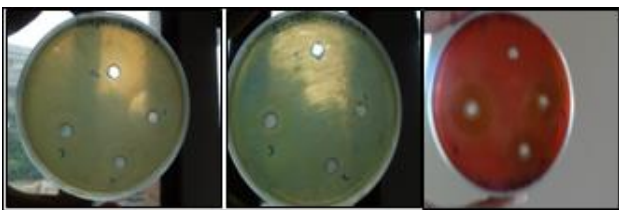


Figure 6: Antibacterial activity of Jowai region garlic by well diffusion method for GPC.

DISCUSSION

The maximum zone of inhibition with Tamil Nadu garlic was observed in GNR, *V. cholerae* in all the three concentrations (100 μ L, 50 μ L, 25 μ L) (Figure 3). In the GPC, *Staphylococcus aureus* exhibited maximum diameter with 32mm, 30mm, 20mm with 100 μ L, 50 μ L, 25 μ L concentrations (Figure 5).

The Jowai region garlic extract had its maximum zone of inhibition with all the gram positive cocci: *Staphylococcus aureus*, *Enterococcus* species and β hemolytic streptococcus (Table 3) (Figure 6).

Among the gram negative bacilli again maximum diameter was seen with 100 μ L of Jowai region garlic extract in *Vibrio cholerae* which had 23mm, and *Pseudomonas aeruginosa* 25mm. These two GNR exhibited the maximum diameter zone (Table 2) (Figure 4) followed by *Staphylococcus aureus*, *Enterococcus faecalis* which showed 25mm in 100 μ L (Table 3). The only bacteria which showed a better zone of inhibition with Jowai region than Tamil Nadu garlic was *Pseudomonas aeruginosa* (Table 2).

CONCLUSION

A total number of 9 isolates were processed for susceptibility testing with aqueous extraction of Tamil Nadu and Jowai region garlic cloves, with the concentration of 1 gram by well diffusion technique as per standard protocol. It was found out that maximum zone of inhibition was observed in *Staphylococcus aureus* and *Vibrio cholerae* with Tamil Nadu garlic & Jowai region garlic cloves.

Our study is the first of its kind to analyze the antibacterial activity of the Jowai region garlic cloves which has been proved to have a strong sensitive pattern against both Gram positive & Gram negative bacteria as shown in our analysis. Therefore the effectiveness of either the Tamil Nadu or Jowai region garlic cloves is proved beyond doubt that it not only has edible quality in preparing mouth-watering spicy food but also antibacterial activity against both GPC & GNB.

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