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ANTIMICROBIAL POTENTIAL OF GARLIC EXTRACT- A NEW APPROACH TO COMBAT CLINICAL PATHOGENS

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ABSTRACT

Garlic (*Allium sativum*) has been used for a long time as a spice or traditional medicine. Therefore, aim of the present research work was to evaluate antimicrobial potential of Garlic extract to combat clinical pathogens. Antimicrobial activity of Garlic was evaluated against clinical pathogens such as *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa* and *Salmonella typhi.* Antibiotic susceptibility profile showed that except two strains of *Staphylococcus aureus* (resistant to Tetracycline) all other clinical pathogens were found to be sensitive to all tested antibiotics. Garlic extract was found to be effective against all clinical pathogens studied. Phytochemical analysis showed that Tannins, Saponins, Flavonoids, Steroids, Reducing sugars, Terpenoids and Phlobatannins were found to be present in garlic extract.

KEYWORDS: Garlic Antimicrobial Activity.

INTRODUCTION

The use of plants in medicine goes as far back as thousands of years and still continues today. Garlic (*Allium sativum*) is one of the edible plants which have generated a lot of interest throughout human history as a medicine. Garlic has been consumed as a spice and medicine for thousands of years (Arora and Kaur, 1999; Cavallito and Bailey, 1944). It has a higher concentration of sulfur compounds than any other *Allium* species which are responsible both for garlic's pungent odor and many of its medicinal effects. One of the most biologically active compounds in garlic is allicin (Lawson and Bauer, 1998).

The antibacterial properties of crushed garlic have been known for a long time. Various garlic preparations have been shown to exhibit a wide spectrum of antibacterial activity against

Gram-negative and Gram-positive bacteria including species of *Escherichia, Salmonella, Staphylococcus, Streptococcus, Klebsiella, Proteus, Bacillus*, and *Clostridium.* Even acid-fast bacteria such as *Mycobacterium tuberculosis* are sensitive to garlic (Uchida et al., 1975). Garlic extracts are also effective against *Helicobacter pylori,* the cause of gastric ulcers (Cellini et al., 1996). Therefore, aim of the present research work was to evaluate antimicrobial potential of garlic extract to combat clinical pathogens.

MATERIALS AND METHODS

I. Aqueous Garlic (*Allium sativum*) Extract (AGE) preparation

Fresh garlic bulbs were purchased from market. Then the garlic bulbs were peeled, weighted (100gm), and cleaned garlic were taken and surface sterilized using ethanol. The ethanol was allowed to evaporate in a sterile laminar air flow chamber, and the garlic was homogenized aseptically using a sterile mortar and pestle. The homogenized mixture was filtered through sterile cheesecloth (French et al., 2005; Patel et al., 2011).

II. Test organisms

Total 4 types of clinical bacterial pathogens (n=3 each) were collected from Pathology Laboratory at Nagpur such as *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa* and *Salmonella typhi* and identified on the basis of morphological, cultural and biochemical characteristics (Collee and Marr, 1996).

III. Antibiotic sensitivity test

Antibiotic sensitivity test was performed by Kirby Bauer Disc Diffusion method (Bauer *et al*., 1966). Six different types of antibiotics were used in the study (Table 1). Strains of clinical pathogens were grown on nutrient agar at 37^0C for 24 hours and the colonies were suspended in sterile saline water equivalent to a 0.5McFarland standard (1.5X108CFU/ml). Hi-sensitivity agar plate was uniformly seeded by adding 100µl inoculated broth and was spread by means of spreader. The discs were placed on each inoculated Hi-sensitivity agar plate. The plates were incubated at 37^0C for 18 hours. The diameter of the zone of inhibition was observed in mm and the isolates were classified as “resistant” or “sensitive” based on the standard interpretative chart according to Clinical and Laboratory Standards Institute (CLSI) guidelines (Ross et al., 2001; CLSI, 2007).

IV. Antibacterial activity of Garlic extract against clinical pathogens

Antibacterial activity of Garlic extract was performed by well diffusion technique: Strains of clinical pathogens were grown overnight on nutrient agar at 37^0C, and the colonies were suspended in sterile saline water equivalent to a 0.5 McFarland standard (1.5×108 CFU/ml). The suspension (100 µL) was spread over the Hi-Sensitivity agar. The wells of 6 mm diameter were cut into the agar medium with a sterilized cork borer. Then 20µl each of the extracts were added separately into the separate wells. The plates were incubated at 37^0C for 18 hours. The diameter of the zone of inhibition around each well was measured and recorded (Jonkers, et al., 1999; Patel et al., 2011).

Table 1: Antibiotics used in the study.

Antibiotics	Abbreviation	Concentration
Amikacin	AK	30mcg
Ciprofloxacin	CIP	10mcg
Gentamicin	GEN	30mcg
Levofloxacin	LE	5mcg
Norloxacin	NX	10mcg
Tetracycline	TE	30mcg

IV. Phytochemical screening of Garlic (*Allium sativum*)

For the phytochemical screening of Garlic following tests were performed (Doss, 2009).

a. **Test for Tannins:-** A 1ml of garlic extract was treated with few drops of 1% ferric chloride and observed for brownish green or a blue-black coloration which shows presence of tannins.

b. **Test for Saponins:-** About 2 ml of sodium bicarbonate (1%) was added to 1 ml of garlic extract and shaked. Lather like formation remains constant for some time is indicative of the presence of saponins.

c. **Test for Flavonoids:** The 5 ml of garlic extract was taken, to which 1 ml of sodium hydroxide solution (10%) was added. Added two drops of concentrated hydrochloric acid at the side of the beaker. The yellow color changes to colorless which shows the presence of flavonoids.

d. **Test for Steroids:** About 100 µl fresh garlic extract was taken in a test tube and 400 µl of acetic anhydride was added. Then, added 1 or 2 drops of concentrated sulfuric acid. Brown ring at the boundary of mixture shows the presence of steroids.

e. **Test for Reducing Sugars:** About 1ml of garlic extract was mixed with mixture of 1ml of Fehling's solution A and B. The solution was kept in water bath at 60^0C. The presence of reducing sugars was indicated by formation of brick red precipitation.

f. **Test for Terpenoids:** About 2 ml of the garlic extract was taken. Then dissolved in 2 ml of chloroform and let it evaporated to dryness. Add 2 ml of concentrated sulfuric acid and heated for about 2 minutes. Development of a grayish color indicates the presence of terpenoids.

g. **Test for Phlobatannins:** About 2 ml of garlic extract was taken and 1% aq. HCl was added to it followed by boiling. Formation of red colored precipitate indicates the presence of phlobatannins.

RESULTS AND DISSCUSSION

The present study was conducted to study antimicrobial activity of garlic extract against clinical pathogens such as *S. aureus, E.coli, P. aeruginosa* and *S. typhi*. Antibiotic susceptibility profile was also carried out against 6 different antibiotics (Table 1). Antibiotic susceptibility profile showed that except two strains of *Staphylococcus aureus* (resistant to Tetracycline) all other clinical pathogens were found to be sensitive to all tested antibiotics (Table 2). Garlic extract was found to be effective against all clinical pathogens studied (Table 2). Arora and Kaur (1999) observed a significant bactericidal effect of garlic extract against *Staphylococcus epidermidis*, *Salmonella typhi* and various yeasts. It was reported that aqueous extract of garlic inhibited the growth of enteric pathogens; *E. coli, S. typhi and S. flexneri* at low concentrations (Shobana et al., 2009).

Phytochemical analysis showed that Tannins, Saponins, Flavonoids, Steroids, Reducing sugars, Terpenoids and Phlobatannins were found to be present in garlic extract (Table 3).

Table 2: Antimicrobial activity of garlic extract on clinical pathogens.

Sr. No.	Clinical Pathogens	Zone of Inhibition (mm)						
		Garlic Extract	Amikacin	Ciprofloxacin	Gentamicin	Levofloxacin	Norloxacin	Tetracycline
1.	*Staphylococcus aureus 1*	25	11	29	11	26	23	12
2	*Staphylococcus aureus 2*	24	14	28	12	31	24	R
3	*Staphylococcus aureus 3*	17	13	28	11	28	23	R
4	*Escherichia coli 1*	22	11	30	12	26	22	12
5	*Escherichia coli 2*	24	11	30	13	21	27	13
6	*Escherichia coli 3*	21	11	28	11	26	22	14

7	*Pseudomonas aeruginosa 1*	18	12	11	11	11	14	11
8	*Pseudomonas aeruginosa 2*	18	23	27	22	25	22	11
9	*Pseudomonas aeruginosa 3*	22	13	11	17	22	25	15
10	*Salmonella typhi 1*	16	11	28	11	28	32	10
11	*Salmonella typhi 2*	16	30	30	26	33	22	28
12	*Salmonella typhi 3*	22	15	29	20	31	25	22

Table 3: Phytochemical screening of garlic extract.

Sr. No.	Test	+ve/-ve
1.	Tannins	+ve
2.	Saponins	+ve
3.	Flavonoids	+ve
4.	Steroids	+ve
5.	Reducing sugars	+ve
6.	Terpenoids	+ve
7.	Phlobatannins	+ve

CONCLUSION

The study revealed that Garlic extract was found to be effective against all clinical pathogens studied such as *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella typhi*. Phytochemical analysis showed that Tannins, Saponins, Flavonoids, Steroids, Reducing sugars, Terpenoids and Phlobatannins were found to be present in garlic extract. However, further research studies are required in order to analyse these active substances and their mechanism of activity in detail.

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