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Anti-bacterial Effect of Garlic (*Allium sativum L)* Extract on Different Pathogenic and Non-pathogenic Bacteria.

Shaloo Verma, Sonpreet Kaur, Joginder Singh, and Akshay Garg*

Department of Biotechnology, Lovely Professional University, Phagwara, Punjab, 144411, India.

ABSTRACT

Garlic (*Allium sativum)* has important dietary and medicinal role since history. The present study focused on the significant antibacterial activity of Garlic extract on various pathogenic and non-pathogenic bacteria (gram positive as well as gram negative):*Staphylococcus aureus*, *Escherichia coli,Bacillus megaterium*, and *Pseudomonas aeruginosa.* The bacterial culture were obtained from the laboratory of the Lovely professional University and was revived in nutrient broth media and then sub-cultured in Nutrient agar media,Mannitol salt agar, Pseudomonas isolation agar and Eosin methylene blue agar media.The Garlic extract of different concentration: 100%, 75%, 50%, and 25% was used to check the effectiveness of garlic extract on bacteria by measuring the zone of inhibition. The zone of inhibition produced against specific bacteria using garlic extract of different concentration showed the concentration dependent antibacterial activity. Zones of inhibition of different treatment groups were measured by agar-well-diffusion assay and compared with control. The comparison of garlic extract with distilled water control showed the significant antibacterial property of garlic extract. Highlight: Antibacterial effect of different concentration of Garlic Extract was tested on different pathogenic *(S.aureus*, *E. coli,* and *P.aeruginosa)* and non pathogenic (*B.megaterium*) bacterial cultures by spread plate and pour plate technique using agar well diffusion method.

Keywords: Pathogenic bacteria, zone of inhibition, aqueousextract and antibacterial property.

Corresponding author

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INTRODUCTION

Allium sativum, commonly known as garlic, is a bulb, species of onion genus*Allium*, belonging to Liliaceae family Garlic extracts have been used to treat infections for thousands of years[1]. Its typical pungent odor and antibacterial activity depend on allicin, which is produced by enzymatic (alliinlyase) hydrolysis of alliin after cutting and crushing of the cloves[2]. Whenever the cellular walls separating them are damaged, some of the enzyme comes into contact with the amino acid and this sets off a chemical reaction that causes sulfenic acid to form instantly but sulfenic acid is unstable and reacts with itself and breaks down at a steady rate into another unstable compound called allicin[3]. Allicin is the "magic bullet" in garlic from which its many benefits are derived. Allicin has a half-life in air of about 18 hours as it slowly deteriorates into other smelly, sulfurous things. Adding allicin to water somewhat stabilizes it and preserves its antibiotic properties and extends its half life to about two months.

Naturally occurring plants have played an important role in the discovery of new therapeutic agents[4].Its therapeutic uses include beneficial effects on the cardiovascular system, antibiotic, anticancer, anti-inflammatory, hypoglycemic, and hormone-like effects[5]. Studies had shown that consuming garlic generally had the following physical effects: lowers blood pressure and LDL Cholesterol;reduce atherosclerotic buildup within the arterial system; lowers and regulate blood sugar; prevent blood clots formation, thus reducing the possibility of strokes and thromboses; prevent cancer, especially of the digestive system, also prevents certain tumors from growing larger by reducing the size of tumor; help to remove heavy metals such as lead and mercury from the body. The great herbalists and physicians of the ancient world record garlic historical use. "Garlic has powerful properties and is of great benefit against changes of water and of residence," wrote Pliny the elder, the first century Roman naturalist (23-79 AD) [6, 7]. Garlic has been used from the ancient times in India and China for a valuable effect on the heart and circulation, cardiovascular disease [8, 9, 10, 11]and regular use of garlic may help to prevent cancer, to treat malaria, and to raise immunity.Garlic has also proposed to treat asthma, candidiasis, colds, diabetes, and antibacterial effect against food borne pathogens like *Salmonella*, *Shigella* and *S. aureus*[12].

Almost all antibiotics are subjected to the problem of bacterial resistance. Therefore, newer herbal antibacterial compounds from plants and their semi-synthetic derivatives are under investigation to overcome the antibiotic resistance.Raw garlic is a potent natural antibiotic that works differently than modern antibiotics and kills some strains of bacteria, like *Staphylocoocusaureus*, that have become immune or resistant to modern antibiotics.

MATERIALS AND METHODS

Source of bacterial strains

The Gram-positive *S.aureus* and *B.megaterium* and gram-negative *E. coli* and *P.aeruginosa*were obtained from the stock maintained in Biotechnology laboratory of Lovely Professional University, Phagwara (Punjab), India and was revived in nutrient broth media and then sub-cultured in nutrient agar media. Further the cultures were grown on their specific media: Mannitol salt agar for *S.aureus*, Pseudomonas isolation agar for *P.aeruginosa* and Eosin methylene blue agar media for *E. coli*and were kept in incubator for 18-48 hours at 37˚C. Gram staining and various biochemical tests of each culture were also done for confirmation of isolates.

Preparation of aqueous garlic extract

Fresh garlic bulbs were purchased from local market of Deepnagar, Jalandhar, Punjab, India. The bulbs were peeled, weighed (100 gm) and cleaned. Cleaned cloves were surface-sterilized by immersing them into 70% (v/v) ethanol for 60seconds[13].Residual ethanol on surface was evaporated in sterile laminar airflow chamber followed by homogenizing aseptically in sterile mortar and pestle. The homogenized mixture was filtered through sterile cheesecloth. This extract was considered as the, Mother extract (100% concentration of the extract). The concentrated Mother extract was further diluted to make 75%, 50% and 25% concentrate by mixing with appropriate amount of sterile distilled water[14].

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Testing of antibacterial activity using agar well diffusion method

The bacterial strains were inoculated into 10 ml of sterile nutrient broth and incubated at 37 °C for 18 h. Each culture was then swabbed on the surface of sterile nutrient agar plate and also pour-plated in nutrient agar media to perform the test in triplet, one with the control and the other two with the test sample. In two agar plate of all four sets, four wells were prepared with the help of sterilized cork borer. In the wells of two plates of each set, 10µl test samples of following concentrations: (1) 100% sterile garlic extract; (2) 75% sterile garlic extract; (3) 50% sterile garlic extract; and (4) 25% sterile garlic extract; were added by using micropipette. In the third plate of all four sets, single well was prepared with the help of sterilized cork borer and 10µl distilled water was added.

RESULT AND DISCUSSION

Thezones of inhibition of different treatment groups measured by agar-well-diffusion assay and compared with the control. The comparison of garlic extract of different concentrations with distilled water control as stated in Table1 and Figure 1 had shown the significant effect of garlic extract. The effect of the crude garlic extract on pathogenic strains was observed maximum for the*P.aeruginosa* followed by *S. aureus* and*E. coli*and it shows the least effect on *B.megaterium*which isnon pathogenic bacteria. Hence from this observation it was found that crude garlic extract had proved its significant antibacterial activity on both pathogenic and nonpathogenic bacterial cultures.

Table 1: Inhibition of bacterial growth by different concentration of Garlic extract

Bacterial strains	Zone of Inhibition (in cm)				
	Control (sterile Distilled Water)	100% Garlic extract	75% Garlic extract	50% Garlic extract	25% Garlic extract
***E. coli* (spread plate)**	0.0	1.6	1.4	1.1	0.9
***E. coli* (pour plate)**	0.0	2.0	1.8	1.3	1.1
***B. megaterium* (spread plate)**	0.0	1.3	1.1	0.9	0.6
***B. megaterium* (pour plate)**	0.0	1.5	1.3	1.2	1.0
***P. aeruginosa* (spread plate)**	0.0	2.6	1.8	1.6	1.3
***P. aeruginosa* (pour plate)**	0.0	2.4	2.1	1.8	1.5
***S. aureus* (spread plate)**	0.0	2.1	1.9	1.6	1.4

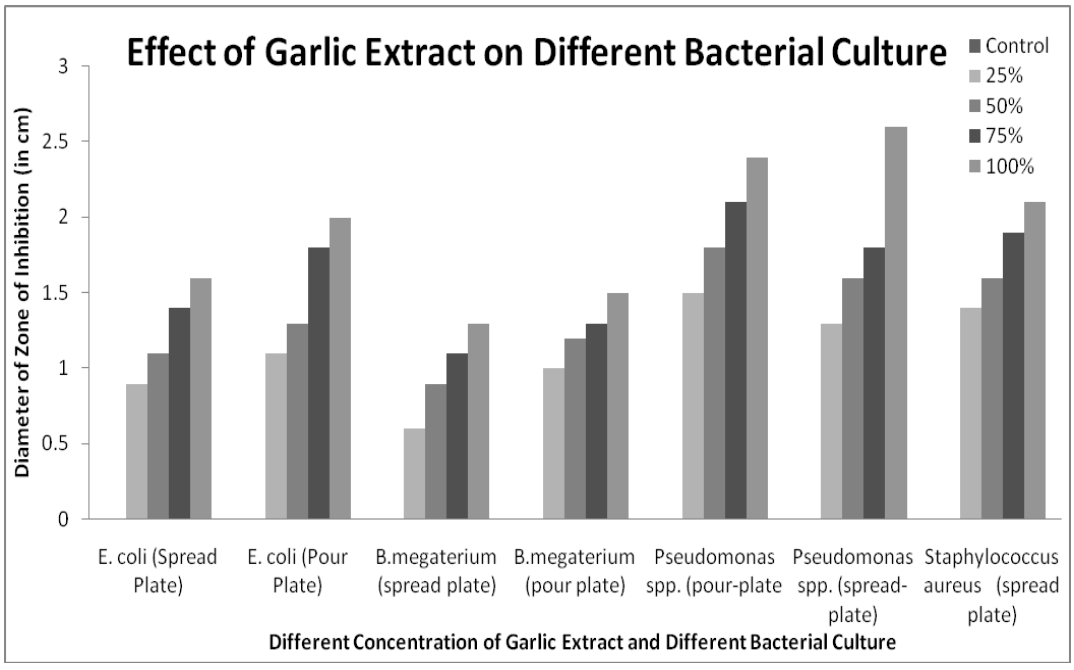

Figure 1: Graph showing the effect of different concentration of garlic extract on different Bacterial Culture

According to Onyeagba and his colleague, (2004) the crude extracts of garlic and ginger applied singly and in combination did not exhibit any *in vitro* inhibition on the growth of test organisms including *Staphylococcus* spp. [15]. In contrast to findings of Onyeagba our study has clearly shown that for garlic extract in concentration of 25%-100% was capable of causing the inhibition of growth of bacteria *S. aureus, E. coli, B. megaterium and P.aeruginosa*(Table 1). Garlic hadshown bactericidal effect at the lowest concentration of 25%. However, this concentration level may vary as observed by [16]. This might be due to the garlic species variation in different country, the processing difference on the garlic species and the inoculums densities. The bactericidal effect of garlic might be due to the structural characteristics of organisms which play a role in the bacterial susceptibility to garlic constituents [17], particularly the lipid content of the membranes will have an effect on the permeability of allicin and other garlic constituents. Hence this phenomenon may favor the destruction of the cell wall and genetic materials.

The findings of this study reveal the distinct antibacterial profile of *Allium sativum* against *S. aureus*, *E. coli*, *P.aeruginosa and B.megaterium*as witnessed from prominent zones of inhibition.*E. coli* is a common pathogenic bacterium for urinary tract infection; *S. aureus* is the cause of pneumonia and several infections in gut, urinary tract, etc.; *P.aeruginosa* is the cause of pneumonia, septic shock, urinary tract infection, gastrointestinal infection, skin and soft tissue infection, etc; and *B.megaterium*is non pathogenic microorganism.Use of garlic extract solely is fruitful and helps to prevent the pathogenic organism from developing resistance against antibiotic.

Several studies [18, 19, 20] have confirmed that *S. aureus*is an important cause for both nosocomial and community acquired infections, which result in substantial morbidity and mortality. Although scientific antimicrobials were of help in the initial phase of their development but the emergence of drug resistantstrains have created a problem in the control and treatment of various infections [21, 22, 23]. Thus there is a need to develop alternatestrategies. Because garlic is known to act synergisticallywith antibiotics, and resistance has not been reported forgarlic, more dose-response preclinical studies andeventually clinical studies should be done to assess theuse of an antibiotic/garlic combination for bacteria thatare difficult to eradicate.

On consideration of the above problems the study was focusedon the effectof garlic on bacteria has shownthat aquous solutions of garlic at various concentrations can completely inhibit thegrowth of*S. aureus, E. coli, B. megaterium and P.aeruginosa.*

CONCLUSION

The results of the present study clearly demonstrated that fresh garlic can provide superior antibacterial effect.Garlic extract have inhibitory activity on various pathogenic bacteria.A major problem with pharmaceutical antibiotics is that they can promote the development of resistant strains of bacteria. Initially the antibiotic kills most of the bacteria being attacked. With repeated exposure, however, few bacteria those by chance are genetically resistant to the antibiotic and begin to multiply. Eventually a recurring infection becomes completely resistant to that antibiotic. After a half century of the massive use of antibiotics, and the indiscriminate over-prescription of them in North America, potentially serious medical problems exist from resistant strains of bacteria. Garlic does not seem to produce such resistant strains, and may be effective against strains that have become resistant to pharmaceutical antibiotics. Garlic is the plant necessary in everyday life from the past until the present days. It contains active compounds that are responsible for its effect on almost every part of the human body. It has been used for medical treatment of everything, from ancient civilizations to date., it can be concluded that administration of garlic should not be avoided; on the contrary, its intake should be as much as possible since it underlies human health.

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