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ANTIMICROBIAL ACTIVITY OF *ALLIUM SATIVUM* AGAINST HUMAN PATHOGENS

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ABSTRACT

Garlic (*Allium sativum* L.) is one of the oldest cultured vegetation and is used ever since ancient times both as diet and as drug. The present study aimed at assessing the antibacterial activity of garlic by disc diffusion method against ten MTCC bacterial species. Various solvent extracts of garlic inhibited the growth of bacterial species at the concentrations of 100, 200, 300, 400 and 500µg. All the four extracts showed maximum activity against *Staphylococcus aureus*. Among the solvent extracts, methanol extract of garlic showed better result in antimicrobial activity. This may be due to the presence of active phytochemicals in the solvent extracts. The extracts showed concentration dependent antibacterial activity against bacterial cultures. The traditional use of *Allium* species for infectious diseases and for controlling bacterial infection appears to be justified.

INTRODUCTION

Garlic (*Allium sativum* L.) belong to the family Liliaceae, a common vegetable used widely in almost all parts of the world. Various species of genus *Allium* have been used as spices and vegetables, and as drugs for curing various diseases for many centuries. Plant physiologists and chemists were attracted towards the powerful and unusual flavours of these vegetables and their medical applications¹⁻⁵. On account of its culinary and medicinal properties, it has been cultivated for centuries all over the world^{6,7}.

Garlic has a long folklore history as a treatment for many respiratory diseases and also it is reported to strengthen the immune system⁸. At the present time, the *Allium* family has over 500 members each differing in morphology, taste and colour, but close in phytochemical and neutraceutical content. In addition to their nutritional effects, the antibacterial activity against a variety of gram negative and gram positive were continue to be investigated⁹. The objective of this study is to determine the antimicrobial activity of garlic collected from the cultivation site (Pannaikadu, Kodaikanal).

MATERIALS AND METHODS

Collection of plant materials

Allium sativum used in this study was collected from Pannaikadu village of Kodaikanal district and brought to the laboratory for further analysis.

Processing of plant materials

The collected *A.sativum* bulb was cleaned thoroughly and dried under shade. The dried bulb was blended into fine powder and stored in air tight container at room temperature.

Preparation of extracts

The organic solvents such as petroleum ether, chloroform, methanol and distilled water was used for the extracting the bioactive compounds from *A.sativum* bulb. The extraction was done using soxhlet apparatus. The extract dried using vacuum evaporator and stored in air tight containers.

Collection of bacterial cultures

Ten different bacterial cultures used in this study were collected from microbial type culture collection (MTCC). The cultures used were *Klebsiella pneumoniae*, *E.coli*, *Streptococcus pyogenes*, *Pseudomonas aeruginosa*, *Enterobacter aerogenes*, *Staphylococcus aureus*, *Proteus vulgaris*, *Salmonella typhi*, *Bacillus typhi* and *Aeromonas hydrophila*. The cultures were revived in nutrient agar medium and stored as slant cultures.

Determination of antimicrobial activity

The Muller Hinton agar (MHA) plates were swabbed with bacterial pathogens and well of 8mm diameter was punched into the MHA medium and filled with 10-50µl (100-500µg) of solvent extract. The plates were incubated at 37°C for 24 hours. After incubation period, the diameters of zone of inhibition produced by the extract with different human bacterial pathogens in different plates were measured and recorded.

RESULTS

The solvent extracts of garlic, garlic exhibited good antibacterial activity against all the ten MTCC cultures tested. Petroleum ether, methanol, water and chloroform extract showed maximum activity against *Staphylococcus aureus* and the antibacterial activity determined was 24.5 ± 0.4 mm, 24.3 ± 0.2 mm, 23 ± 0.5 mm and 18.5 ± 0.4 mm zone of inhibition respectively in highest concentrations. Methanol and water extract of garlic showed maximum activity against *Pseudomonas aeruginosa* in the range of 21.2 ± 0.2 mm zone of inhibition and 18.3 ± 0.2 mm zone of inhibition in 500 µl concentrations respectively. Petroleum ether extract of garlic showed maximum of 14.5 ± 0.4 mm in 500 µl concentrations against *Pseudomonas aeruginosa*. *Pseudomonas aeruginosa* showed resistant to least concentration of petroleum ether and chloroform extract of garlic. 10.6 ± 0.4 mm were the maximum antibacterial inhibition zone observed for chloroform extract of garlic against *Pseudomonas aeruginosa* (table 1).

Klebsiella pneumonia showed maximum sensitivity (15.5 ± 0.5 mm zone of inhibition in 500 µl concentrations) to methanol extract and exhibited no sensitivity to the least concentration of petroleum ether, chloroform and water extract and showed less activity towards the highest concentration of chloroform extract of garlic (10.8 ± 0.6 mm zone). Water and petroleum ether extract exhibited 14.1 ± 0.2 mm zone of inhibition in 500 µl concentration and 12.8 ± 0.2 mm zone in 500 µl concentrations respectively. Methanol and petroleum ether extract exhibited 17.8 ± 0.2 mm zone and 16.1 ± 0.2 mm zone of inhibition against *E.coli* in 500 µl concentrations followed by water and chloroform extract of garlic.

Table 1. Antimicrobial activity of garlic against bacterial pathogens

Clinical Pathogens	Zone of Inhibition (mm)/Concentration of extract (µg)																			
	100				200				300				400				500			
	Petroleum ether	Chloroform	Methanol	Water	Petroleum ether	Chloroform	Methanol	Water	Petroleum ether	Chloroform	Methanol	Water	Petroleum ether	Chloroform	Methanol	Water	Petroleum ether	Chloroform	Methanol	Water
<i>K.pneumoniae</i>	0	0	10.5±0.5	0	0	0	11.3±0.2	10.1±0.2	9 ± 0.4	8.8±0.2	14.1±0.2	12.3±0.5	10.5 ± 0.4	9.8±0.6	15.3±0.2	13.3±0.2	12.8±0.2	10.8±0.6	15.5±0.5	14.1±0.2
<i>E.coli</i>	9 ± 0.4	0	11±0.5	8.6±0.5	10.1±0.2	8.8±0.2	13.3±0.2	11.1±0.2	11.3± 0.4	10 ± 0.4	16.1±0.2	13.1±0.2	14 ± 0.4	12 ± 0.4	16.3±0.2	14±0.5	16.1±0.2	13.3±0.2	17.8±0.2	14.1±0.2
<i>S.pyogenes</i>	8.8± 0.2	8.8± 0.2	10.3±0.5	10.1±0.2	9.5 ± 0.4	11 ± 0.4	10.5±0.5	13±0.5	11.3± 0.4	12.5 ± 0.4	13.3±0.5	15.1±0.2	13.5 ± 0.4	14.1±0.2	14.1±0.2	17.1±0.2	15.3±0.4	16.1±0.2	14.5±0.5	18.3±0.2
<i>P.aeruginosa</i>	0	0	16.6±0.7	12.8±0.2	10 ± 0.8	0	18.1±0.2	13.3±0.2	11.1± 0.2	8.8±0.2	18.3±0.2	14.8±0.2	12.6±0.4	10.1±0.2	20.1±0.2	16.1±0.2	14.5 ± 0.4	10.6±0.4	21.2±0.2	18.3±0.2
<i>E.aerogenes</i>	9.1± 0.2	0	14±0.5	12±0.5	11.6 ± 0.4	8.8±0.2	17.1±0.2	13.3±0.2	13.5 ± 0.4	10 ± 0.4	17.5±0.5	15.1±0.2	15.3±0.4	11.1±0.2	20.1±0.2	16.1±0.2	18.1±0.2	11.1±0.2	21.3±0.5	18.1±0.2
<i>S.aureus</i>	12.8± 0.2	10.3± 0.4	11.8±0.2	12.1±0.2	14.8±0.2	12.5 ± 0.4	15.1±0.2	15.3±0.2	16.5 ± 0.4	14.5 ± 0.4	18.5±0.5	16.1±0.2	20.3±0.4	15.1±0.2	21.3±0.5	19.3±0.5	24.5±0.4	18.5 ± 0.4	24.3±0.2	23±0.5
<i>P.vulgaris</i>	0	11 ± 0.4	12.1±0.2	11.8±0.2	8.8±0.2	12 ± 0.4	12.5±0.5	13.8±0.	11.8± 0.6	13.3 ± 0.2	15.1±0.2	15.3±0.2	14 ± 0.4	15.3 ± 0.2	15.3±0.2	16.1±0.2	15.5±0.4	15.1 ± 0.2	19.1±0.2	16.3±0.2
<i>S.typhi</i>	8.8± 0.2	0	11.8±0.2	9.1±0.2	9 ± 0.4	0	12.3±0.2	9.3±0.2	10.5± 0.7	9.5 ± 0.5	14.1±0.2	11.1±0.2	12.1±0.2	10 ± 0.4	16.8±0.7	13.3±0.5	13.8±0.2	11.1 ± 0.2	17.3±0.5	14.3±0.2
<i>B.subtilis</i>	0	0	9.1±0.2	0	0	9 ± 0	9.3±0.2	9.3±0.2	9.8± 0.2	9.8 ± 0.6	11.1±0.2	10.1±0.2	11.1±0.2	11.1±0.2	13.1±0.2	11.1±0.2	11.8±0.6	11.5±0.5	13.3±0.2	11.5±0.5
<i>A.hydrophila</i>	0	9.3 ± 0.2	9.1±0.2	0	8.8±0.2	11.1±0.2	10.17±0.2	9.1±0.2	10.8± 0.2	12 ± 0.4	13.3±0.5	10.3±0.2	12.8±0.2	14.5 ± 0.5	14.1±0.2	10.5±0.5	14.1±0.2	15 .3±0.2	14.5±0.5	11.3±0.2

Streptococcus pyogenes was sensitive to water, chloroform, petroleum ether and methanol extract of garlic was 18.3 ± 0.2 mm, 16.1 ± 0.2 mm, 15.3 ± 0.4 mm and 14.5 ± 0.5 mm zone of inhibition respectively in 500 μ l concentrations. Methanol extract of garlic showed maximum antibacterial activity against *Enterobacter aerogenes* in the range of 14 ± 0.5 mm to 21.3 ± 0.5 mm in 100 to 500 μ l concentrations. Water and petroleum ether extract showed the maximum activity of 18.1 ± 0.2 mm zone of inhibition in 500 μ l concentrations. *Enterobacter aerogenes* was resistant to the least concentration of chloroform extract. It showed maximum of 11.1 ± 0.2 mm zone of inhibition in 500 μ l concentration. 19.1 ± 0.2 mm zone for methanol extract and 16.3 ± 0.2 mm zone of inhibition for water extract 500 μ l concentrations, 15.3 ± 0.2 mm zone for chloroform extract and 15.5 ± 0.4 mm zone for petroleum ether extract of garlic was observed against *Proteus vulgaris* in various concentrations respectively.

Methanol and water extract showed maximum activity against *Salmonella typhi* was 17.3 ± 0.5 mm and 14.3 ± 0.2 mm zone respectively in 500 μ l concentrations. Petroleum ether and chloroform extract of garlic showed maximum of 13.8 ± 0.2 mm and 11.1 ± 0.2 mm zone in 500 μ l concentrations respectively. *Bacillus subtilis* was sensitive to methanol extract of garlic in the range between 9.1 ± 0.2 mm and 13.3 ± 0.2 mm zone of inhibition in 100 and 500 μ l concentrations. Chloroform, water and petroleum ether extract does not showed antibacterial against *Bacillus subtilis* in the least concentrations. It showed 11.5 ± 0.5 mm of maximum zone of inhibition for both chloroform and water extract against *Bacillus subtilis* in 500 μ l concentrations. 11.8 ± 0.6 mm zone was the maximum zone observed for petroleum ether extract against *Bacillus subtilis* in highest concentration. Chloroform extract of garlic showed maximum activity (15.3 ± 0.2 mm zone in highest concentration) against *Aeromonas hydrophila*. 14.5 ± 0.5 mm of inhibition zone for methanol extract and 14.1 ± 0.2 mm of inhibition zone for petroleum extract in 500 μ l concentrations were observed against *Aeromonas hydrophila* (table 1). The maximum activity exhibited by water extract was 11.3 ± 0.2 mm zone of inhibition in 500 μ l concentrations and showed less activity for water extract in higher concentration.

DISCUSSIONS

Proteus mirabilis was sensitive to aqueous extracts of *Ophioscordon* variety of *Allium* at higher concentration (400 and 500mg). *E.aerogenes* was not susceptible to the aqueous extract of garlic varieties, while *S.typhi* was susceptible to the extracts of garlic varieties. Ethanolic extract of *sativum* was highly effective against all the bacterial species that was

taken for the whereas *E.coli*, *S.typhi* and *S.flexineri* were sensitive to ethanolic extract of *Ophioscordon* variety in Shobana et al¹⁰ study. Also they reported that alcoholic extract of *A.sativum* was highly effective against all the bacterial species that was taken for the study.

In Benkeblia¹¹ reported that ethanolic extract of garlic exhibited different inhibition levels against *S.aureus* and *S.entritidis*. In dose response study, the inhibition zone increased with increasing concentration of extracts. Low concentrations (50 and 100ml/l) inhibited weakly the development of bacteria; however *S. entritidis* was more sensitive than *S.aureus*. Kyung et al¹² reported that allicin of garlic extract showed strong antibacterial activity against *S.aureus* at 150ml/l concentration. This antibacterial activity was enhanced and highest when garlic extract was heated for 45 minutes at 121°C. According to Onyeagba and his colleagues¹³ the crude extract of garlic did not exhibit any invitro inhibition on the growth of test organisms including *Staphylococcus* sp. In Karuppiah and Rajaram¹⁴ investigation, the garlic cloves extracts exhibited high degree of inhibitory activity against most of the seven tested organisms. Among the clinical pathogens, *P.aeruginosa*, *E.coli*, *Bacillus* sp., *S.aureus* and *Enterobacter* sp. were the least inhibited by garlic extracts. The diameter of zone of growth inhibition varied between 7mm and 19mm in garlic. The garlic cloves alcoholic extract showed highest diameter of zone of inhibition of 19.45mm against *P.aeruginosa* followed by *E.coli* (18.50mm) and *Bacillus* sp. (16.5mm). It showed similar zone of inhibition of 13.50mm in diameter against *Proteus* sp., *Enterobacter* sp. and *S.aureus*.

At 100%, the maximum zone of inhibition was observed against *B.subtilis* (54mm) a gram positive organism and the minimum was observed against *Proteus* sp. (22mm), a gram negative organism in Durairaj et al¹⁵ study. This indicated that aqueous garlic extract has the potential of a broad spectrum of activity against both gram positive and gram negative bacteria. All the four solvent extracts of *Allium* extract completely inhibits the growth of the microorganisms used in this study. This may due to the extract preparations, different concentrations of the active compounds present in the extract and their interactions in the culture media. Antibiotics were used for therapy, but many of the pathogenic bacteria are resistant. Natural products of higher plants may offer a new source of antibacterial agents and from this result it is clear that the medicinal value of *Allium* sp. is comparable to the present day antibiotics.

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