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Anti-Bacterial Activities of *Allium sativum* Against *Escherichia coli*, *Salmonella* Ser. Typhi and *Staphylococcus aureus*

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Abstract: The objective of this study was to observe the inhibitory effect of garlic (*Allium sativum*) against various bacterial strains. The crude extract and methanolic extract of garlic were tested against *Escherichia coli, Staphylococcus aureus* and *Salmonella* ser. Typhi. In result we found that in the well diffusion method, the crude and methanolic extract both gave the same inhibition zone towards *E. coli,* the crude extract gave the maximum inhibition zone towards *S. aureus* and the methanolic extract gave the maximum inhibition zone towards *S.* ser. Typhi. In growth inhibition in broth, methanolic extract of garlic was found to give best result in *E. coli* and crude extract of garlic was found to give best result in *S.* ser. Typhi. Overall result shows that the crude extract was most powerful against the all pathogenic bacteria in comparison to methanolic extract, though both were effective. This result gives us a confidence that conventional medicinal approach can be used for treating several ailments.

Key words: Antibacterial Activity · *Allium sativum* · Crude and Methanolic Extract · *E. coli* · *S. Aureus* and *S.* ser. Typhi.

INTRODUCTION

Medicinal plants have been used as traditional treatments for numerous human diseases. In rural areas of the developing countries, they continue to be used as the primary source of medicine [1]. It is estimated by the World Health Organization that approximately 75-80% of the world's population use plant medicines either in part or entirely [2]. Neanderthal man valued herbs as medicinal agents 60,000 years ago [3]; this conclusion is based on a grave in Iran in which pollen grains of eight medicinal plants were found.

According to Kim [4] there are approximately 500,000 plant species occurring worldwide, of which only 1% has been phytochemically investigated, there is great potential for discovering novel bioactive compounds. Mohanty [5] stated that since ancient time, naturally occurring plants have played an important role in the discovery of new therapeutic agents.

Garlic (Allium sativum) has an important dietary and medicinal role for centuries [6]. Its therapeutic uses include beneficial effects on the cardiovascular system, antibacterial, anticancer, anti-inflammatory, hypoglycaemic and hormone-like effects [7]. It's typical pungent odor and antibacterial activity depend on allicin, which is produced by enzymatic (alliin lyase) hydrolysis of alliin after cutting and crushing of the cloves. Growing numbers of American health care consumers are turning to plant medicines for many reasons - low cost and seeking natural alternatives with fewer side effects are commonly cited. Nature has got remedies for all the ailments. Vast numbers of living organisms exist in nature; we can use the strategy of nature to cure disease.

Thus in the present study we have taken garlic (*A. sativum*) to see their antibacterial effect against three diseases causing bacteria. The aim of the present study was to identify whether the plants we have chosen are effective against the bacteria under the study causing specific diseases.

MATERIALS AND METHODS

Selected Bacterial Species: *Escherichia coli, Salmonella* ser. Typhi and *Staphylococcus aureus* were selected for

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the present experiments. Lyophilized cultures of bacterial species were collected from laboratory of Index Medical College, Indore.

Selected Plant Species: *A. sativum* (Lehsun) was selected for access their antibacterial activity. It was purchased from the local vegetable market.

Extract Preparation:

Methanol Extract: The methanol extract was prepared by method explained by Sarvanan [8]. Fresh plant material was being collected and then washed under tape water for 2-3 times for the removal of extra debris, mud, etc. It was then cut into fine pieces and again washed with the distilled water for clearing of material. Now 90% solution of methanol (90ml of methanol with 10ml of distilled water) was taken into which 10g of plant material was added. It was then crushed with pestle and mortar till fine paste was obtained. It was then filtered with the help of Watman filter paper no.1, then the filtered solution was centrifuged at 5000rpm for 5minutes. Pellet was discarded and supernatant was be used for experimental work.

Crude Extract: The crude extract was prepared by method explained by Abubakar [9]. Fresh plant material was being collected and then washed under tape water for 2-3 times for the removal of extra debris, mud, etc. It was then cut into fine pieces and again washed with the distilled water for clearing of material. It was then crushed with pestle and mortar till fine paste was obtained. It was then filtered with the help of Watman filter paper no.1, filtered solution was centrifuged at 10000rpm for 10minutes. Pellet was discarded and supernatant was be used for experimental work.

Media: The two types of media were used. Muller Hinton media was used for well diffusion method [10]. Nutrient broth media was used for MIC method as previously done by Srinivasan [11].

Inoculum Preparation: From collected pure bacterial sample, 4-5 colonies were taken with a wire loop. These colonies were transferred to 10ml of physiological saline in test tube.

Well Diffusion: The susceptibility of bacteria against different parts of medicinal plants was tested by well diffusion method explained by Chitravadivu [12].

Determination of MIC (Minimal Inhibitory Count): MIC of garlic extract against the test cultures were determined by tube dilution method. The test bacterial cultures were inoculated with various concentrations of garlic extract, incubated for 24 h at 37°C which can be observed visually [13].

RESULT AND DISCUSSION

The antibacterial activity of garlic bulb (*A.sativum*) crude and methanolic extract against *S. typhi,S. aureus*and *E. coli* was assessed on the basis of well-diffusion method in MHA media and growth inhibition in nutrient broth media method. Results of antibacterial activity of garlic bulb against *S.* ser Typhi*, S. aureus* and *E. coli* are summarized in Tables 1-2.

Well-diffusion Method in MHA Media: The crude extract of garlic was most efficient against the *S. typhi* followed by *S. aureus* and then *E. coli*. The inhibition zones were 10, 15 and 22 mm against *E. coli, S. aureus* and *S.*ser. Typhi respectively.

The methanolic extract of garlic bulb was also effective against all the three bacteria under study. It gave maximum inhibition zone against *S. aureus* followed by *E. coli* and then *S.* ser. Typhi. The inhibition zones were 10, 12 and 9 mm against *E. coli, S. aureus* and *S.* ser. Typhi respectively.

On comparison it was observed that the antibacterial activity of garlic bulb on *E. coli* was maximum and equally effective in both crude and methanolic extract. *S. aureus* exhibited maximum inhibition with the crude extract of garlic bulb. The inhibition zones were 15mm and 12mm in crude and methanolic extract respectively. The *S.* ser. Typhi shows maximum inhibition with crude extract of garlic bulb. The inhibition zones were 22mm and 9mm in crude and methanolic extract respectively.

Growth Inhibition in Nutrient Broth Media Method: The crude extract of garlic was most efficient against the *S. aureus* followed by *S.* ser. Typhi and then *E. coli.* O.D. after 50h was 0.095, 0.160 and 0.565 for S. *aureus*, *S.* ser. Typhi and *E. coli* respectively.

The methanolic extract of garlic bulb was also effective against all the three bacteria under study. It gave maximum inhibition against *S.* ser. Typhi followed by *S. aureus* and then *E. coli.* O.D. after 50h was 0.200, 0.280 and 0.340 for *S.* ser. Typhi, *S. aureus* and *E. coli* respectively.

Table 1: Antibacterial activity of garlic bulb extract against different bacteria shown by well diffusion method

S. No.	Organism	Inhibition zone (diameter) mm		
		Water (Negative control)	10% Crude extract	10% Methanolic extract
1.	*E. coli*	00	10	10
2.	*S. aureus*	00	15	12
3.	*S.* ser. Typhi	00	22	09

Table 2: Antibacterial activity of garlic bulb extract against different bacteria shown by growth inhibition in nutrient broth media method

	10% Crude extract O.D. at Different Hrs.			10% Methanolic extract O.D. at Different Hrs.			Negative control (Water) O.D. at Different Hrs.		
Organism	24h	40h	50h	24h	40h	50h	24h	40h	50h
E. coli.	0.390	0.400	0.565	0.460	0.300	0.340	0.460	0.610	0.988
S. aureus	0.010	0.008	0.095	0.268	0.206	0.280	0.185	0.355	0.460
S. typhi	0.006	0.005	0.160	0.190	0.160	0.200	0.230	0.450	0.960

On comparison it was observed that the antibacterial activity of garlic bulb on *E. coli* was more effective in methanolic extract than crude. O.D. after 50h was 0.340and 0.565 for methanolic and crude extracts respectively. The *S. aureus* was maximum inhibited in crude extract of garlic bulb. O.D. after 50h was 0.095 and 0.280 for crude and methanolic extracts respectively. The *S.* ser. Typhi was also maximum inhibited in crude extract of garlic bulb. O.D. after 50h was 0.160 and 0.200 for crude and methanolic extracts respectively.

Daka [14] studied Garlic (*A. sativum*) and reported its antimicrobial properties against *S. aureus.* It has both a bacteristatic and bactericidal activity when tested *in vitro* using crude preparation of garlic. Therefore, garlic may be used successfully for treating food poisoning causative agent like *S. aureus*. Allium vegetables such as garlic have been used throughout history for their medical properties, for instance in treatment of bacterial infections. Moreover, Dorant [15] observed a decreased risk of gastric cancer with an increasing consumption of allium vegetables, possibly by an effect on Helicobacter pylori as this organism is associated with gastric cancer. The antimicrobial effects of aqueous garlic extract (AGE) against 133 multidrug-resistant Gram-positive and Gram-negative bacterial isolates, including *S.aureus, S.epidermidis, Streptococcus pneumoniae, Streptococcus pyogenes, Haemophilus influenzae, S.typhi, Pseudomonas aeruginosa, E.coli, Shigella* spp. and *Proteus* spp. and against 10 *Candida* spp. were studied. The results of this study support the use of garlic in health products and herbal remedies in Nigeria [13]. The results of present investigation show the antibacterial activity of garlic bulb against different studied bacteria and are in good agreement with the reports of earlierinvestigators.

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