International Journal of Scientific Engineering and Research (IJSER)
ISSN (Online): 2347-3878
Index Copernicus Value (2015): 62.86 | Impact Factor (2015): 3.791

Assessment of Antibacterial Activity of Garlic (*Allium sativum* L.): An *in Vitro* Study

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Abstract: ***Garlic (Allium sativum L.) has an important dietary and medicinal role for centuries. It is a large annual plant of the Liliaceae family, which is used in traditional medicine for infectious diseases. The present study was designed to evaluate the antibacterial activity of fresh extract of garlic. Five bacteria were chosen to study antibacterial activity of garlic. The zones of inhibition exhibited by the extract against Bacillus cereus, Staphylococcus aureus, Escherichia coli, Klebsiella sp. and Proteus sp. were compared with the reference antibiotic chloramphenicol (1%). Antibacterial activity of the garlic extract against the tested bacteria was found significant (p < 0.05). Results showed that garlic extract produced the highest susceptibility (mm) toward B. cereus. So, it was set up as the most sensitive bacteria, followed by S. aureus and E. coli. All these three bacteria showed greater potency than 1% chloramphenicol with high significances (p < 0.05). The observation suggested that B. cereus was inhibited strongly by garlic when applied.***

Keywords: Garlic; Antibacterial activity; *Allium sativum*; Antibiotic

1. Introduction

Many infectious diseases have been known to be treated with herbal remedies throughout the history of mankind. Natural products, either as pure compounds or as standardized plant extracts, provide unlimited opportunities for new drug leads because of the unmatched availability of chemical diversity. *Allium* species such as garlic (*Allium sativum* L.) is used as foodstuff, condiment, flavouring, and folk medicine. Garlic has been revered throughout the time not only for its culinary use, but also for its therapeutic properties (Skrinjar and Nemet, 2009). Garlic bulbs contain a good number of phytochemicals, most of which are hydrocarbons and their derivatives (Griffiths *et al.*, 2002). Several authors have reported pharmaceutical activity of extracts of garlic including antitumor, antidiabetic, antioxidant, antibacterial, antiallergic and molluscicidal activity (Helen *et al.*, 2000; Lampe, 1999). *In vitro* studies have shown garlic to possess antibacterial, antiparasitic, and antifungal activity (Rose *et al.*, 2005; Zohri *et al.*, 1995).

Garlic can be used as herbal medicine and has attracted particular attention of modern medicine because of its widespread health use around the world, and the cherished belief that it helps in maintaining good health, warding off illnesses and providing more vigour (Banerjee and Maulik, 2002). Modern scientific research has revealed that garlic possesses numerous therapeutic properties that are similar to onion, including antioxidant effects, an ability to inhibit the formation of inflammatory compounds, and direct anti-inflammatory effects (Banerjee and Maulik, 2002; Rose *et al.*, 2005). The allicin is considered to be most potent antibacterial agent and also exhibits antiparasitic activity against major human intestinal parasites. Another constituents of garlic known as ajoene may play a great role as topical antifungal agent. The sulfur constituents in garlic have been verified for antiviral activity (Gebreyohannes and Gebreyohannes, 2013). Garlic derived organo sulfur compounds such as diallylsulfide, diallyldisulfide and diallyltrisulfide provide significant protection against carcinogenesis (Das *et al.*, 2007). Similarly, other pharmaceutical effects of garlic include antiulcer (Jiang *et al.*, 2008), antidiabetic (Sheela *et al.*, 1995), antihypertensive (Caro, 1978), lipid lowering agents (Cerella *et al.*, 2011), neuro-regenerative (Mathew and Biju, 2008), enhances male reproduction (Wilkes *et al.*, 2009), topical application in warts (Dehghani *et al.*, 2005) and probiotic effects (Jaime *et al.*, 2001). Therefore, keeping in view the pharmaceutical potential of garlic, present study was undertaken to evaluate antibacterial activity of fresh extract of this household specie.

2. Materials and Methods

2.1 Extract preparation

Fresh garlic bulbs were purchased from the local market, Singramau, Jaunpur, Uttar Pradesh and it was verified from National Botanical Research Institute (NBRI), Lucknow, Uttar Pradesh. Skins of garlic bulbs were peeled out, washed with sterilized water and air dried for about 2 h and sliced. Then the garlic pieces were ground in an electric blender (Philips, India) separately and filtered using the clean and dry muslin cloths. The crude juice was squeezed out, then filtered through Whatmann filter paper No. 1 under vacuum pressure. The extract was kept in a refrigerator at 4°C for further analysis.

2.2 Preparation of inoculums

About 18 h broth culture of the test bacterial isolates (*Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella* sp. and *Proteus* sp.) were suspended into sterile nutrient broth. They were standardized according to the National Committee for Clinical Laboratory Standards (NCCLS, 2002) by gradually adding normal saline to compare their turbidity to McFarland standard of 0.5 which is expressed in colony forming unit (CFU) per millilitre (ml) and is approximately 1.0×10^6 CFU/ml.

2.3 Antibacterial activity

The antibacterial activity of the crude extract was determined in accordance with the agar well diffusion method described by (Irobi *et al.*, 1994). The bacterial isolates were first grown in a nutrient broth for 18 h before use and standardized to 0.5 McFarland standards (10^6 CFU/ml). 200 μl of the standardized cell suspensions were spread on a Mueller-Hinton agar (Hi-media). Wells were then bored into the agar using a sterile 6 mm diameter cork borer. Approximately 50 μl extract of the garlic was introduced into wells, allowed to stand at room temperature for about 2 h and then incubated at 37°C. Controls were set up in the parallel using 1% chloramphenicol and sterile distilled water was used to reconstitute the extract. After 24 h, the plates were observed for zones of inhibition.

3. Results and Discussion

The present study investigated antibacterial activity of fresh extract of garlic. The five bacteria, such as *B. cereus*, *S. aureus*, *E. coli*, *Kleibsella* sp. and *Proteus* sp. were taken to study antibacterial activities. Extract obtained from the garlic was assessed for the inhibitory effect against the selected five bacteria and 1% chloramphenicol was taken as a reference. From the observation, garlic extract produced the highest susceptibility (mm) toward *B. cereus* (52 ± 0.18). So, it was set up as the most sensitive bacteria, followed by *S. aureus* (38 ± 0.16) and *E. coli* (35 ± 0.47). All these three bacteria showed greater potency than 1% chloramphenicol with high significances ($p < 0.05$). The observation suggested that *B. cereus* was inhibited strongly by garlic when applied (Table 1).

Table 1: Zone of inhibition produced by garlic (*Allium sativum*) against the bacteria

Microorganisms	Zone of Inhibition (mm)	
	Garlic	1% Chloramphenicol
B. cereus	52 ± 0.18	33 ± 0.11
S. aureus	38 ± 0.16	13 ± 0.48
E. coli	35 ± 0.47	22 ± 0.47
Kleibsella sp.	23 ± 0.43	27 ± 0.70
Proteus sp.	19 ± 0.48	22 ± 0.38

Experimental evaluation done by few researchers (Bray and Bettger, 1990) suggested that the garlic had been effective against diseases, in the pathophysiology of which oxygen free radicals (OFRs) have been implicated. Effectiveness of garlic could be due to its ability to scavenge OFRs. The major antibacterial effect of garlic is due to allicin (Abdou *et al.*, 2001) so that, garlic extracts have been found to possess antibacterial property against *S. typhimuium*, *E. coli* No 1, *S. epidermidis*, *S. Aureus* (Ani *et al.*, 2006; Ankri and Mirelman, 1999). Some of the advantages that herbal preparations have over the synthetic ones are that they do not act directly on bacteria, but create an adverse environment for them, thus threatening their survival and they have also been found to deter the development of resistant strains of microorganisms (Irobi *et al.*, 1994).

4. Conclusion

Garlic (*Allium sativum*) has been used widely as common household spice from the ancient period and has also been regarded as traditional healer. Garlic has traditional dietary and medicinal applications as an anti-infective agent. In the present *in vitro* study, Garlic was found to be more potent against selected bacterial species. From the observation, garlic extract produced the highest susceptibility (mm) toward *B. cereus*. So, it was set up as the most sensitive bacteria, followed by *S. aureus*, *E. coli*, *Kleibsella* sp. and *Proteus* sp. The observation suggested that *B. cereus* was inhibited strongly by garlic when applied. Thus, it can be concluded that the antibacterial activity of garlic making it one of the potent therapeutic phytomedicines.

Acknowledgements

The author (BKS) is thankful to the Principal, R.H.S.P.G. College, Singramau, Jaunpur, U.P. for providing the laboratory facilities. The author is also thankful to National Botanical Research Institute (NBRI), Lucknow, U.P. for the verification of garlic bulbs.

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