

Original Research Article

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Antibacterial Activity of Fresh Garlic Juice against *Vibrio* sp. Isolated from Shrimp Farm Water: An *in vitro* Study

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ABSTRACT

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Antibacterial activity of garlic was investigated on inhibition of *Vibrio* sp. isolated from shrimp farm water samples by well diffusion method. 100%, 50%, 25%, 10%, 5% of Fresh garlic juices were prepared from 100g garlic bulbs. Results indicated that the isolated *Vibrio* sp. showed different levels of sensitivity to different concentrations fresh garlic juice. The zone of inhibition of the bacterial growth increased with increasing concentration of fresh garlic juice. The results revealed that fresh garlic juice was effective against the test organism.

Introduction

Consumption of protein rich fish and fishery products has been dramatically increased all over the world. Among these, shrimps are in utmost demand as it is delicious and protein rich. In spite of reduced supply from capture side which is not up to the level to meet the increasing demand, aquaculture is focusing on the culture of shrimp in intensive manner. Intensification of production system has been followed by number of diseases. Whilst various forms of diseases have been reported in aquatic animals, most were caused by common bacteria (Morales, 2004; Holmstrom *et al.*, 2003). To reduce the occurrence of

diseases antimicrobial agents have been practiced in aquaculture. Unfortunately, excessive use of antibiotics led to the emergence of antibiotic-resistance among shrimp pathogens. This reduced the effectiveness of the prophylactic use of antibiotics in aquaculture (Sorum, 2000; Sorum, 2006). In addition to antibiotic resistance, presence of antibiotic residues has also been reported in shrimp and shellfish products (Wang *et al.*, 2017; Cabello *et al.*, 2013). This could eventually affect the beneficial microbes present in pond water and sediments, resulting in changes in microbial diversity in the shrimp pond (Hunter *et al.*, 2005; Matyar *et al.*, 2008).

In this view, the development of effective and safe drug is necessary for shrimp health management. Natural products can be a valuable source for maintaining the health of the animal under culture. Garlic is reported to act as a strong antibacterial agent against various kinds of bacteria such as *Escherichia coli*, *Shigella senteriae*, *Staphylococcus aureus*, *Salmonella* sp., *Streptococcus* sp., *Klebsiella* sp., *Proteus mirabilis*, *Pseudomonas aeruginosa* and *Helicobacter pylori* (Indu *et al.*, 2006).

The aim of this study is to detect the *in vitro* activity of Fresh Garlic Juice in the growth inhibition of *Vibrio* sp. isolated from shrimp farm water sample. Bacteria of the *Vibrio* genus are the causative agent for the majority of the diseases caused by bacteria in shrimp farm. If garlic shows some antimicrobial property against the disease causing *Vibrio* species, it could help to curb their growth in shrimp farm.

Materials and Methods

Preparation of fresh garlic juice

Fresh garlic was purchased from local market. Garlic was skinned and sliced; 100g of sliced garlic pieces were crushed in sterile mortar and pestle and then the mixture was filtered through a sterile cloth. This filtrate was considered 100% fresh garlic extract was stored at -20°C, and was thawed before use. The 100% garlic juice (undiluted) was inoculated on nutrient agar media and incubated at 37°C overnight and was found to be sterile. The concentrated (100%) juice was further diluted to 5%, 10%, 25% and 50% by mixing with distilled water.

Culture medium

Culture medium was prepared according to the producing companies instructions and after

incubation at 37°C for 24 hours, used for culture of bacteria used in this study.

Sample collection

Shrimp farm water samples were collected from shrimp farms at Tiruvallur district, Tamil Nadu. 100 µl of water sample was spread into TCBSagar plate with the help of a glass spreader. The plates were incubated at 37°C for overnight.

Isolation of pure culture

A nichrome inoculating loop was used to inoculate the isolated green colonies on TCBS agar. The inoculum was streaked into four quadrants of the nutrient agar plate with the help of an inoculation loop. The loop was sterilized between each successive quadrant streak. The purpose of this technique was to dilute the inoculum sufficiently on the surface of agar medium so that well-defined colonies of bacteria can be obtained.

Preparation of bacterial culture

All the bacterial isolates were picked up with the help of inoculating loop and were separately inoculated in a liquid media Tryptone Soy Broth (TSB) and incubated at 37°C for overnight. These bacterial cultures were further used for antibacterial assay.

Well diffusion method screening

Well diffusion technique was performed according to Saeed and Tariq, 2005. Three replicates Mueller Hinton Agar (MHA) was poured in petriplates of 9–10 cm; depth of agar was 3–4 mm. A sterilised cotton swab was dipped into the suspension. The inoculum was then swabbed over the entire agar surface. The plate was allowed to dry for 3–5 min. Wells of 5 mm diameter were cut on the surface of the agar. Thirty microliter of 5%,

10%, 25%, 50%, and 100% solutions (v/v) of Fresh Garlic Juice was added to different wells and in one well, normal distilled water was added as a control. The plates were incubated at 37°C for 24 h. The antibacterial activity was observed through zone of inhibition by measuring the diameter in millimeter (mm) including disc diameter.

Results and Discussion

Inhibitory effect of Fresh garlic juice

To deal with the bacterial infections effective drugs need to be given attention. Unfortunately these effective drugs (antibiotics) has accompanied with unsafe indications, i.e. emergence of antibiotic resistance. This phenomenon of resistance has paved the way to safe and effective drugs for

the treatment of bacterial infections. In this study garlic showed antibacterial activity against *Vibrio* sp. and the sensitivity was gradually increased with increasing concentration of fresh garlic juice. Inhibitory effect of garlic juice was tested against *Vibrio* sp. using agar well diffusion method where distilled water was used as a control. There was no zone of inhibition observed against distilled water. The inhibition zone was observed against garlic juice of different concentrations. The sensitivity of the bacteria against Fresh Garlic Juice gradually increased with the increase in concentration. The zone of inhibition at different concentrations of fresh garlic juice is presented in Figure 1 and Table 1. It was observed that five different concentrations of fresh garlic juice showed varied level of antibacterial activity against *Vibrio* sp.

Fig.1 Inhibitory effect of different concentrations of fresh garlic juice on growth of *Vibrio* sp.



Table.1 Zone of inhibition at different concentration of fresh garlic juice against the growth of *Vibrio* sp.

Conc. (%)	5	10	25	50	100
Zone of Inhibition (mm)	6.5 ± 0.03	8.0 ± 0.02	12.0 ± 0.05	14.0 ± 0.01	16.0 ± 0.02

Many reports have documented that garlic can effectively eliminate principal pathogenic bacteria including *Pseudomonas* sp., *Edwardsiella* sp. and *Vibrio* sp. Zhang (2003) has studied the inhibitory effects of garlic on two isolates of *Aeromonas hydrophilla* *in vitro*. It was shown to be effective with minimum inhibitory concentration of 15.6 mg/l and 1.95 mg/l respectively for AH1 and AH2. Khashan (2014) has reported that garlic is effective against the growth of *Staphylococcus aureus* which is due to the allicin compound in the garlic extract. The sensitivity of the bacterium increased with increasing concentration of garlic. Yadhav *et al.*, (2015) demonstrated an *in vitro* study to test the antibacterial effect of fresh garlic juice against five bacterial pathogens *E. coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*. It was found to be effective against five bacterial pathogens. Since the use of antibiotics has been banned for use in aquaculture for disease inhibition in many developing countries effective therapeutic strategies need to be standardized. Drugs of herbal sources could serve as an effective measure to reduce the impact of diseases in aquaculture. It is apparent that fresh garlic juice could be a good antibacterial agent against *Vibrio* sp.

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References

Cabello, F.C., Godfrey, H.P., Tomova, A., Ivanova, L., Dolz, H., Millanao, A., and Buschmann, A.H. 2013. Antimicrobial use in aquaculture re-examined: its relevance to antimicrobial resistance

and to animal and human health. *Environmental Microbiology*, 1917: 42.

Holmstrom, K., Graslund, S., Wahlstrom, A., Pongshompoo, S., Bengtsson, B. E., and Kautsky, N. 2003. Antibiotic use in shrimp farming and implications for environmental impacts and human health. *International Journal of Food Science and Technology*, 38: 255-266.

Hunter-Cevera, J., Karl, D., and Buckley, M. 2005. Marine microbial diversity: the key to earth's habitability. (A report from the American Academy of Microbiology) Colloquium held 8–10 April 2005, San Francisco, CA, USA: Marine Microbial Diversity. Washington, DC, USA: American Academy of Microbiology.

Indu, M. N., Hatha, A.A.M., Abirosh, C. 2006. Antimicrobial activity of some of the south-Indian spices against serotypes of *Escherichia coli*, *Salmonella*, *Listeria monocytogenes* and *Aeromonas hydrophila*. *Brazilian Journal of Microbiology*, 37: 153-158.

Khashan. 2014. Antibacterial activity of garlic extract (*Allium sativum*) against *Staphylococcus aureus* *in vitro*. *Global journal of bio-science and biotechnology*. 3: 346-348.

Matyar, F., Kaya, A., and Dinçer, S. 2008. Antibacterial agents and heavy metal resistance in Gram-negative bacteria isolated from seawater, shrimp and sediment in Iskenderun Bay, Turkey. *Science of the Total Environment*, 407: 279-285.

Morales, C. M. S. (Ed). 2004. *Enfermedades del camaron*, Editorial Trillas, ISBN-968-24-7112-5, Mexico, D. F.

Saeed, S., and Tariq, P. 2005. Antibacterial activities of *Menthapiperita*, *Pisum sativum* and *Momordica charantia*. *Pakistan Journal of Botany*, 37: 997-1001

- Sorum, H. 2000. Farming of Atlantic salmon – an experience from Norway. *Acta Veterinaria Scandinavica Supplementum*, 93: 129–134.
- Sorum, H. 2006. *Antimicrobial Resistance in Bacteria of Animal Origin*. Aarestrup, F.M. (ed.). Washington, DC, USA: American Society for Microbiology Press, p. 213–238.
- Wang, H., Ren, L., Yu, X., Hu, J., Chen, Y., He, G., and Jiang, Q. 2017. Antibiotic residues in meat, milk and aquatic products in Shanghai and human exposure assessment. *Food Control*, 80:217–225.
- Yadav, S., Trivedi, N.A., Bhatt, J.D. 2015. Antimicrobial activity of fresh garlic juice: An in vitro study. *Ayu*, 36(2): 203–207.
- Zhang, L. 2003. Pharmacodynamics research of allicin on *Aeromonas hydrophila*. *Water Conservancy Related Fisheries*, 23:49-51.

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