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Antibacterial activity of chitosan from freshwater prawn, *Macrobrachium dayanum* (Henderson, 1893) against *Escherichia coli* and *Staphylococcus aureus*

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ABSTRACT

In the present study, chitosan has been extracted from *M.dayanum*. Further the antibacterial activity of different concentrations (25%, 50%, 75%, 100%) of chitosan was tested against Gram-positive bacteria, *Staphylococcus aureus* (ATCC 6538) and Gram-negative bacteria, *Escherichia coli* (ATCC8739) by Agar Well Diffusion method. The result of the present study suggests that chitosan has concentration dependent antibacterial activity against both bacterial strains indicating its possibility of using as antibacterial agent.

Key Words - *M.dayanum,* Antibacterial, Chitosan, *S.aureus,E.coli.*

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INTRODUCTION

Growth of pathogens is usually prevented by using preservatives which are chemical in nature. These chemical preservatives serve as antimicrobial compounds that inhibit the growth of pathogenic microorganism. To minimize the toxic effect of chemicals, emphasis is given on the alternative natural sources for antimicrobial compounds. Natural antimicrobial are derived from many, including animals (chitosan) (Tiwari *et al.* 2009).

Chitosan is derived by partial N-deacetylation of chitin, is a straight chain polymer of glucosamine and N-acetyl glucosamine.(Muzzarelli *et al.* 1997) Chitosan is derived from chitin, which is found in the shells of crustaceans like freshwater and marine crabs, shrimps and crawfish. Chitosan have attracted the industrial fields due to its properties like analgesic, antitumor, antioxidant, haemostatic, hypocholes terolemic, biodegradability a biocompatibility (Raafat 2009).

The objective of this present study was to assess antibacterial activity of chitosan, extracted from shell of freshwater prawn, *Macrobrachium dayanum* against Gram negative, *Escherichia coli* Gram-positive, *Staphylococcus aureus*.

MATERIALS AND METHODS

Collection of the animals:

Macrobrachium dayanum were purchased from local market of Ranchi, Jharkhand. Shells were scraped free of loose tissues from the prawn in laboratory, washed thoroughly with tap water to remove impurities. They were dried at 60^{o}C and pulverized using pestle and mortar for further analysis.

Preparation of chitosan:

Chitin and Chitosan were prepared from *Macrobrachium dayanum* shell according to Takiguchi (1991a,b) with some modifications (Yateendra *et al.* 2012) for purification of chitosan. The production of chitosan from crustaceans shell generally consists of three basic steps demine ralization, deproteinization and deacetylation.

Preparation of stock solution:

In the preparation of chitosan solutions (15mg) chitosan was dissolved in 3ml of 0.2% (w/v) aqueous acetic acid solution. From this 0.25,0.50,0.75 and 1.0 ml was taken and made up to 1.0 ml by adding 0.2% acetic acid to prepare various concentrations containing 1.25 mg, 2.50

mg, 3.75 mg and 5 mg of chitosan sample corresponding to 25,50,75 and 100% respectively.

Bacterial Strains:

The antibacterial activity of the prepared chitosan from *Macrobrachium dayanum* was tested against two strains, *Escherichia coli* (ATCC 8739) and *Staphylococcus aureus* (ATCC 25923) obtained from the Department of Microbiology, Yugantar Bharti Analytical and Environmental Engineering Laboratory, Sidroul, Namkum, Ranchi, Jharkhand.

Preparation of bacterial culture:

Nutrient broth medium was prepared and sterilized in an autoclave at 15 lbs pressure. Two bacterial species were incubated in the nutrient broth and incubated at 34°C for 24 hours. Nutrient agar medium was also prepared, autoclaved and transferred aseptically in to sterile Petri dishes. On this, 24 hours bacterial broth cultures were inoculated by using a sterile cotton swab.

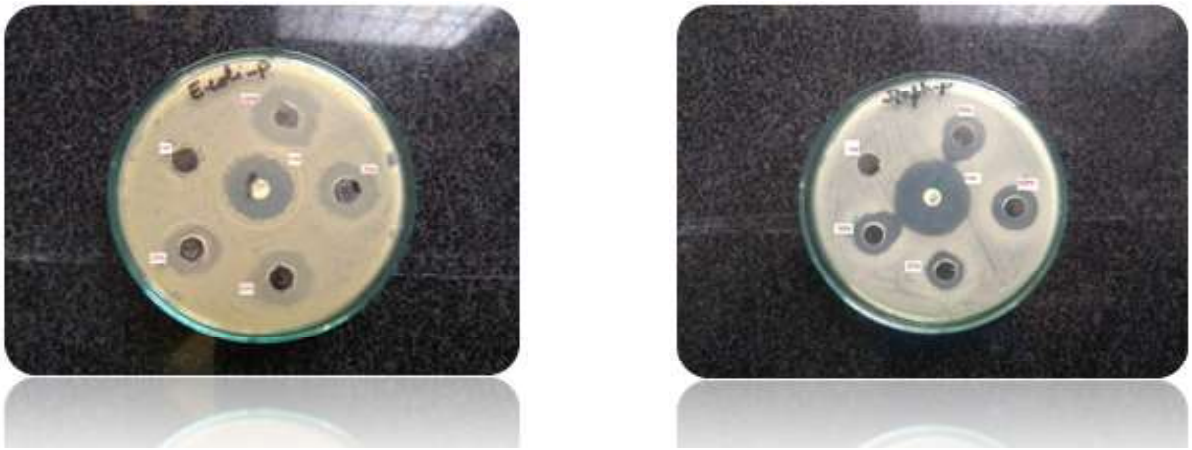
Antibacterial assay:

The antibacterial activity of the individual bacterial strains was tested using Agar Well Diffusion method (Ramasamy *et al.* 2011). Well of 6mm diameter were made aseptically in the plates. 24 hours old bacterial broth cultures were inoculated using a sterile cotton swab on sterile Nutrient Agar plates. Using micropipette, solution of different concentration of chitosan and 0.2% acetic acid as negative control was loaded in the respective wells. Ciprofloxacin (5µg) disc was placed using sterile forceps, as positive control. The plates were incubated at 34°C for 24 hours in upright position. The antibacterial assay was carried out in triplicate. After incubation at 34°C for 24 hours, zone of inhibition was measured in millimeters.

RESULT

Sl.No.	Microorganisms	Concentration of chitosan					
		(5mg) 100%	(3.75mg) 75%	(2.5mg) 50%	(1.25mg) 25%	Positive control	Negative control
1.	*Escherichia coli* (ATCC 8739)	21.1±0.10	20.03±0.15	19.03±0.05	18.1±0.17	22.06±0.05	
2.	*Staphylococcus aureus* (ATCC 6538)	17.2 ±0.20	16.16 ±0.15	15.16± 0.11	14.03 ±0.20	28.03 ±0.05	-

(-) = No zone of inhibition, Results indicate zone of inhibition in mm, Values are given as mean ± SD of three experiments, Positive control (Ciprofloxacin 5µg), Negative control (0.2% acetic acid).



Escherichia coli ***Staphylococcus aureus***

Fig. 1:- Antibacterial activity of Chitosan from *M.dayanum*

The chitosan extracted from *M.dayanum* showed vital antibacterial activity against both pathogenic bacteria *Escherichia coli* and *Staphylococcus aureus* were depicted in Table 1 and Fig 1. Chitosan showed wide spectrum of antibacterial activity against both pathogenic bacteria. The antibacterial effect of chitosan on the pathogenic bacteria showed that the highest activity (21.1 ± 0.10 mm) was observed against *E.coli* with highest (100%) concentration. At the same concentration, *S.aureus* showed 17.2 ± 0.20 mm zone of inhibition. Regarding 25%, 50%, 75% concentration, maximum activity (18.1 ± 0.17 mm, 19.03 ± 0.05 mm, 20.03 ± 0.15 mm) was found against *S.aureus*. The antibacterial activity was found to be concentration dependent with no activity in negative control.

DISCUSSION & CONCLUSION

The present research investigation was made in search of antibacterial activity of chitosan extracted from shell of *Macrobrachium dayanum* against pathogenic bacteria *Escherichia coli* and *Staphylococcus aureus*.

Chitosan (5mg/ml) in the present study showed a wider spectrum of antibacterial activity of 17.2 ± 0.20 mm and 21.1 ± 0.10 mm zone of inhibition and at 1.25 mg/ml 14.03 ± 0.20 mm and 18.1 ± 0.17 mm zone of inhibition in *Staphylococcus aureus and Escherichia coli* respectively. When compared to this finding, chitosan from shrimp shell (Kamala *et al.* 2013) reported at 1mg formed 8.4 mm and 8.9 mm zone of inhibition and at 500µg/ml 5.2 mm and 6.4 mm zone of inhibition in *Escherichia coli* and *Staphylococcus aureus* respectively. K.Prabhu *et. al.* (2012) reported that *Escherichia coli* and *Staphylococcus aureus* showed inhibition zone of 13.04 ± 1.11 mm and 11.06 ± 0.32 mm respectively with chitosan at 500µg/ml and no zone of inhibition against *Staphylococcus aureus* at 1.25µg/ml.

Several hypotheses have been proposed to explain the mechanism of antimicrobial activity of chitosan. One of the hypotheses is that the mechanism involves interactions of chitosan positively charged molecules with negatively charged constituents of microbial cell walls and membranes interrupting with normal cell metabolism (Savard *et al.* 2002). Several authors indicated that chitosan may directly affect cell membrane function (J.L.Leuba and P.Stossel, 1986). Result of the present study revealed that chitosan has concentration dependent antibacterial activity against *Escherichia coli* and *Staphylococcus aureus*. This result is in accordant with the work of Jeon *et.al* (2001).

This study revealed that chitosan from fresh water prawn, *Macrobrachium dayanum* could be used to inhibit Gram positive (*Staphylococcus aureus*) and Gram negative (*Pseudomonas aeruginosa*) bacterium and need to be explored in the development of new pharmaceuticals.

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