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In vitro antibacterial activity of chitosan from crab, *Maydelliathelphusa masoniana* (Henderson, 1893) against *Staphylococcus aureus* and *Pseudomonas aeruginosa*

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Abstract : In the present study, chitosan has been extracted from *M.masoniana.* 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, *Pseudomonas aeruginosa* (ATCC10145) by Agar Well Diffusion method. The result of the present study suggests that as the concentration of chitosan increased the antibacterial effect was strengthened against both bacterial strains indicating its possibility of using as drug.

Keywords : *M.masoniana,* antibacterial, chitosan, *S.aureus, P.aeruginosa*

INTRODUCTION

Nature has played an instrumental role in providing effective therapeutic entities.[1] In the present era, people are suffering from several bacterial diseases and the most common medicine suggested are synthetic antibiotics. The antibiotics are compound that inhibit the growth of undesirable microorganisms. Thus to minimize these effects, more emphasis is given on alternative source which are naturally derived antimicrobial agents. Natural antimicrobial are derived from many, including animals (chitosan).[2]

Chitosan is a copolymer of glucosamine and N-acetyl glucosamine units linked by 1, 4 glycosidic bonds and it is obtained through the alkaline hydrolysis of chitin.[3] Chitosan is derived from chitin, which is found in the shells of crustaceans like freshwater and marine crabs and shrimps. Chitosan has gained attention of the scientific community due to its functional properties such as film-forming capabilities, mineral-binding properties, hydrolipidemic activity, biodegradability, antimicrobial activity, immunoadjuvant activity, acceleration of wound healing, and eliciting of phytalexins.[4]

The goal of this study was to evaluate antibacterial activity of chitosan, extracted from shell of freshwater crab, *Maydelliathelphusa masoniana* against food borne and UTI strains, Gram-positive, *Staphylococcus aureus* and Gram negative, *Pseudomonas aeruginosa.*

MATERIALS & METHODS

Collection of the animals:

Maydelliathelphusa masoniana were purchased from local market of Ranchi, Jharkhand. Shells were scraped

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free of loose tissues from the crab wastes in laboratory, washed thoroughly with tap water to remove impurities. They were dried at 60°C and pulverized using pestle and mortar for further analysis.

Preparation of chitosan:

Chitin and Chitosan were prepared from *Maydelliathelphusa masoniana* shell waste according to *Takiguchi*[5, 6] with some modifications[7] for purification of chitosan. The production of chitosan from crustaceans shell generally consists of three basic steps demineralization, 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 *Maydelliathelphusa masoniana* was tested against two strains, *Staphylococcus aureus* (ATCC 25923) and *Pseudomonas aeruginosa* (ATCC 27853), obtained from the Department of Microbiology, Yugantar Bharti Analytical and Environmental Engineering Laboratory, Sidroul, Namkum, Ranchi, Jharkhand. *Staphylococcus aureus*, a Gram positive bacterium and *Pseudomonas aeruginosa,* a Gram negative bacterium were chosen because both are commonly associated with food products and Urinary tract infection.

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:

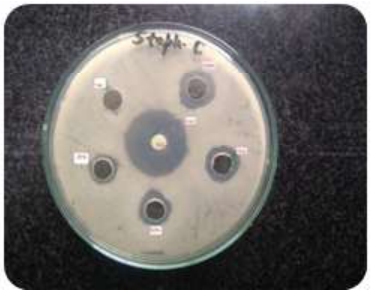
The antibacterial activity of the individual bacterial strains was tested using Agar Well Diffusion method.[8] Well of 6mm diameter were made aseptically in the plates. 24 hours old bacterial broth cultures were inoculated using a sterile cotton swab on sterile Nutrient Agar plates. Using micropipette, 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

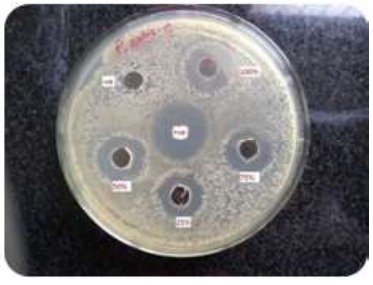
Table 1: Antibacterial activity of chitosan of *M.masoniana*

Sl. No.	Microorganisms	Concentration of chitosan					
		(5mg) 100%	(3.75mg) 75%	(2.5mg) 50%	(1.25mg) 25%	Positive control	Negative control
1.	*Staphylococcus aureus* (ATCC 6538)	15.13 ±0.15	14.16 ±0.15	13.03± 0.05	12.0 ±0.2	25.03 ±0.05	-
2.	*Pseudomonas aeruginosa* (ATCC 10145)	19.83± 0.25	18.8 ±0.20	17.10± 0.26	15.26± 0.25	24.96 ±0.15	-

(-) = 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)



Staphylococcus aureus



Pseudomonas aeruginosa

Fig. 1. Antibacterial activity of chitosan from *M. masoniana*

The chitosan from *M. masoniana* showed good antibacterial activity against both pathogenic bacteria. (Fig 1). The antibacterial activity was found to be concentration dependent. At the same time the activity was absent in negative control (Table 1). Effect of chitosan on pathogenic bacteria revealed that, the highest activity (19.83±.25mm) was observed against *Pseudomonas aeruginosa* with highest (100%) concentration. At the same concentration, *Staphylococcus aureus* showed 15.13±0.15 mm zone of inhibition. Regarding at 25, 50, 75% concentration maximum activity (15.26±0.25, 17.10±0.26, 18.8±0.20 mm) was found against *Pseudomonas aeruginosa.* The lowest activity (12.20±0.2 mm) was found with 25% concentration against *Staphylococcus aureus.*

DISCUSSION & CONCLUSION

In this study, antibacterial activity of chitosan prepared from crab shell waste was tested against two strains *Staphylococcus aureus* and *Pseudomonas aeruginosa.*

The present investigation showed a higher antibacterial activity in 100% concentration with the maximum inhibition zone of 19.83±0.25 mm clear zone recorded against *Pseudomonas aeruginosa* and in 25% concentration with 12.0±0.2 mm inhibition zone against *Staphylococcus aureus*. When compared to this finding, chitosan from *Sepia kobiensis* reported the maximum inhibition of above 16mm against *Pseudomonas aeruginosa* at the highest concentration of 5mg/ml and minimum inhibition between 11-15 mm against *Staphylococcus aureus*.[9] Chitosan from, *Podophthalmus vigil* reported to have inhibition zone of 8.17±1.21 mm against *Staphylococcus aureus* at only highest concentration of 500µg/ml and no zone of inhibition against *Pseudomonas aeruginosa* at all concentrations.[1]

According to literature[10] chitosan possesses antimicrobial activity against a number of Gram-negative and Gram-positive bacteria. The antibacterial mechanisms of chitosan suggested being, the positive charge of the amino group at C-2 resulted in a polycationic structure which can be expected to interact with the predominantly anionic component of the microorganism surface.[11] The interaction resulted in great alteration of the structure of outer membrane[12], which caused release of major proportion of proteinaceous material from the cells.[13] According to our results, as the concentration of chitosan increased, the antibacterial activity was strengthened. This result is consistent with the work of Jeon *et al.*[10] who have also reported the increased antibacterial activity with increase in the concentration of chitosan.

This study demonstrate that chitosan from fresh water crab, *Maydelliathelphusa masoniana* have excellent antibacterial activity against Gram positive (*Staphylococcus aureus*) and Gram negative (*Pseudomonas aeruginosa*) bacterium. Chitosan may be used against bacterial infection.

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