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Antibacterial activity of chitosan of freshwater crab, *Maydelliathelphusa masoniana* (Henderson, 1893) against *Staphylococcus aureus* and *Escherichia coli.*

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Abstract : Chitosan is known for its diverse functional properties due to its biological activities and application in the pharmaceutical and food industries. Antibacterial properties of chitosan extracted from shell waste of freshwater crab, *Maydelliathelphusa masoniana* of Jharkhand were determined against Gram negative bacteria, *Escherichia coli* (ATCC 25922) and Gram positive bacteria, *Staphylococcus aureus* (ATCC 25923). The antibacterial activity of chitosan (5mg/ml) in 0.2% acetic acid, was tested by Well-Diffusion method. Zone of inhibition measured for *E.coli* was 19.33±0.57 mm and 18.66±0.77 mm for *S.aureus*. Oflaxacin (5mcg) drug showed 31.66±0.57 mm for *S.aureus* and 29.66±0.57 mm for *E. coli*. Comparative statistical analysis by Student's t-test showed that the antibacterial efficacy of chitosan solution was similar both for *E.coli* and *S.aureus*. Negative control (0.2% acetic acid) does not show any zone of inhibition. These results indicate that chitosan from shell waste of freshwater crab *Maydelliathelphusa masoniana* could be used as an effective antibacterial agent against *E.coli* and *S.aureus.*

Keywords- *M.masoniana*, Antibacterial, Oflaxacin, *E.coli, S.aureus*

INTRODUCTION

Crab belongs to the largest distributed Phylum Arthropoda among invertebrates under the animal Kingdom. It is well known due to its consumption as food and also as medicine against many health problems. Generally people consume its meat and throw its shell as waste. In recent times, shell wastes are used as a source of biopolymer, Chitosan. Chitosan is mainly known for usage in food, pharmaceuticals and cosmetics industries Chitosan is non-toxic, biodegradable, polymer of D-glucosamine, linked by 1,4 -glycosidic bonds, obtained by deacetylation of Chitin, a polysaccharide of exoskeleton of freshwater as well as marine arthropods and molluscs.

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Traditional antimicrobials have been used as reliable preservatives to control microbial hazards in the food industry for decades[1]. But these widely accepted compounds are synthetic with harmful side effects. To minimize these effects, there is a need of natural and healthy source of medicines. Due to the negative impact from chemical preservatives, attention has shifted to the use of naturally derived antimicrobial agent to control food borne pathogen[2]. With the increasing claim for food safety and health standards, consumers have been more concerned about the occurrence of chemical residues in the food products[3], therefore natural antimicrobials are considered better than traditional synthetic antimicrobials. Natural antimicrobials are derived from many, including animals (chitosan).[4]

Chitosan have attracted the interest of many researchers, medical, pharmaceutical and industrial fields due to its properties like analgesic, antitumor, antioxidant, haemostatic, hypocholesterolemic, biodegradability and biocompatibility.[5] Chitosan was shown to have several advantages over other disinfectants, as it possesses a higher antibacterial activity, a broader spectrum of activity, a higher kill rate, and lower toxicity towards mammalian cells[6,7]. In the present study, Chitosan is extracted from the shell of one such locally found freshwater crab of Jharkhand*, Maydelliathelphusa masoniana* to determine its antibacterial activity.

MATERIALS & METHODS

- **Collection of animals**

Maydelliathelphusa masoniana were purchased from local market of Ranchi, Jharkhand. Shells were scraped free of loose tissue 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 and chitosan solution**

Chitin and chitosan were prepared from *Maydelliathelphusa masoniana* shell waste according to *Takiguchi*[8,9] with some modifications[10] for purification of chitosan. The production of chitosan from crustaceans shell generally consists of three basic steps demineralization, deproteinization and deacetylation. In the preparation of chitosan solutions 0.5% (w/v) chitosan were dispersed in 0.2% (v/v) acetic acid solution.

- **Bacterial Strains**

The antibacterial activity of the prepared chitosan from *Maydelliathelphusa masoniana* was tested against two strains, *Staphylococcus aureus* (ATCC 25922) and *Escherichia coli* (ATCC 25922), obtained from the Department of Microbiology, Rajendra Institute of Medical Science, Ranchi, Jharkhand. *Staphylococcus aureus,* a Gram positive bacterium and *Escherichia coli,* a Gram negative bacterium were chosen because both are commonly associated with food products and Urinary tract infections.

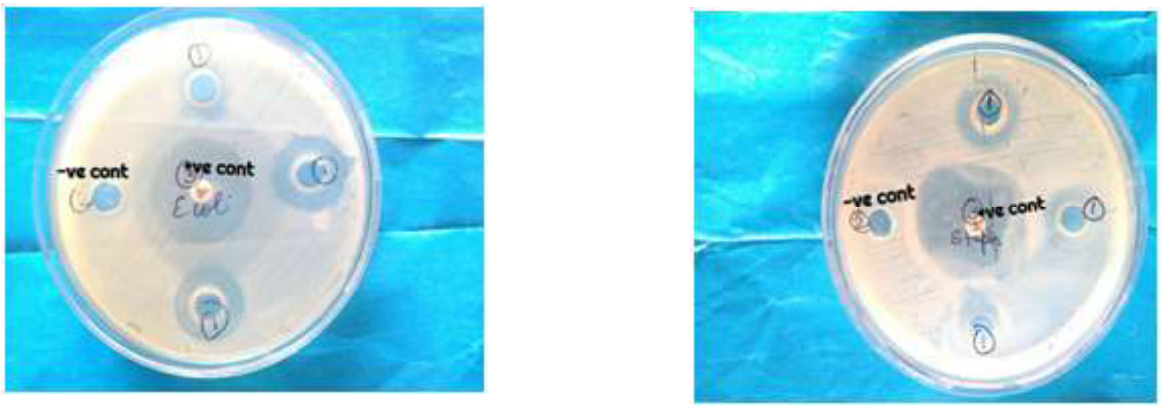
Nutrient broth was prepared and sterilized in an autoclave at 15 lbs pressure for 15 min. Individual species of bacteria were inoculated in the sterile nutrient broth and incubated at 37°C for 24 hrs. Muller Hilton Agar (MHA, Himedia) medium was prepared, sterilized in an autoclave at 15lbs pressure for 15 min and poured into sterile petridishes and incubated at 37°C for 24 hrs. The antibacterial activity of the individual bacterial strains was tested using Agar Well diffusion method[11]. Wells of 6mm diameter were made aseptically in the inoculated plates. Bacterial cultures were emulsified in normal saline and turbidity was matched with 0.5% McFarland turbidity standards. 24 hrs old nutrient broth cultures of test bacteria were aseptically swabbed on sterile MHA plates. Using micropipette, solution of chitosan (5mg/ml) in 0.2% acetic acid and 0.2% acetic acid as negative control was loaded in the respective wells. Ofloxacin disc (5mcg) was placed using a sterile forcep, as positive control. The plates were incubated at 37°C for 24 hrs in upright position. The antibacterial assay was carried out in triplicate. After incubation at 37°C for 24 hrs, zone of inhibition was measured in millimetres.

RESULTS

Table 1: Antibacterial activity and inhibition zone of chitosan (mm)

Sl. no.	Bacterial strains	Zone of inhibition (mm)				Negative control	t-test value
		I	II	III	Mean ± sd		
1.	*Staphylococcus aureus* (ATCC 25923)	19	18	18	18.66±0.70	---	25.4***
	Positive control	31	32	32	31.66±0.57		
2.	*Escherichia coli* (ATCC 25922)	20	19	19	19.33±0.57	---	21.9***
	Positive control	29	30	30	30.29±0.57		

(---) No zone of Inhibition,0.2% acetic acid - Negative control, Ofloxacin (5mcg)-Positive control,
*** $p<0.001$ or significant at 0.1%



Escherichia coli *Staphylococcus aureus*

Figure 1: Antibacterial activity of chitosan from *Maydelliathelphusa masoniana*

In Table 1, chitosan solution (5mg/ml) showed zone of inhibition of 18.66±0.70 mm against *S.aureus* (ATCC 25923) and 19.33±0.57 mm against *E.coli* (ATCC25922) whereas with negative control (0.2% acetic acid) gave no inhibition zone. Positive control, Ofloxacin (5mcg) showed 31.66±0.57 mm and 29.66±0.57 mm against *S.aureus* and *E.coli* respectively. Statistical analysis revealed that there was no significant difference between zone of inhibition against *S.aures* and *E.coli* advocating that chitosan solution have similar antibacterial efficacy for both the strains.

DISCUSSION & CONCLUSION

According to Jean[12], Ueno[13], chitosan possesses antimicrobial activity against a number of Gram-negative and Gram-positive bacteria. Similar investigations were also made by Md. Monarul Islam[14] that with 1% chitosan solution (in 1% acetic acid) treated against *S.aureus* and *E.coli.* Zone of inhibition observed was 13mm and 10mm. respectively. Annaian Shanmugam[15] also examined antibacterial activity of 5% chitosan solution (0.2% acetic acid) and observed 11-15 mm inhibition zone against *S.aureus* and *E.coli.*

0.5% chitosan solution of the present study showed wider spectrum of antibacterial activity than reported zone of inhibition of 3mm against *S.aureus* and *E.coli* from chitosan (50mg/ml) in 0.1% acetic acid extracted from shell waste of *Portunus pelagicus* by P. Raja[16]. K.Prabu[18] reported that *S.aureus* showed inhibition zone of 8.17±1.21mm and 13.04±1.11 mm was formed against *E.coli* with chitosan solution (500mcg/ml) in 1% acetic acid, from another marine crab, *Podophalamus vigil.*

Whereas chitosan of the present study showed narrower spectrum of antibacterial activity than reported zone of inhibition of 55.4±0.71 mm and 49.7±0.31 mm against *E.coli* and *S.aureus* respectively from 5% chitosan in 0.1% acetic acid extracted from shell waste of crab obtained from Cario, Egypt.[2] A.Shanumugam[17] reported very good antibacterial activity against *E.coli* with zone of inhibition above 16mm diameter and good activity against with 11-15mm diameter against *S.aureus* by chitosan (5mg/ml) in 0.2% acetic acid extracted from cuttlebone of *Sepia kobiensis*.

In the present study, *S.aureus* and *E.coli* showed more sensitivity against chitosan than the previously mentioned reports. In the previous investigation, chitosan was extracted from cuttlebone of *Sepia kobiensis* and marine crab and shrimp but in present study chitosan was extracted from freshwater crab. Thus this can be the reason for better antibacterial efficacy of chitosan extracted from freshwater crab *Maydelliathelphusa masoniana.* In conclusion, the present investigation revealed that the chitosan from *Maydelliathelphusa masoniana* inhibits growth of human pathogenic bacterial strains, *S.aureus* and *E.coli.*

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