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Antibacterial activity of chitosan of freshwater crab, *Sartoriana spinigera* (Wood Mason,1871) against human pathogens

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Abstract : Antibacterial properties of chitosan extracted from shell waste of freshwater crab, *Sartoriana spinigera* of Jharkhand were determined against Gram-negative bacteria, *Pseudomonas aeruginosa* (ATCC 27853) 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 *P. aeruginosa* was 16.66±0.57 mm and 18.66± 0.70 mm for *S. aureus*. Ofloxacin (5mcg) drug showed 31.66±0.57 mm for *S. aureus* and 24.66±0.57 mm for *P. aeruginosa*. Statistical analysis by Student's t-test showed that the antibacterial efficacy of chitosan in *S. aureus* was significantly more than in *P. aeruginosa* at 5% level. Negative control (0.2% acetic acid) does not show any zone of inhibition. .These results indicate that chitosan from shell waste of freshwater crab *Sartoriana spinigera* could be used as an effective antibacterial agent against *P. aeruginosa* and *S. aureus.*

Keywords : ***S. spinigera,*** **Antibacterial, Ofloxacin,** ***P. aeruginosa, S. aureus***

INTRODUCTION

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) [3].

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[4]. Chitosan is an important derivative of shells of crustaceans like freshwater and marine crabs and shrimps. Chitosan have attracted the interest of many researchers, medical, pharmaceutical and industrial fields due to its properties like analgesic, antitumor, antioxidant, haemostatic, hypocholesterolemic, biodegradability & 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].

Hence the aim of the study was to determine the antibacterial activity of chitosan extracted from freshwater crab, *Sartoriana spinigera* against food borne and UTI strains, Gram-positive, *Staphylococcus aureus* and Gram-negative, *Pseudomonas aeruginosa*.

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MATERIALS & METHODS

Collection of animals:

Sartoriana spinigera 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 *Sartoriana spinigera* 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 *Sartoriana spinigera* was tested against two strains, *Staphylococcus aureus* (ATCC 25923) and *Pseudomonas aeruginosa* (ATCC 27853), obtained from the Department of Microbiology , Rajendra Institute of Medical Science, 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 infections.

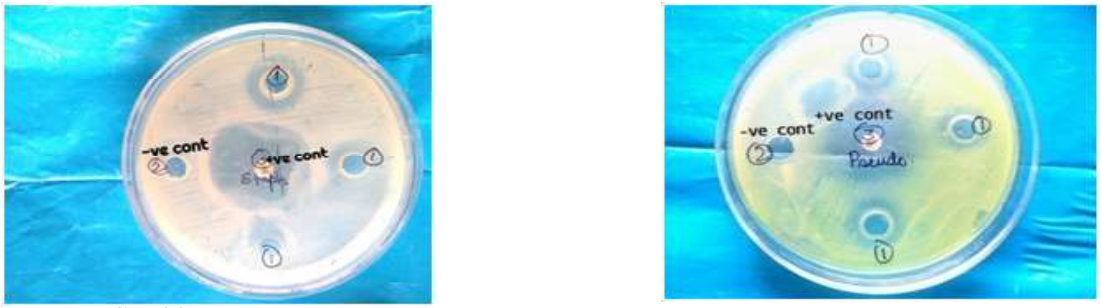
Nutrient broth was prepared and sterilized in an autoclave at 15 lbs pressure for 15 min. Individual species of bacteria were inoculated in the sterile nutrient broth and incubated at 37 °C for 24 hrs. Muller Hilton Agar (MHA, Himedia) medium was prepared, sterilized in an autoclave at 15lbs pressure 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 hr 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	---	3.92*
	Pseudomonas aeruginosa (ATCC 27853)	16	17	17	16.66±0.57	---	
2.	*Staphylococcus aureus* (ATCC 25923)	19	18	18	18.66±0.70	---	25.4***
	Positive control	31	32	32	31.66±0.57		
3.	*Pseudomonas aeruginosa* (ATCC 27853)	16	17	17	16.66±0.57	---	17.0***
	Positive control	25	25	24	24.66±0.57		

(—) – No zone of Inhibition,0.2% acetic acid – Negative control, Ofloxacin (5mcg)-Positive control,*p<0.05 or significant at 5%,***p<0.001 or significant at 0.1%



Staphlococcus aureus ***Pseudomonas aeruginosa***

Figure 1: Antibacterial activity of chitosan from *Sartoriana spinigera*

Chitosan exhibited a wide range of bioactivity as an antibacterial agent against Gram-positive and Gram-negative bacteria. The results illustrated in Table 1 and Figure 1 showed the inhibition zone made by chitosan (5mg/ml) were 18.66±0.70 mm and 16.66±0.57 mm against *S. aureus* and *P. aeruginosa* respectively.

Statistical analysis by Student's t-test revealed that antibacterial efficacy of chitosan in *S. aureus* was significantly more than in *P. aeruginosa* at 5% level.

DISCUSSION & CONCLUSION

The overall goal of the present investigation is to compare the ability of antibacterial activity of chitosan prepared from shell waste of *Sartoriana spinigera* against bacterial strain, *Staphylococcus aureus* and *Pseudomonas aeruginosa.* The results of the present investigation clearly showed (Table 1 and Figure 1) that good antibacterial activity was exhibited by chitosan against human pathogenic strain, *S. aureus* and *P. aeruginosa*.

According to Jean[12], Ueno[13], chitosan possesses antimicrobial activity against a number of Gram- negative and Gram-positive bacteria. Similar investigation were also made by Md. Monarul Islam[14,15] on crab and shrimp chitosan and reported that with 1% chitosan solution (in 1% acetic acid) treated against *S. aureus, E. coli and S. paratyphi* , zone of inhibition observed was 13mm,10mm and 16mm respectively.

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 *P. aeruginosa* from chitosan (50mg/ml) in 0.1% acetic acid extracted from shell waste of *Portunus pelagicus* by P. Raja [16]. Varadharajan D[17] reported that *S. aureus* and *P. aeruginosa* showed 10.4±0.12 mm and 7.2±0.15 mm zone of inhibition respectively by chitosan extracted from marine Crab, *Scylla serrata*. K. Prabu[18] reported that *S. aureus* showed inhibition zone of 8.17±1.21mm but no zone was formed against *P. aeruginosa* 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±0.71mm and 33.4±0.53 mm against *P. aeruginosa* and *S. aureus* respectively from 5% chitosan in 0.1% acetic acid extracted from shell waste of crab obtained from Cairo, Egypt.[2] On the contrary, A. Shamugam[19] reported very good antibacterial activity against *P. aeruginosa* 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*.

The reason for better antibacterial activity in present study could be the source of chitosan extracted from freshwater crab, *Sartoriana spinigera* rather than shell wastes of marine crabs, as in other reported cases. In conclusion, the present investigation revealed that the chitosan from *Sartoriana spinigera* inhibits growth of human pathogenic bacterial strains, *S. aureus* and *P. aeruginosa*.

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