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**Nageswararao PV**
Department of Zoology, Division of Aquaculture and Fisheries, Andhra University, A.U. P.O., Visakhapatnam, Andhra Pradesh, India

**Babu DE**
Department of Zoology, Division of Aquaculture and Fisheries, Andhra University, A.U. P.O., Visakhapatnam, Andhra Pradesh, India

**Correspondence**
**Nageswararao PV**
Department of Zoology, Division of Aquaculture and Fisheries, Andhra University, A.U. P.O., Visakhapatnam, Andhra Pradesh, India

# Assessment of anti-microbial activity of chitin, chitosan and shrimp shell of *Litopenaeus vannamei* from bhimavaram farms, west Godavari district, Andhra Pradesh

**Nageswararao PV and Babu DE**

**Abstract**
Chitin and chitosan are valuable marine biopolymers, which is an abundant by-product of the shrimp processing industry. Chitosan is an amino polysaccharide prepared by processing shrimp waste (Shell) which involves partial deacetylation of chitin. The chitin and chitosan were isolated from shrimp exoskeleton. The Extracted chitosan have antimicrobial properties were determined by employing several microbial organisms like *Escherichia coli, Bacillus cereus, Pseudomonas aeruginosa* and *Staphylococcus aureus*. The current study demonstrates the extracted chitosan exhibit potent anti-microbial activity.

**Keywords:** shrimp shell, chitin, chitosan, anti-microbial activity

## Introduction
Chitin is composed of repeating monomer units of 2-acetamido-2-deoxy-β-Dglucose connected through β (1→4) linkage, resembling cellulose, except that the hydroxyl groups in position 2 have been substituted by acetamido groups [Fernandez-Kim, 2004; Kumar, 2000] [5, 11]. Chitin, poly (β-(1→4)-N-acetyl-D-glucosamine), first identified in 1884, is a naturally occurring polysaccharide consisting of amino sugars. This biopolymer is synthesized by an extremely large number of living organisms and considering the amount of chitin produced annually in the world, it is the second most plentiful natural polymer, behind only cellulose [Fernandez-Kim, 2004; Kumar, 2000; Rinaudo, 2006] [5, 11, 17].

Chitosan, a cationic polysaccharide with a high molecular weight, linear polymer that comprises -1, 4- linked glucosamine (GLCN) 1 with various amounts of N-acetylated GLCN residues. It is typically obtained by the alkaline deactivation of chitin extracted from an abundant source of shellfish exoskeletons or the cell walls of some microorganisms (Hirano *et al*., 1976) [6]. It has attracted marked interest as a biomedical material, because it has unique biological activities, such as antitumor (Saiki *et al*., 1990) [18], immune stimulatory (Meyer and Isaksen, 1995) [15] and antibacterial (Tokoro *et al*., 1989; Kobayashi *et al*., 1990) [22, 10], activities. Both Chitin and chitosan are considered as low cost adsorbent for the removal of toxic heavy metals. Chitin and chitosan are reported wide range of applications in the various fields due to their good biocompatibility, biodegradability, metal-binding capacity, wound healing capacity and capacity to form membranes, beads, fibers, scaffold and gels (Tripathi *et al*., 2009; Elwakeel, 2010) [23, 4].

The antimicrobial property has been established beside diverse genus of microbes, which required extensive care especially with some strains of pathogenic microscopic organism (Li *et al*., 1992; Khanafari *et al*., 2008; Limam *et al*., 2011) [9, 13]. The effects against microbial property of chitosan substance are significantly based on its material appearances, greatest particular molecular mass, and deacetylation status (DD). Chitosan that contains greater deacetylation degree has a larger ability as inhibitory property against bacterial strains (Acharya *et al*., 2005) [1]. Few years ago, many studies reported concerning chitosan that it could be used as bio product compound (Li *et al*., 1992; Limam *et al*., 2011) [12, 9]. Nevertheless, chitosan has great molecular weight, which affects reduced solubility and also becomes high viscosity solution, restricts its utilization as supplementary nutrition, foundation, farming and medicine manufacturing (Xie *et al*., 2001) [27]. The shrimp waste has been used

Primarily for the preparation of chitin and chitosan. The potential applications of chitin and chitosan depend on their physico-chemical properties and biological activities. However, the application of chitin, chitosan and shrimp shell in terms of microbial inhibition on a few microbial organisms is reported

## Material and Methods

### Sample collection

The shrimp (*Litopenaeus vannamei*) specimens collected from Bhimavaram farms, West Godavari District, Andhra Pradesh, the shell and operculum were detached from the animal. The collected exoskeletons were placed in Ziploc covers and refrigerated overnight. Around 1000gms of exoskeleton wet samples were taken and crushed into small pieces using a meat mincer. The crushed samples were oven fried for 4 days at 65˚C. The obtained dried samples were made into 4 equal parts for further process (Toan, 2009) [21].

### Chitin, chitosan extraction, deacetylation and demineralization

40% NaOH (w/v) was added to shrimp shell to dissolve the proteins and carbohydrates and the process was led to 72 hrs. After deproteination by NaOH, these shells were washed with water and again treated with 4% Conc. Hydrochloric acid in order to remove the minerals (Calcium carbonate) from shells and the demineralization time was 20- 24 hours thereafter washed with water and dried (Trung *et al*., 2006) [24]. The deprotonated and decalcified shells were the chitin. The demineralized shrimp shell samples were then treated for one hour with 50 ml of a 2% NaOH solution to decompose the albumen into water soluble amino-acids. The remaining chitin is washed with deionized water, which is then drained off.

The chitin was further converted into chitosan by the process of deacetylation (Huang *et al*., 2004) [8]. To produce chitosan, the above dried deproteinated and decalcified chitins were moisturized with water. For deacetylation, the moisturized chitin was put into 20 M NaOH solution and stirred. Deacetylation process was carried out for 48 hours. After deactivation, chitosan flakes were washed, squeezed and dried in a forced air oven at 60- 70°C. Chitosan were extracted from shrimp shell waste and the obtained chitosan are used for further antioxidant assays.

### Anti-microbial activity

Four strains of micro-organisms *viz*., *Escherichia coli, Bacillus cereus, Pseudomonas aeruginosa* and *Staphylococcus aureus* were got from the Institute of Microbial Technology, Chandigarh, India. The test microorganisms were cultured on special culture media (Nutrient agar slants) and Incubate at room temperature. The agar slants were maintained at 4°C. Antibacterial activity was determined by the following method (Holder and Boyce, 1994) [7]. By using DMSO (Dimethyl sulfoxide) as negative control, samples of micro-organism strains were prepared in replicates and tested with incubated bacterial strains. Finally, antibacterial action was detected by measuring the inhibition area (Agwa *et al*., 2000) [2].

## Results and Discussion

In the present investigation, the antimicrobial activity of shrimp shell, chitin and chitosan were studied and is represented in table 1. In general, the anti-microbial effectiveness of chitosan and its derivative against Gram-positive and Gram-negative bacteria is somewhat controversial. In some published works, the literature presents that unmodified chitosan generally acts stronger on Gram-negative than on Gram-positive strains (No *et al.*, 2002; Silva *et al*., 2010) [16, 20]. Such better anti-microbial efficiency has been attributed to bacterium wall characteristics, considering that the Gram-negative cell wall is thinner and consequently more susceptible than the Gram-positives.

**Table 1:** Antibacterial activity of chitin, chitosan and shrimp shell extracts against some bacteria

| Mean diameter of inhibition ±Standard error mean (SEM) | | | | |
|---|---|---|---|---|
| Extract used | *E. coli* | *B. cereus* | *P. aeruginosa* | *S. aureus* |
| Streptomycin | 28.23±0.23 | 21.00±0.06 | 25.00±0.03 | 19.65±0.09 |
| Chitin | 31.15±0.05 | 16.18±0.12 | 18.35±0.05 | 17.45±0.24 |
| Chitosan | 18.64±0.11 | 21.35±0.24 | 19.45±0.24 | 18.00±0.19 |
| Shrimp Shell | 24.51±0.24 | 9.55±0.36 | 21.00±0.11 | 10.55±0.35 |

The data of table 1 showed the results of the antibacterial influence of shrimp shell, chitosan, and chitin methanolic extract, which were resolved by anti-microbial resistance test methods beside intact microorganisms' strains e.g. *E. coli, B. cereus, P. aeruginosa* and *S. aureus* at sample extracts concentrations 200 mg/ml, chitin extracts presented greater effect beside the verified strains than chitosan and shrimp shell. Chitosan might be an important substance of medicines, which could be beside infection of bacteria. The antibacterial influence of shrimp shell, chitosan and chitin methanolic extracts were seemed to alter actually in efficiency, then bacterial strains revealed greatly unaffected and some strains of tested bacteria further affected by methanol extracts of shrimp shell, chitosan and chitin. Antimicrobial activities of regular positive antibiotic controller (Streptomycin) presented an inhibitory influence on all verified bacteria. These findings were in accordance with Shital (2010) who recommended that there is a continuous and critical necessity to determine recent anti-microbial materials with various chemical composition and novel mechanism of action as a worrying increase in the occurrence of new and re-evolving infectious diseases (Benhabiles *et al*., 2012) [3]. The antibacterial action of chitosan is more than that of chitin, all of them inhibit the growth of cultured microorganisms, and this is referred to that chitosan is higher with polyatomic amines (Wu *et al*., 2006) [26]. This is coordinated with findings of (Tsai and Su, 1999) [25] who examined the efficiency of chitosan derived from shrimp with different concentrations as inhibitor of some strains of microorganisms as Escherichia coli (Liu *et al*., 2006) [14].

## Conclusion

Chitosan in comparison with chitin is soluble in acidic media, which is applied for improvement of processing methods. In fact, chitosan may be easily processed as fiber, film, sponge, bead, gel or solution. Additionally, its cationic charge provides the possibility to form electrostatic complexes and/or multilayer structures. The results of this work demonstrate the extracted chitosan exhibit potent anti-microbial activity. The current study similarly existing that several drugs could be produced extracting from chitosan and chitin substance that have great antimicrobial action. It also showed the prospect of developing chitosan as an effective substance for bacteria inhibition.

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