



IJPPR

INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals

ISSN 2349-7203



Human Journals

Research Article

August 2015 Vol.:4, Issue:1

© All rights are reserved by D. Sakthivel et al.

Extraction of Chitin and Chitosan from Mangrove Crab *Sesarma plicatum* from Thengaithittu Estuary Pondicherry Southeast Coast of India



IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals

ISSN 2349-7203



D. Sakthivel*, N. Vijayakumar and V. Anandan

Department of zoology, Kanchi Mamunivar Centre for
Post Graduate Studies, Lawspet, Pondichery –
605 008, India.

Submitted: 26 July 2015
Accepted: 1 August 2015
Published: 25 August 2015



HUMAN JOURNALS

www.ijppr.humanjournals.com

Keywords: Mangrove crab, *S. plicatum*, Chitin, Chitosan and antimicrobial activity

ABSTRACT

Chitin and chitosan are unique and typical marine polysaccharides waiting for future development and have been attracted the interest of many researchers from various disciplines. Chitin and its derivatives exhibit a variety of physicochemical and biological properties resulting in numerous applications. Chitin and chitosan are manufactured commercially on a large scale from the outer shells of shrimps, lobsters and crabs. In this present study to find out a novel source for natural antimicrobial 'chitosan', from the shell of mangrove crab *Sesarma plicatum* which was characterised by FT-IR spectroscopy. The present study represents the first attempt to investigate chitin and chitosan extracted from mangrove crab *S. plicatum* shell and the yield of chitin and chitosan from the *S. plicatum* shell was found as 18.46% and 41.37% respectively. The FT-IR spectrum of chitin 11 major peaks between 407 and 3404 cm^{-1} and chitosan 6 peaks between 408.04 and 3914.81 cm^{-1} was isolated from the mangrove crab of *S. plicatum* was obtained and compared with that of standard chitin 15 major peaks lying between 530.11 and 3766.27 cm^{-1} and chitosan 8 major peaks lying between 523.90 and 3434.37 cm^{-1} . The antibacterial activity of chitin and chitosan was showed the maximum zone of inhibition (7mm) was recorded in *Micrococcus sp* strain and minimum zone of inhibition (1mm) was observed in *V. parahaemolyticus* bacterial strain. The antifungal activity of maximum and minimum zone of inhibition (5mm & 2mm) was observed in *Rhizopus sp* fungal strain. From the above observation it is clear that the body shell of *S. plicatum* with rich chitin and chitosan value it can be used for alternate source as agriculture, medicine, pharmaceuticals, food processing, environmental protection, and biotechnology industry.

INTRODUCTION

Chitin, which is the second most abundant natural polysaccharide on earth after cellulose, is the structural component of the exoskeletons of crustaceans, insects, mushrooms and cell walls of certain fungi and green algae [1, 2]. The amount of chitin with respect to dry weight is the highest in crustaceans. Hence, crustacean shells are regarded the main source of chitin for the chemical industry [3]. Chitin is insoluble in water, in every common organic solvent and in acidic, basic and neutral aqueous solutions [4]. Chitosan (poly- β -(1 \rightarrow 4) N-acetyl-D-glucosamine), is the N-deacetylated derivative of chitin. A precise nomenclature with respect to the degree of N-deacetylation has not been defined between chitin and chitosan [5]. Chitosan is insoluble at neutral and alkaline pH, but is soluble in inorganic and organic acids including acetic, formic, lactic, hydrochloric, and glutamic acids. However, the chemical and biochemical reactivity of chitosan is higher than that of chitin since chitosan has free amino groups distributed regularly in its molecular chain [6]. Chitosan exhibits a variety of physicochemical and biological properties and therefore can be used in various fields such as the edible film industry, as additives to enhance nutritional quality of foods, for the recovery of solid materials from food processing wastes, and in the purification of water [2]. The properties that make chitosan commercially important are its biodegradability, biocompatibility in both, plant and animal tissues, non-toxicity and allergenicity, and the ability to transform into gels, beads fibers, colloids, films, flakes, powders and capsules [7, 8]. Additional exclusive characteristics of chitosan are its nondigestibility and bland taste that make it an excellent choice as a food additive component, predominantly in the preparation of low-calorie foods [9]. Chitosan and chitosan-based materials can be used as edible films or coatings. So far, edible coatings made from chitosan have been used on various foods such as tomatoes [10], Emmental cheese [11] and raw shrimps [12]. Chitosan films are tough, long-lasting, flexible, and difficult to tear [13]. In a study completed by [14], chitosan coatings, on fruits and vegetables, modified the internal atmosphere, thereby delaying ripening and decreasing transpiration. [15] Proven that chitosan films have moderate water permeability and could enhance the storage life of fresh products and foodstuff with high water activity.

Chitin and its derivatives are interest of research because they have various biological activities and can be used in various applications such as immunoadjuvant, wastewater treatments, and agrochemicals uses [16]. Chitin and chitosan are manufactured commercially on a large scale from the outer shells of shrimps, lobsters and crabs [8].

Shrimp and crab shells contain chitin, protein and inorganic compounds (mainly composed of calcium carbonate) [17, 16]. Chitosan, the deacetylated derivative of chitin, is one of the abundant, renewable, non-toxic and biodegradable carbohydrate polymers, and available largely in the exoskeletons of shellfish and insects. Therefore, chitosan has received much attention as a functional biopolymer for diverse applications, especially in pharmaceuticals, foods and cosmetics. These functions have been revealed to be dependent not only upon their chemical structure but also their molecular size. Especially in medicine and food industry, the application of the natural polysaccharide is limited since its high molecular weight results in low solubility in acid free aqueous media. Traditional methods for the preparation of chitin include demineralization and deproteinization of the raw materials with strong acids and bases (e.g. HCl and NaOH) [18, 16]. The production of chitin and its hydrolyzed derivatives, such as acetylglucosamine and chitooligosaccharide, from waste of the shellfish industry has been limited due to the high cost of chitinase and the shrimp and crab shell pretreatment process [18].

Chitin and chitosan oligomers are known to have various biological activities including antitumor activities [19], immune-enhancing effects [20], protective effects against infection with some pathogens [21], anti-fungal activities and antimicrobial activities [21]. Chitosan can inhibit the growth of a wide range of bacteria. This is due to the fact that chitosan possesses a high antibacterial activity, a broad spectrum of activity, a higher killing rate, and lower toxicity toward mammalian cells [22]. Several researches have been carried out to assess the antimicrobial activity of crab shell extract on various microbes but none has been done to assess its effect on fish pathogens. Therefore, this study not only aims to isolation of chitin and chitosan from mangrove crab *S. plicatum* and its characterization; to detect the antimicrobial activity of crab shell extract on fish pathogens, but also to highlight the economic importance of crab shell which will bring into notice to the Government of India.

MATERIALS AND METHODS

Extraction of chitin and chitosan

Mangrove crab *S. plicatum* species was collected from Thengaithittu Pondicherry region (Lat.11° 54' 23.1 N, Log 79° 48; 54.7"E); east coast of India. The shell obtained after the removal of body tissue was washed, air-dried and pulverised using pestle and mortar. Chitin was extracted from the shells of a mangrove crab, *S. plicatum* by demineralization and deproteinization. The pulverised shell was treated with 2N HCl for 24 hours to remove the mineral content and then treated with

1N NaOH at 80°C for 24 hours to remove protein [23]. The extracted chitin was converted into chitosan through deacetylation process [24]. Chitin was deacetylated in 40% aqueous NaOH by heating under reflux for 6 hours at 110°C and was cooled at room temperature. The obtained precipitate was washed with distilled water and stood for 12 hours at room temperature with constant stirring in 10% acetic acid solution. The pH was adjusted to 10 with 40% NaOH solution. The solution was dialyzed against deionized water for 24 hours. The product was centrifuged at 10,000 rpm for ten minutes and lyophilized to give chitosan.

FTIR (Fourier Transform-Infra Red) spectrum analysis

The dry samples of Chitin and chitosan (10mg) was mixed with 100mg of dried potassium bromide (KBr) and compressed to prepare as a salt disc. The disc was then read spectrophotometrically (Bio-Rad FTIR-40-model, USA). The frequencies of different components present in each sample were analyzed.

Antimicrobial assay

Chitin and chitosan sample was tested for inhibition against the fish pathogenic bacteria (*Aeromonas hydrophila*, *Vibrio parahaemolyticus*, *V. cholerae*, *Micrococcus sp.*, and *Streptococcus sp.*) and fungi (*Aspergillus flavus* & *Rhizopus sp.*). Microbial assay were carried out by disc diffusion technique [25]. Pathogenic strains were inoculated in sterile nutrient broth and incubated at 37°C for 24h. Pathogens were swabbed on the surface of the Muller Hinton Agar and Czapek Dox Agar plates and discs (Whatman No.1 filter paper with 6 mm diameter) were impregnated with the 50 µl of Chitin and chitosan sample on the surface. Control discs were placed with antibiotic on pathogens. The plates were incubated at 37°C for 24 h and the antimicrobial activity was measured based on the inhibition zone around the disc impregnated.

RESULTS

The present study represents the first attempt to investigate various physiochemical properties of chitosan extracted from mangrove crab *S. plicatum* shell. The yield of chitin and chitosan is varying in different animals of the same group or different groups. In the present study the yield of chitin and chitosan from the *S. plicatum* shell was found as 18.46% and 41.37% respectively.

The FT-IR spectrum of chitin and chitosan was isolated from the mangrove crab of *S. plicatum* was obtained and compared with that of standard chitin and chitosan. The FT-IR

spectrum of standard chitin showed 15 major peaks lying between 530.11 and 3766.27 cm^{-1} ; whereas the FT-IR spectrum of chitin of *S. plicatum* recorded only 11 major peaks between 407 and 3404 cm^{-1} (Fig.1 and 2). The FT-IR spectrum of the standard chitosan reported 8 major peaks lying between 523.90 and 3434.37 cm^{-1} ; whereas the FT-IR spectrum of the chitosan sample from mangrove crab of *S. plicatum* recorded 6 peaks between 408.04 and 3914.81 cm^{-1} (Fig. 3 and 4).

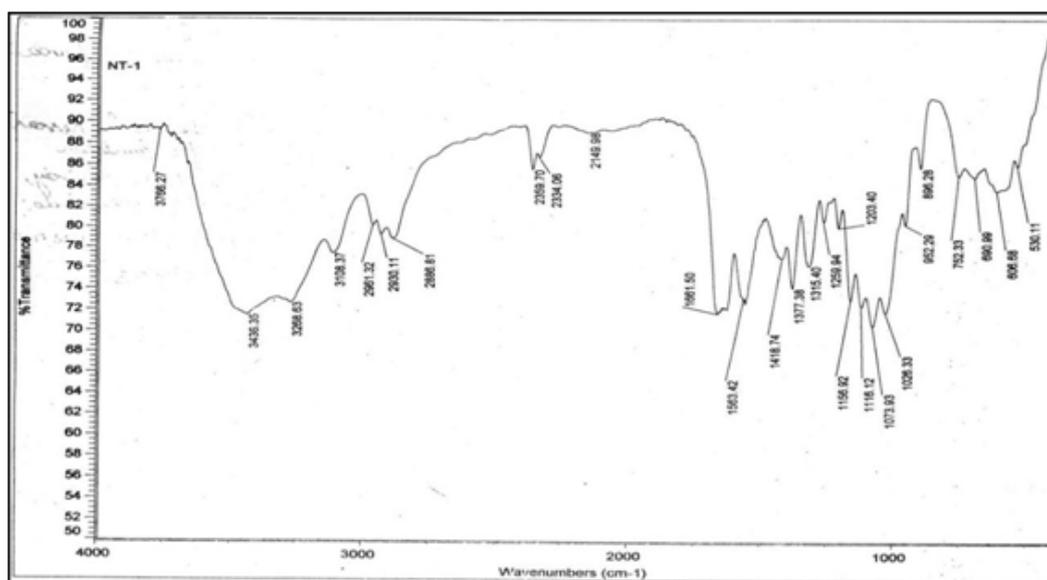


Figure 1. The FT - IR spectrum of standard chitin

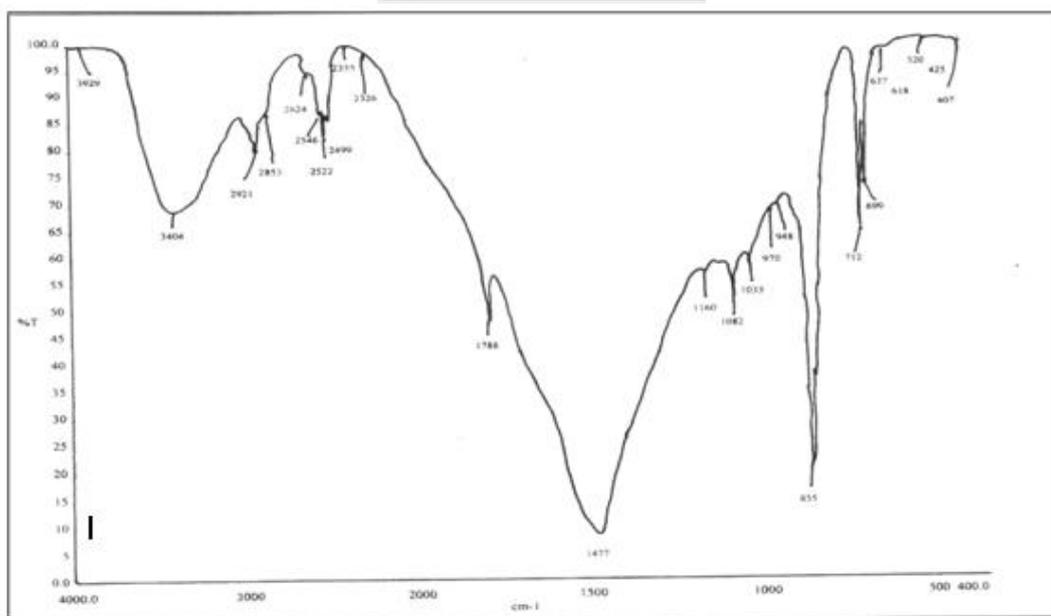


Figure 2. The FT - IR spectrum of chitin from *S. plicatum* mangrove crab

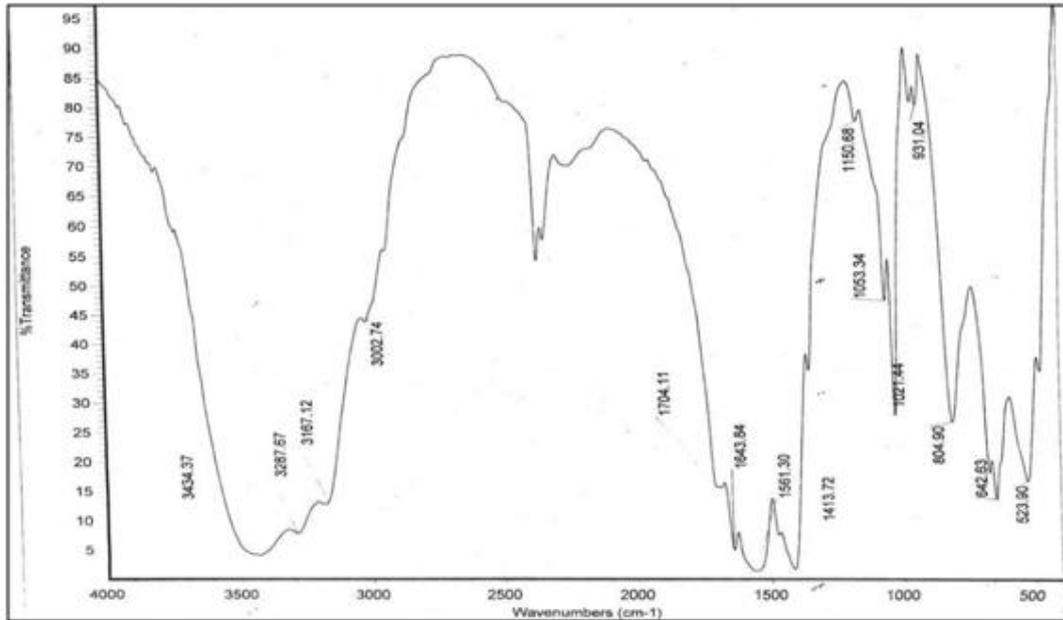


Figure 3. The FT - IR spectrum of standard chitosan

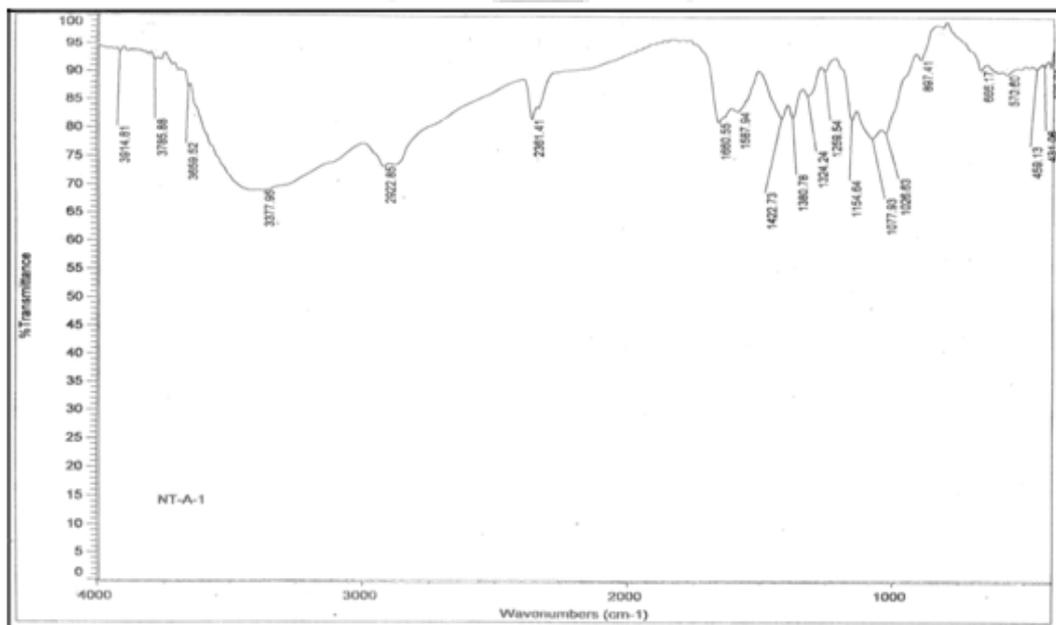


Figure 4. The FT - IR spectrum of chitosan from *S. plicatum* mangrove crab

In present study the zone of inhibition in different bacterial strains against chitin and chitosan was showed in (Fig. 5). Among the various bacterial strains maximum zone of inhibition (7mm) was recorded in *Micrococcus sp* strain and minimum zone of inhibition (1mm) was observed in *V. parahaemolyticus* bacterial strain. The antifungal activity of maximum and minimum zone of inhibition (5mm & 2mm) was observed in *Rhizopus sp* fungal strain. The positive control (antibiotic) was observed activity against all the microbial strains tested; the maximum activity against *V. parahaemolyticus* (6mm).

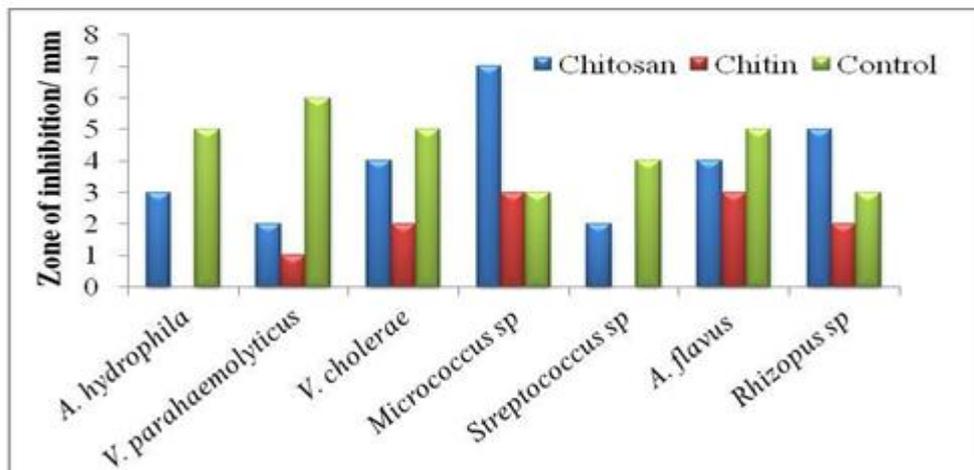


Figure 5. Antimicrobial activity of chitin and chitosan



B1- Chitosan, B2- Chitin, C- Control

Figure 6. Plate 1 antibacterial & plate 2 antifungal activities

DISCUSSION

Natural and non-toxic biopolymers chitin and chitosan are now widely produced commercially from crab and shrimp waste shells. During the past few decades, chitin and chitosan have attracted significant interest in view of a wide range of proposed novel applications. Their unique properties, biodegradability, biocompatibility and non-toxicity make them useful for a wide range of applications [26]. Chitin is mainly used as the main raw material to produce chitin derived products, such as chitosans, oligosaccharides, and glucosamine [27]. There are now over 2,000 concrete applications, and the field of nutrition is the largest user of chitosan with 1000 tons consumed in 2000. The industrial worldwide

production of these derivatives in year 2000 is estimated to be above 10,000 tons [28]. Chitin and chitosan show excellent biological properties such as non-toxicity [29], which is illustrated by a dose limit of $17 \text{ g kg}^{-1} \text{ day}^{-1}$ [30], antibacterial [31], since it offers excellent biocompatibility and biodegradation profile in physiological environment. Chitin is also used as an excipient and drug carrier in film, gel or powder form for applications involving mucoadhesivity.

Crabs are defined as a biological indicator in the ecological studies. Among the 4500 species of crab over the world, only 22 crab species have been economically evaluated [32]. Many researchers have been carried out on biological, systematic, anatomic and morphological characteristics and food chemistry of crabs [33]. The exoskeleton of crustacean is the most important industrial source for production of chitin now-a-days. All over the seas of the world, the amount of this biopolymer is estimated to be 1.560 million tons. In every year, about 100 billion tones of chitin are produced by crustaceans, mollusks, insects, and fungi. Annual synthesis of this polysaccharide in freshwater ecosystem is estimated to be about 600 million tons [34]. The crabs whose 1000 species exist in the marine water can be evaluated as an important source of chitin indicated that the production of chitin and chitosan is currently based on crab discarded by [35]. The present study represents the first attempt to investigate various physiochemical properties of chitosan extracted from mangrove crab *S. plicatum* shell. The yield of chitin and chitosan is varying in different animals of the same group or different groups. In the present study the yield of chitin and chitosan from the *S. plicatum* shell was found to be 18.46% and 41.37% respectively. [36] Reported that the isolation of chitin and chitosan from shells of crab *Sylla cerrata*, lobster *Panulirus ornatus*, prawn *Paeneaus indicus* was 12.0%, 23.0%, 15.7% and 28.0% respectively; the yield of chitosan was 66.0%, 74.6% 74.3% and 75.0% from chitin. [37] Recorded that the chitosan yield of crab shell was determined as 4.65% from grinded crab shell after demineralization (yield is 34.32%), deproteinization (yield is 7.25%), decoloration (yield is 6.83%) and deacetylation processes. [38] Reported that chitin content of crab (*Chionoecetes opilio*) is around 10.6%. Content of chitin was determined as 14% in blue crab [39], in *Chionoecetes opilio* and *Pandalus borealis* between 17 and 32.2% [40]. It has been reported that the differences in the quantities of chitin between different crabs varied according to the species and season [41]. It is generally accepted that 20-30% of crustacean waste is chitin [42]. The yield of chitin and chitosan from mangrove crab *S. plicatum* was high and low when compared to that of other crustaceans, which may be due to the high chitosan content of the crab shell.

The FT-IR spectrum of chitin and chitosan was isolated from the mangrove crab of *S. plicatum* was obtained and compared with that of standard chitin and chitosan. The FT-IR spectrum of standard chitin showed 15 major peaks lying between 530.11 and 3766.27 cm^{-1} ; whereas the FT-IR spectrum of chitin of *S. plicatum* recorded only 11 major peaks between 407 and 3404 cm^{-1} . The FT-IR spectrum of the standard chitosan reported 8 major peaks lying between 523.90 and 3434.37 cm^{-1} ; whereas the FT-IR spectrum of the chitosan sample from mangrove crab of *S. plicatum* recorded 6 peaks between 408.04 and 3914.81 cm^{-1} . [43] Reported that the infrared spectrum of chitin showed the absorbance bands of 3419.67 cm^{-1} & 858.98 cm^{-1} and chitosan showed the absorbance bands at 3431.29 cm^{-1} & 608.22 cm^{-1} . The region between 3000 cm^{-1} and 3500 cm^{-1} shows the stretching of OH groups. This band is broad because of the hydrogen bonds. The OH band overlaps the stretching band of NH. Another significant change is observed in the region from 1000 cm^{-1} to 1200 cm^{-1} . In this region chitosan presents a broad band centered at 1084.93 cm^{-1} associated with the stretching of C=O. The studies in the literature about FTIR spectroscopy related with chitosan showed some characteristic peaks, which are at 2940 cm^{-1} (-CH₃, -CH₂), 1655 cm^{-1} (C=O stretch vibration of secondary amide I band), 1555 cm^{-1} (N-H bending vibration of amide II band), 1570 cm^{-1} (N-H bending vibration of primary amides) and 1070 cm^{-1} (C-O stretching) [44]. FTIR analysis reveals the presence of antimicrobial compound signals at different ranges. The research of the mangrove crab *S. plicatum* chitosan has medicinal value due to high quality of antimicrobial compounds.

Chitosan can inhibit the growth of a wide range of bacteria. This is due to the fact that chitosan possesses a high antibacterial activity, a broad spectrum of activity, a higher killing rate, and lower toxicity toward mammalian cells [22]. In present study the zone of inhibition in different bacterial strains against chitin and chitosan was showed. Among the various bacterial strains maximum zone of inhibition (7mm) was recorded in *Micrococcus sp* strain and minimum zone of inhibition (1mm) was observed in *V. parahaemolyticus* bacterial strain. The antifungal activity of maximum and minimum zone of inhibition (5mm & 2mm) was observed in *Rhizopus sp* fungal strain. The positive control (antibiotic) was observed activity against all the microbial strains tested; the maximum activity against *V. parahaemolyticus* (6mm). [45] Studied the antimicrobial effects of chitosan in a liquid medium after incubation at 30°C for 24 hrs, *Pseudomonas fragi*, *B. subtilis* and *S. aureus* were inhibited by 0.01% chitosan whereas *E. coli* was inhibited at 0.1%. *Lactobacillus plantarum* and *P. pentosaceus* were also inhibited at 0.1% chitosan. [46] Reported that the antimicrobial activity of crab

shell extract the average minimum inhibitory concentration of *Klebsiella pneumoniae* was determined to be 10.42 µg/ml and the activity of the 1% acetic acid used. Minimum lethal concentrations MLC can be defined as the lowest concentration of a toxic substance in an environmental medium that kills individual organisms or test species under a defined set of conditions [47]. [48] Studied the effect of three different concentrations of chitosan (0.5, 1.0 and 2.0%) on four different bacteria (*L. monocytogenes*, *S. entericserovar*, *S. aureus*, and *S. cerevisiae*) using a suspension test and a surface test procedure. [49] Studied the antimicrobial effects of chitosan using chitosan acetate, a derivative of chitosan. Chitosan acetate exhibited significant inhibitory activity against various food-borne enteropathogenic bacteria including *E. coli*, *V. vulnificus*, *Shigella sonnei*, *S. typhi* and *S. enteritidis* by measuring the minimum bacterial growth inhibitory concentrations (MICs) and bacterial survival fraction. To improve the antimicrobial action of chitosan films, other preservatives can be incorporated, such as organic acids. Chitosan was exhibited significant inhibitory activity against various pathogenic bacterial and fungal strains. Chitosan is antimicrobial against a wide range of target organisms. Activity varies considerable with the type of chitosan, the target organism and the environment in which it is applied.

Pharmaceutical industry is in need of different types of chitosan presently available in the market which are to be refined further more to meet the required standards. For instance Chitosan used in the wound healing and scaffolds must be in the form of oligomeres having low molecular weight in former case whereas later ones need more proliferated structure and high molecular weight for tissue engineering. The importance of the biopolymer chitin and chitosan resides in their biological (biodegradability, biocompatibility and non-toxicity) and physicochemical properties (degree of acetylation and MW). These unique properties offer much potential applications in many fields. The recovery of chitin by chemical method using concentrated acids and bases in order to deproteinize and to demineralize shellfish shells (the most industrially exploited) at high temperature can deteriorate the physicochemical properties of this biopolymer and consequently their biological properties and gives rise to products of varying quality, not reproducible and non homogeneous. Studies on chitin and chitosan from crab in India are limited particularly in mangrove crabs. This might be due to lack of awareness on benefits of these chitosan particularly from mangrove crab. The results of the present study provide information about the chitin and chitosan composition, but also suggest the consumption of this mangrove crab tissue. It is rich in chitin and chitosan. Further, the presence of chitin and chitosan in *S. plicatum* shell

adds more value through the possibility. From the above observation it is clear that the shell body of *S. plicatum* with rich chitin and chitosan value can be used for alternate source as a agriculture, medicine, pharmaceuticals, food processing, environmental protection, and biotechnology industry.

ACKNOWLEDGEMENT

Authors are thankful to Prof. V. Anandan Director, Kanchi Mamunivar Centre for Post Graduate Studies for giving facilities and encouragement during the study period.

REFERENCES

- [1] Muzzarelli RAA, Jeuniaux C and Gooday GW. Natural chelating polymers, Pergamon Press, New York; 1973.
- [2] Shahidi F, Arachchi JKV and Jeon YJ Food applications of chitin and chitosan. *Trends in Food Science and Technology* 1999; 10: 37-51.
- [3] Jeuniaux C, Voss-Foucart MF, Poulieck M and Bussers JC. Sources of chitin, estimated from new data on chitin biomass and production. In Chitin and chitosan: sources, chemistry, biochemistry, physical properties and applications. Elsevier Science Publishers Ltd., London and New York. 1989; 3-9.
- [4] Madhavan P. Chitin, Chitosan and their Novel Appli- cations, Science Lecture Series, CIFT, Kochi, 1992; 1.
- [5] Zikakis JP (Ed). Chitin, chitosan and related enzymes. Academic Press, Orlando, Fl; 1984.
- [6] Ogawa, K., Yui, Toshifumi, Y., and Okuyama, K. Three D Structures of chitosan. *Int J of Biological Macromolecules* 2004; 34(1-2):1-8.
- [7] Ravi Kumar MNV. A review of chitin and chitosan applications. *Reactive and functional polymers* 2000; 46: 1-27.
- [8] Senel S and McClure SJ. Potential applications of chitosan in veterinary medicine. *Advanced drug delivery reviews* 2004; 56: 1467-1480.
- [9] Muzzarelli RAA. Chitosan - based dietary foods. *Carbohydrate Polymers* 1996; 29: 309-316.
- [10] El Ghaouth E, Arul J, Grenier J and Asselin A. Chitosan coating to extend the shelf life of tomatoes. *Hort Science* 1992; 27 (9):1106-1018.
- [11] Coma V, Gros-Martial A, Garreau S, Copinet A, Salin F and Deschamps A. Edible antimicrobial films based on chitosan matrix. *J Food Sci* 2002; 67 (3): 1162-1169.
- [12] Simpson BK, Gagne N, Ashie INA and Noroozi E. Utilization of chitosan for preservation of raw shrimp (*Pandalus borealis*). *Food Biotechnology* 1997; 11(1): 25-44.
- [13] Jeon YJ, Kamil JYVA and Shahidi F. Chitosan as an edible film for quality preservation of herring and Atlantic cod. *Journal of Agricultural and Food Chemistry*, 2002; 50 (18):5167-5178.
- [14] Arai L, Kinumaki Y and Fujita T. Toxicity of chitosan. Bull. Tokai Reg. *Fish Res Lab* 1968; 56:89.
- [15] Kittur FS, Kumar KR and Tharanathan RN. Functional packaging properties of chitosan films. *Unters. Forsch. Berlin, New York, Springer-Verlag* 1998; 206:44-47.
- [16] Wang SL and Chio SH. Deproteinization of shrimp and crab shell with the protease of *Pseudomonas aeruginosa* K-187. *Enzyme and Microbial Technology* 1998; 22: 629-633.
- [17] Carroad PA, Tom RA. Bioconversion of shellfish chitin waste: process conception and selection of microorganism. *J Food Sci* 1978; 527:58-60.
- [18] Cosio IG, Fisher RA and Carroad PA. Bioconversion of shellfish waste: waste pretreatment, enzyme production, process design, and economic analysis. *J food Sci* 1982; 47: 901.
- [19] Pae HO, Seo WG, Kim NY, Oh GS, Kim GE, Kim YH. Induction of granulocytic differentiation in acute promyleotic leukemia cells (HL-60) by water soluble chitosan oligomer. *Leukemia Res* 2001; 25:339-46.
- [20] MacLaughlin FC, Mumper RJ, Wang J, Tagliaferri JM, Gill I, Hinchcliffe M., Chitosan and depolymerized chitosan oligomers as condensing carriers for in vivo plasmid delivery. *J Control Release* 1998; 56:259-72.

- [21] Wang XH, Li DP, Wang WJ, Feng QL, Cui FZ, Xu YX, Song XH and van der Werf M. Crosslinked collagen/chitosan matrix for artificial livers. *Biomaterials* 2003; 24:3213–3220.
- [22] Takemono K, Sunamoto J and Akasi M. *Polymers & Medical care*. Chapter IV. Tokyo, Mita 1989.
- [23] Takiguchi Y, Chen RH and Chen HC. Physical properties of chitinous materials. In: advances in chitin science. (Eds.), Vol. III Proceeding from the third Asia-Pacific Chitin, Chitosan Jikken manual chapter 1, Gihodou Shupan Kabushki -Kasisha, Japan, 1991a; 1-7.
- [24] Takiguchi Y, Otakara A and Yabuki M. Preparation of chitosan and partially deacetylate chitin. In: Chitin, chitosan - Jikken manual (Eds.), chapter 2, Gihodou Shupan Kabushki Kaisha, Japan, 1991b; 9 – 17.
- [25] Kelman D, Kashman Y, Rosenberg E, Ilan M, Iirach I and Loya Y. Antimicrobial activity of the reef sponge *Amphimedon viridis* from the Red Sea evidence for selective toxicity. *Aquat Microb. Ecol* 2001; 24: 9-16.
- [26] Wang SL, Chang TJ and Liang TW. Conversion and degradation of shellfish wastes by *Serratia* sp. TKU016 fermentation for the production of enzymes and bioactive materials. *Biodegradation* 2010; 10532-009-9303.
- [27] Einbu A, Varum KM. Characterization of chitin and its Hydrolysis to GlcNAc and GlcN. *Biomacromolecules* 2008; 9, 1870-1875.
- [28] Kurita K, Chitin and Chitosan. Functional biopolymers from marine crustaceans. *Marine Biotechnol* 2006; 8, 203- 226.
- [29] Morimoto M, Saimoto H, Yoshihiro S. Control of functions of chitin and chitosan by chemical modification. *Trends Glycosci Glycotechnol* 2002; 14, 205–222.
- [30] Crini, G., Guibal E, Morcellet M, Torri G and Badot PM. Chitine et chitosane. Préparation, propriétés et principales applications. In: chitine et chitosane. Du biopolymère à l'application, 1st Ed., Presses universitaires de Franche-Comte, France, 2009; 19-54.
- [31] Muzzarelli C, Francescangeli O, Tosi G, Muzzarelli, RAA. Susceptibility of dibutyl chitin and regenerated chitin fibres to deacylation and depolymerization by lipases. *Carbohydr Polym* 2004; 56: 137–146.
- [32] Cumberland N, Ng PKL, Yeo DCJ, Magalhaes C, Campos MR, Alvarez F, Naruse T, Daniels SR, Eser LJ, Attipoe FYK, Clotilde-Ba FL, Darwal W, Mclvor A, Baillie JEM, Collen B and Ram M. Freshwater crabs and the biodiversity crisis: Importance, threats, status, and conservation challenges. *Biol Conserv* 2009; 142: 1665-1673.
- [33] Gulle P, Turna II and Gulle I. Some reproductive and population characteristics of fresh water crab (*Potamon potamios* Olivier. 1804) in Egirdir Lake. Univ Suleyman Demirel. *J Sci Inst* 2007; 11: 134-139.
- [34] Cauchie HM. An attempt to estimate crustacean chitin production in the hydrosphere. In: Advances in Chitin Science: Vol 2: (Domard A, GAF Roberts, KM Varum, eds): Jacques Andre Publisher, Lyon, France, 1997; 32.
- [35] Felicity burrows, Clifford louime, Michael Abazinge and Oghenekome Onokpise,. Extraction and evaluation of chitosan from crab exoskeleton as a seed fungicide and plant growth enhancer. *J Agri & Envi Sci* 2007; 2(2), 103-111.
- [36] Peter Michael Oduor-Odote, Marcin Struszczyk H and Martin Peter G. Characterisation of Chitosan from Blowfly Larvae and Some Crustacean Species from Kenyan Marine Waters Prepared Under Different Conditions. *J Mar Sci* 2005; 4 (1): 99–107.
- [37] Yildiz Bolat, Şengul Bilgin, Ali Gunlu, Levent Izci, Seval Bahadır Koca, Soner Çetinkaya and Habil Ugur Koca. Chitin-Chitosan Yield of Freshwater Crab (*Potamon potamios*, Olivier 1804) Shell. *Pakistan Veterinary Journal* 2010; 0253-8318.
- [38] Hertrampf JW and Piedal-Pascual F. Handbook on ingredients for aquaculture feeds. Kluwer Academic Publishers, Dordrecht, the Netherlands, 2000; 109-113.
- [39] Tharanathan RN and FS Kittur,. Chitin-the undisputed biomolecule of great potential. *Critical Rev Food Sci Nutr* 2003; 43: 61–87.
- [40] Shahidi F and Synowiecki J. Isolation and characterization of nutrients and value added products from snow crab (*Chionoecetes opilio*) and shrimp (*Pandalus borealis*) processing discards. *J Agri Food Chem* 1991; 39: 1527-1532.
- [41] Cho YI, No HK and Meyers SP. Physicochemical characteristics and functional properties of various commercial chitin and chitosan products. *J Agric Food Chem* 1998; 46 3839-3843.

- [42] Johnson EL and Peniston QP. Utilization of shellfish waste for chitin and chitosan production. In *Chemistry and Biochemistry of Marine Food Products*; AVI Publishing: Westport, 1982; 19.
- [43] Shanmugam Annaian, Namasivayam Subhapradhaa, Shankar Sumana, Pasiyappazham, Characterization of Biopolymer “Chitosan” From the Shell of Donacid Clam *Donax Scortum* (Lins, 1758) and Its Antioxidant Activity. *IJPPS* 2012; 4, (2): 0975-1491.
- [44] Choi CY, Kim SB, Pak PK, Yoo D and Chung YS. Effect of N-acylation on structure and properties of chitosan fibers. *Carbohydrate Polymers* 2007; 68: 122–127.
- [45] Darmadji P and Izumimoto I. Effect of chitosan in meat preservation. *Meat Science* 1994; 38: 243-254.
- [46] Tracy Adole and Barry A. Omogbai. Antibacterial Effect of Crab Shell Extract on *Klebsiella pneumoniae* and *Proteus mirabilis*. *Journal of Pharmacy and Biological Sciences*.2012; 01-06.
- [47] McNaught AD and Wilkinson A. Compendium of chemical terminology: IUPAC recommendations. 2nd ed. Oxford, Malden, MA; 1997.
- [48] Knowles J and Roller S. Efficacy of chitosan, carvacrol, and a hydrogen peroxide based biocide against foodborne microorganisms in suspension and adhered to stainless steel. *J Food Prot* 2001; 64(10):1542-1548.
- [49] Park JH, Cha B and Lee YN. Antibacterial activity of chitosan acetate on food borne enteropathogenic bacteria. *Food Sci Biotechnol* 2003; 12(1): 100-103.

