Comparative Study on Yielding Capacity of Chitin and Chitosan Derived from Two Marine Crab Shell Wastes and their Antimicrobial Activity

B. Sangeetha, V. Indra and D. Sheela

Research Journal of Agricultural Sciences
An International Journal

P- ISSN: 0976-1675
E- ISSN: 2249-4538

Volume: 12
Issue: 04

Res Jr of Agril Sci (2021) 12: 1408–1412

CARAS

Res. Jr. of Agril. Sci. (July-Aug 2021) 12(4): 1408–1412
www.rjas.org

Full Length Research Article

Comparative Study on Yielding Capacity of Chitin and Chitosan Derived from Two Marine Crab Shell Wastes and their Antimicrobial Activity

B. Sangeetha[1], V. Indra*[2] and D. Sheela[3]

Received: 17 May 2021 | Revised accepted: 19 Jul 2021 | Published online: 14 Aug 2021

A B S T R A C T

Chitin and Chitosan is a natural biopolymer have become of great interest not only as underutilized resources, but also as new functionalized materials of high potentials in various fields. Although synthesized by various organisms crustacean's shells dominate the production. The present study was aimed to focus on the extraction of chitin and chitosan from two marine crabs i.e., *Portunus sanguinolentus* and *Scylla serrata* by following chemical extraction method and yielding capacity was compared and antibacterial potential of the end product was investigated. The chemical extraction method resulted in 23.8% (in *P. sanguinolentus*) and 20.3% (in *S. serrata)* chitin yield and 28.5% *(P. sanguinolentus*) and 19.5% (*S. serrata)* chitosan on dry weight basis respectively. FTIR and XRD patterns displayed bands corresponding to the stretching and vibration of OH, NH and CO band confirming the presence of chitin and chitosan. The percentage yield of chitin and chitosan in *P. sanguinolentus* was higher than the *S. serrata* same trend of results were obtained for antibacterial activity using gram positive and gram-negative strains. The obtained for chitin and chitosan samples were indicated that the chitosan showed zone of inhibition was minimum in (*S. serrata)* and maximum activity in (*P. sanguinolentus)* on all the tested pathogens.

***Key words*:** Chitin, Chitosan, UV-vis, XRD, FT-IR, Antibacterial activity

Chitin is synthesized from many different organisms and is typically isolated from the cell walls of fungi and algae, the exoskeleton of insects, endoskeleton of cephalopods and shells of mollusks and crustaceans [1]. However, the primary commercial sources of chitin are crab and shrimp shells. Crustaceans include crabs, lobsters, crayfish, shrimp, krill, woodlice and barnacles. Traditionally, crustacean shells as by-products of the seafood processing industry constitute the primary and commercial source of chitin. For instance, shrimp wastes contain high concentrations of protein, which stem primarily from the skeletal tissues. This skeletal tissue is comprised of a calcified protein-chitin matrix, which is responsible for the hard shells of crustaceans [2]. The crustacean shells are assembled from three fundamental components namely: (a) chitin, (b) minerals and (c) proteins. Chitin serves as the skeleton which is enriched with minerals, mainly inorganic carbonate salts that strengthen the shells while proteins render the shells as living tissues [3]. On a dry weight basis, crustacean shell waste consists of approximately 40% protein, 35% minerals, 20% chitin and 5% lipids. However, the actual chitin content will vary depending on species, the health of the animals, harvesting season and geographical location. For example, the chitin content in crab shells may be as high as 32% as compared to less than 20% in shrimp shells [4-5]. Extraction of chitin requires the removal of proteins and a tiny amount of pigments and lipids by deproteinization and inorganic calcium carbonate by demineralization. In some cases, an additional step of decolourization is applied to remove the excess residual pigments [6]. The term biopolymer is used to identify polymers which can be synthesized from living organisms. Chitin and chitosan are examples of biopolymers that have received considerable research interests due to their potential applications in agriculture and food, biomedicine and pharmaceutical, papermaking and textile industries, cosmetics and wastewater treatment [7]. Structurally, chitin is a linear polysaccharide, made of N-acetyl-D-glucosamine units connected by β (1→4) linkages. Every year, approximately 100 billion tons of chitin is produced by crustaceans, mollusks, insects, fungi and related organisms every year [8]. When the acetyl-D-glucosamine units in chitin lose its acetyl groups in a process of deacetylation, the molecule is called chitosan.

Although chitin and chitosan can be extracted from various terrestrial and aquatic organisms, commercial chitin and chitosan are mostly extracted and obtained from

* V. Indra
✉ indravadivel@gmail.com
[1-3] Department of Zoology, Presidency College, Chennai - 600 005, Tamil Nadu, India

crustacean wastes (i.e., crabs, shrimps and krill). Hence, most of the studies are based on the α-chitin/chitosan extracted from crustacean shells or animal source. Currently, chitin is commercially extracted using chemical methods which involve the removal of minerals and proteins using strong acids and bases at high temperatures. These processes not only require high energy consumption but create environmental issues as the effluents generated must be neutralized by adequate treatments [9]. Besides, there is a high cost associated with the purification of chitin extracts from crustacean shells as well as the allergen they may possess to individuals who are at high risk to shell and seafood exposure. Furthermore, an examination of the extracts from this method has shown inconsistencies that have led to the production of chitin/chitosan with variable physicochemical properties [10]. As a result, alternate sources of raw materials and extraction methods may mitigate or reduce the drawbacks of the conventional source and process which need to be explored and investigated for their potential to extract chitin/chitosan. Emerging research has examined sources such as insects and fungi as potential raw materials for chitin/chitosan production [11-14]. Depending on the origin of a sample, chitin/chitosan can exhibit a variety of chemical, physical and biological properties [15]. According to elementary studies and analyses of different crustaceans (crab shrimp, lobster, and squid), there was great variability of this composition when chitin amounts were varied from species to species. Hence, there is a need to develop efficient demineralization and deproteinization processes to remove mineral content (20–30%) and protein content of approximately 40% in order to obtain chitin that is free of inorganic and protein content. This study showed that different concentrations of NaOH and demineralization with hydrochloric acid and acetic acid influenced the yield of the extraction process used to obtain chitin and chitosan.

MATERIALS AND METHODS

Marine crabs were purchased from the Kasimedu Fish Landing Centre at Chennai (13° 7′ 31.98″ N, 80° 17′ 51.52″ E), Tamil Nadu. India. They were brought to the laboratory and shells were separated and washed thoroughly using running tap water and finally rinsed with double distilled water. The shells were dried at room temperature for a week and crushed well. They were ground well in the motor and pestle to obtain fine article particle for further studies. Both the crabs were identified as *Portunus*, *Sanguinolentus* and *Scylla serrata* (Fig 1A-B), and authenticated by Chief Scientist, Dr. C. P. Balasubramanian, CIBA, Chennai, Tamil Nadu.

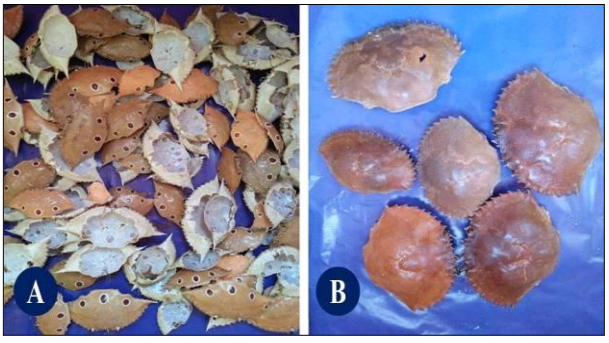

Fig 1 Shells of *Portunus sanguinolentus* and *Scylla serrata*

Demineralization

30grams of course samples was mixed with concentrate 12% HCL and then was in 200ml of double distilled water. Then the sample was stirred at room temperature for 30 minutes. Then washed it thoroughly with double distilled water and filtered until to reach obtain PH 7. After these process completions it was dried on a hot plate for 5 minutes.

$$2HCl + CaCO_3\ CaCl_2 + H_2O + CO_2 \uparrow$$

Deproteinization

The demineralized samples were combined with 15 gram of NaOH in 200 ml of double distilled water and maintained the sample 60°C with 30 minutes on hot plate. Then the sample was washed thoroughly with double distilled water to obtain until pH 7. If pH exceeds more than 7 same procedure was repeated to remove protein molecules.

Decolouration

The same samples were mixed with potassium permanganate ($KMnO_4$) in a glass beaker and maintained for 3 hours in a water bath to removal of pigmentation. And then washed with water 5 to 7 times until to reach pH. 7 with double distilled water. Finally, to get the product is chitin.

Deacetylation

With chitin 80%NaOH with 200 ml of water are mixed together and kept for 4 hours. The samples are then completely rinsed with water until pH 7. The finishing material was grinded as well as the powder name as chitosan for yields for different levels of different crab waste shells.

Chitin and chitosan extraction from crab shells

Chitin and chitosan were extracted by following the Abdulkarim *et al.* [16] and Juárez-de la Rosa *et al.* [17], flow chart representing the schematic procedure in detail in (Fig 2).

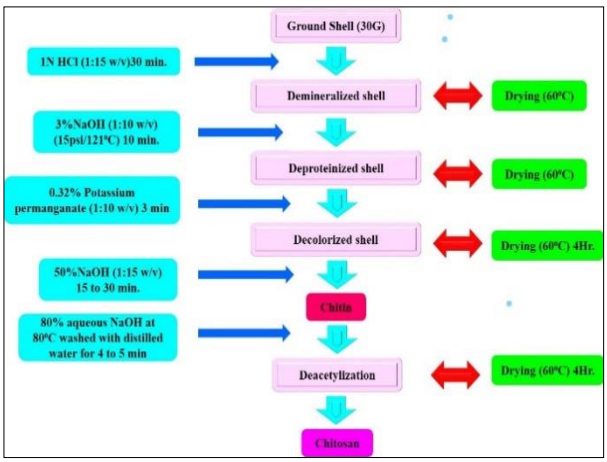

Fig 2 Flow chart represents the schematic procedure for the extraction of chitin and chitosan

Antibacterial activity of chitosan extracted from *P. sanguinolentus* and *Scylla serrata*

To study the antibacterial activity of chitosan of the crab shell wastes gram positive bacterial strains like (*Staphylococcus aureus, Bacillus cereus, Enterococcus faecalis, Bacillus subtilis*) and gram-negative bacterial strains like (*E. coli, Klebsiella pneumonia, Proteus spp, Shigella spp*) strains were purchased from king institute, Chennai.

Disc diffusion method was followed to measure the zone of inhibition and DMSO was used as blank and amikacin as a standard against tested clinical pathogens according to the method of [18].

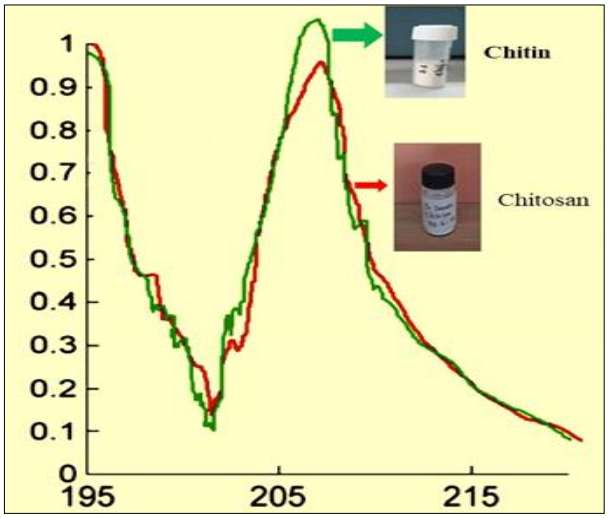

Fig 3 UV spectral analysis of Chitin and Chitosan

Spectral characterization

Spectral characterization of Chitin and Chitosan was done by UV-vis (PHILIPS, T10), FT-IR (Bruker alpha) and XRD (CuK-Alpha: (λ=0.154056 Angstrom).

RESULTS AND DISCUSSION

The nanoparticle formation was monitored by the UV-vis spectra using a UV-vis spectrophotometer. The UV-spectrum of chitin and chitosan was recorded at a wavelength of 215nm within 20 minutes. The formation of AuNPs is due to the excitation of electrons. Present in the chitin and chitosan. The colour change from pale white to bright white which further confirms the formation of AuNPs.

UV-vis spectrum can be used for optical characterization of chitin and chitosan as stated by Juárez-de la Rosa *et al*. [19]. Similar results were observed in chitin and chitosan isolated from *P. sanguinolentus, S. serrata*, [20] F. solani et al. (2015). FT-IR is an important technique for structural analysis of biomolecules and is especially used in determining active functional groups present in the selected from two marine crab samples such as *P.sanguinolentus* and *S.serrata.* The FT-IR spectrum was used to determine the active functional groups of compounds based on the peak value in the area of infrared radiation. In the present study crab *P. sanguinolentus*, *S. serrata* shell powder was conceded into the FT-IR as well as the functional groups. Groups of the components were separated based on the ratio are recorded and identified [21-22]. The presence of amide, alcohol, phenolic, carboxylic acids aldehydes, ketones, alkanes, primary amines, aromatics, esters ,ethers, alkyl amides and aliphatic amine groups. The FT-IR spectrum of present study functional groups of active compounds were identified with standard peaks at 921.6cm-1, 4060 cm-1 as possible groups.

FT-IR peaks values of *P. sanguinolentus* crab shell powder showed 921.6, 1492,1153,1128 showed the presence of alkyl groups; 1641 confirms the presence of alkene, 2999 showed the presence of S1-0 which indicate the aliphatic alkane (CH_2)n 4060cm-1(H-H) stretch, strong banded groups.

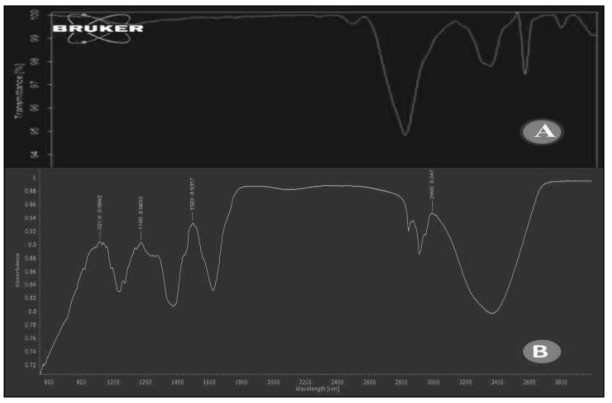

Fig 4 FT-IR spectral image A-Chitin and B-Chitosan

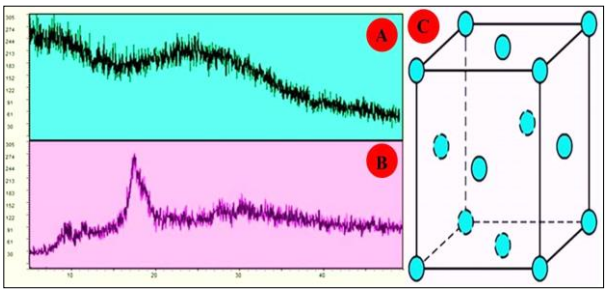

Fig 5 XRD spectrum of A- Chitin, B- Chitosan and C- FCC lattice structure of Chitin and Chitosan

Thus, the XRD pattern chitin and chitosan displayed crystalline in nature (Table 1). In the present study, the yield of chitin was found more in the shell of *Portunus sanguinolentus* (23.8 g) than that of chitin yield present in *Scylla serrata* representing (22.3 g). Whereas, the yield of chitosan was also found to be more in the shell of *Portunus sanguinolentus* than the *Scylla serrata*. The content of chitin and chitosan in crab shell varies depending on species as well as the physicochemical parameters similar results were reported by Das *et al.* [23] in *Scylla serrata* and *Portunus sanguinolentus*.

Table 1 Yield of chitin and chitosan from *Portunus sanguinolentus* and *Scylla serrata*

Name of the crab	Chitin yield (g)	Chitosan yield (g)
P. sanguinolentus	23.8	28.5
S. serrata	20.3	19.5

According to elementary studies and analyses of different crustaceans (crab shrimp, lobster, and squid), there was great variability of this composition when chitin amounts were varied from species to species [24]. Hence, there is a need to develop efficient demineralization and deproteinization processes to remove mineral content (20–30%) and protein content of approximately 40% in order to obtain chitin that is free of inorganic and protein content. This study showed that different concentrations of NaOH and demineralization with hydrochloric acid and acetic acid influenced the yield of the extraction process used to obtain chitin and chitosan.

The chitin and chitosan are used in the preparation of the materials like wound dressing, antibacterial and antifungal agents, dialysis membrane, biomedical beads, fabrics and gauzes. Chitosan is a wound-healing accelerator, and its effectiveness in protecting wounds from bacterial

invasion by suppressing bacterial proliferation. It can effectively counter microorganisms developing typhoid's. Interestingly, their commercial applications are very small despite substantial development in chitin/chitosan research, along with the vast volume of possible uses of chitosan and its derivatives. It is also fair to say that the shrimp and crab manufacturing industries in the world are currently able to meet the demands for raw materials for decades to come at the current industrial use stage of chitin/chitosan. Some also traced the poor growth of the industry to the negative position played by patents, which are more likely to show market development. It is also assumed that in addition to investigating ways to replace products used in farming and industry with chitin/chitosan. More studies on individual applications and benefits will help to broaden the scope. The development of chitosan from the studied organisms was found in the crystallization analysis. Partial decay in chitosan with the deacetylation mechanism was observed; its robust FCC lattice structure also demonstrates its strong crystalline existence. Chitin and chitosan from *Portunus sanguinolentus* are proposed in future experiments to be used as a reduction in synthesis of noble metal nanoparticles.

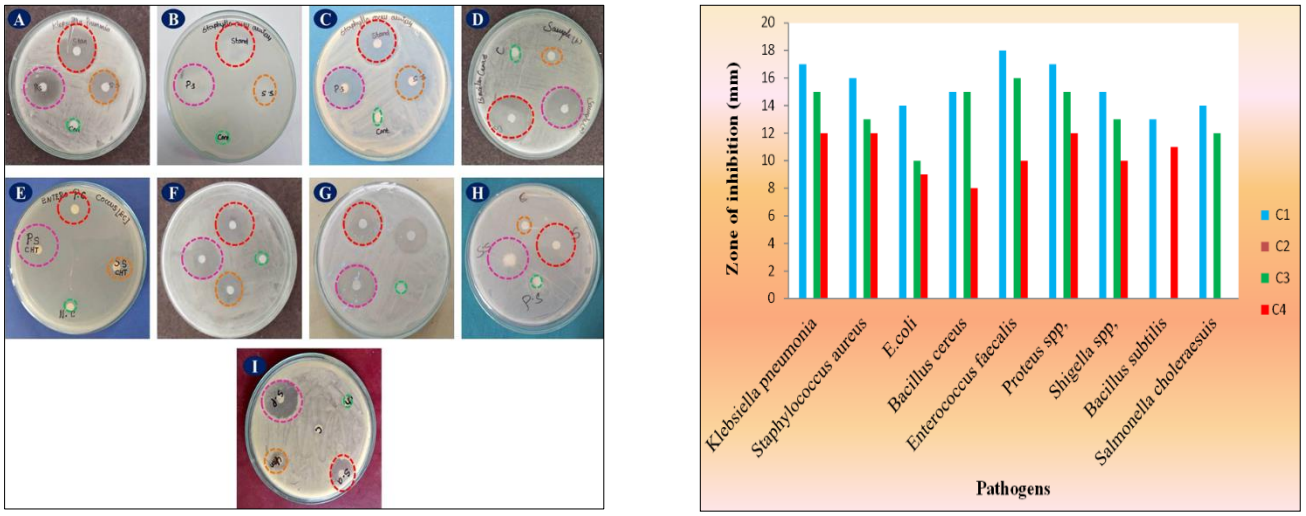

Fig 6 Zone of inhibition induced by *P. sanguinolentus* CHS and *S. serrata* CHS against the tested pathogens

The disc diffusion antimicrobial assay of the chitosan samples is shown. DMSO was used as a blank and it had no inhibitory effect on any of the tested microorganisms. The inhibition zone diameters of the antibiotic (positive control treatment) are also presented in (Table 2). The results obtained for chitosan samples indicated that chitosan showed little or no inhibition on all the tested microorganisms. Only *P. sanguinolentus* showed antimicrobial activity against all tested microorganisms the *S. serrata* sample displayed some antimicrobial activity against *E. coli*, which is evidenced by the observed inhibition zone observed. Although not properly defined, inhibition zones (Fig 6) were observed for the samples against *Klebsiella pneumonia, Staphylococcus aureus, E. coli, Bacillus cereus, Enterococcus faecalis, Proteus spp, Shigella spp, Bacillus subtilis* and *Salmonella choleraesuis;* which was an indication that the samples may have antimicrobial activity against these organisms. It would seem therefore that the chitosan samples may have displayed higher antimicrobial activity against gram-positive than gram-negative bacteria. Though, this was not consistent, and thus may only be a probability, higher antimicrobial activity against gram positive bacteria have been observed in other studies [25-26]. Although the effect of chitosan MW on its antimicrobial activity is yet to be fully determined as earlier mentioned in the results in this assay seem to agree with the inference that *P. sanguinolentus* chitosan displayed greater activity than *S. serrata*, having the highest MW, displayed antimicrobial activity against all the tested microorganisms. Similarly, the antimicrobial activity can be attributed to the high chitin of *P. sanguinolentus* samples, as chitosan samples with higher DD has been reported to possess stronger antimicrobial activity [27]. The antimicrobial activity of chitosan has been noted to be dependent on the presence of a positive charge. Thus, it is expected that chitosan with higher DD would have more effective activity because it would contain a higher concentration of positive charges. However, the initial expectation of clear and defined inhibition zones as observed in other studies was not obtained in this study. The results indicate that the chitosan samples may have minimal antimicrobial activity. It is probable that the chitosan samples in this assay were incapable of fully diffusing through the paper disks, as it was observed that the chitosan droplets formed a thin layer of film on the surface of the paper disc. Consequently, the e chitosan samples were unable to interact with the microbial cell walls and inhibit microbial growth. This assumption is not unlikely as the study by Foster and Butt [28] revealed that films made from chitosan displayed no antibacterial activity but chitosan solutions and gels inhibited the growth of microbial organisms.

The data pertaining to the antibacterial potential of the chitosan are presented in (Fig 6, Table 2). All the growth parameters had a significant effect by treatments which were dose dependent. However, in case of zone of inhibition of *Portunus sanguinolentus* chitosan was consequently increased with increase in concentration (Fig 6 red dotted encircle) when compared to Zone of inhibition of *S. Serrata* chitosan. When compared to *P. sanguinolentus* chitosan other tested samples such as *Scylla serrate* chitosan and negative control showed poor activity. Amikacin showed similar zone of inhibition as observed in *P. sanguinolentus* chitosan this may be due to antibacterial nature of antibiotic. The study shows that chitosan of *P. sanguinolentus* provides an economical and preeminent way for the isolation of chitosan. Our results confirm that the *P. sanguinolentus* chitosan had significant antibacterial activity against the test pathogenic bacteria.

Table 2 Zone of inhibition

Pathogens	Standard Amikacin	*P. sanguinolentus*	*S. serrata*
Klebsiella pneumonia	17	15	12
Staphylococcus aureus	16	13	12
E. coli	14	10	9
Bacillus cereus	15	15	8
Enterococcus faecalis	18	16	10
Proteus spp,	17	15	12
Shigella spp,	15	13	10
Bacillus subtilis	13	-	11
Salmonella choleraesuis	14	12	

CONCLUSION

Chitosan has fascinating antibacterial activity, good biodegradation, outstanding biocompatibility, non-toxicity and excellent physical and chemical properties. As a result, chitosan has been widely used in the field of antibacterial. chitosan and its derivatives show antibacterial activity against gram positive bacteria and gram-negative bacteria. The result of the present study indicates that the source of raw materials for chitosan does play an important role in its physicochemical characteristics. Though the differences may not be quite significant, it is important and crucial to factor in this when comparisons of chitosan are one. Furthermore, the antimicrobial results indicated that the chitosan samples although exhibiting these abilities, the physicochemical properties associated with each chitosan sample influences its activities.

LITERATURE CITED

1. Dhillon GS, Kaur S, Brar SK, Verma M. 2013. Green synthesis approach: extraction of chitosan from fungus mycelia. *Critical Reviews in Biotechnology* 33(4): 379-403.
2. Kristbergsson K, Weiss J. 2006. Sonication-assisted extraction of chitin from North Atlantic shrimps (Pandalus borealis). *Journal of Agricultural and Food Chemistry* 54(16): 5894-5902.
3. Dhillon GS, Kaur S, Brar SK. 2013. Perspective of apple processing wastes as low-cost substrates for bioproduction of high value products: A review. *Renewable and Sustainable Energy Reviews* 27: 789-805
4. Abdou ES, Nagy KSA, Elsabee MZ. 2008. Extraction and characterization of chitin and chitosan from local sources. *Bioresource Technology* 99(5): 1359-1367.
5. Synowiecki J, Al-Khateeb NA. 2003. Production, properties, and some new applications of chitin and its derivatives. *Critical Reviews in Food Science and Nutrition* 43(2): 145-171.
6. Younes I, Rinaudo M. 2015. Chitin and chitosan preparation from marine sources. Structure, properties and applications. *Marine Drugs* 13(3): 1133-1174.
7. Kumar MNR. 2000. A review of chitin and chitosan applications. *Reactive and Functional Polymers* 46(1): 1-27.
8. Muxika A, Etxabide A, Uranga J, Guerrero P, de la Caba K. 2017. Chitosan as a bioactive polymer: Processing, properties and applications. *International Journal of Biological Macromolecules* 105: 1358-1368.
9. Pachapur VL, Guemiza K, Rouissi T, Sarma SJ, Brar SK. 2016. Novel biological and chemical methods of chitin extraction from crustacean waste using saline water. *Journal of Chemical Technology and Biotechnology* 91(8): 2331-2339.
10. No H., Lee KS, Meyers SP. 2000. Correlation between physicochemical characteristics and binding capacities of chitosan products. *Journal of Food Science* 65(7): 1134-1137.
11. Kaya M, Baran T, Asan-Ozusaglam M, Cakmak YS, Tozak KO, Mol A, Mentes A, Sezen G. 2015. Extraction and characterization of chitin and chitosan with antimicrobial and antioxidant activities from cosmopolitan Orthoptera species (Insecta). *Biotechnology and Bioprocess Engineering* 20(1): 168-179.
12. Teng WL, Khor E, Tan TK, Lim LY, Tan SC. 2001. Concurrent production of chitin from shrimp shells and fungi. *Carbohydrate Research* 332(3): 305-316.
13. Wu T, Zivanovic S, Draughon FA, Conway WS, Sams CE. 2005. Physicochemical properties and bioactivity of fungal chitin and chitosan. *Journal of Agricultural and Food Chemistry* 53(10): 3888-3894.
14. Yen MT, Mau JL. 2007. Physico-chemical characterization of fungal chitosan from shiitake stipes. *LWT-Fd Sci. Tech.* 40(3): 472-479.
15. Usman A, Zia KM, Zuber M, Tabasum S, Rehman S, Zia F. 2016. Chitin and chitosan-based polyurethanes: A review of recent advances and prospective biomedical applications. *Int. Jr. of Biological Macromolecules* 86: 630-645.
16. Abdulkarim A, Isa MT, Abdulsalam S, Muhammad AJ, Ameh AO. 2013. Extraction and characterization of chitin and chitosan from mussel shell. *Extraction* 3(2): 108-114.
17. Juárez-de la Rosa BA, Ardisson PL, Azamar-Barrios JA, Quintana P, Alvarado-Gil JJ. 2007. Optical, thermal, and structural characterization of the sclerotized skeleton of two antipatharian coral species. *Materials Science and Engineering*: C, 27(4): 880-885.
18. Ghosh A, Das BK, Roy A, Mandal B, Chandra G. 2008. Antibacterial activity of some medicinal plant extracts. *Journal of Natural Medicines* 62(2): 259-262.
20. Solani F, Krishnaveni B, Ragunathan R. 2015. Extraction and characterization of chitin and chitosan from CBNR BKRR, Synthesis of their bionanocomposites and study of their productive application. *Journal of Pharmaceutical Sciences and Research* 7(4): 197.
21. Pillai LS, Nair BR. 2014. Functional group analysis of *Cleome viscosa* L. and *C. burmanni* W. & A. (Cleomaceae) extracts by FT-IR. *Journal of Pharmacognosy and Phytochemistry* 2(6): 120-124.
22. Liu N, Chen XG, Park HJ, Liu CG, Liu CS, Meng XH, Yu LJ. 2006. Effect of MW and concentration of chitosan on antibacterial activity of *Escherichia coli*. *Carbohydrate Polymers* 64: 60-65.
23. Das NG, Khan PA, Hossain Z. 1996. Chitin from the shell of two coastal *portunid* crabs of Bangladesh. *Indian Jr. Fish* 43(4): 413-415.
24. Tolaimate A, Desbrieres J, Rhazi M, Alagui A. 2003. Contribution to the preparation of chitins and chitosans with controlled physico-chemical properties. *Polymer* 44(26): 7939-7952.
25. Dutta PK, Tripathi S, Mehrotra GK, Dutta J. 2009. Perspectives for chitosan based antimicrobial films in food applications. *Food Chemistry* 114(4): 1173-1182.
26. Romano N, Zeng C. 2007. Effects of potassium on nitrate mediated alterations of osmoregulation in marine crabs. *Aquatic Toxicology* 85(3): 202-208.
27. Kaya M, Cakmak YS, Baran T, Asan-Ozusaglam M, Mentes A, Tozak KO. 2014. New chitin, chitosan, and O-carboxymethyl chitosan sources from resting eggs of *Daphnia longispina* (Crustacea); with physicochemical characterization, and antimicrobial and antioxidant activities. *Biotechnology and Bioprocess Engineering* 19(1): 58-69.
28. Foster LJR, Butt J. 2011. Chitosan films are NOT antimicrobial. *Biotechnology Letters* 33(2): 417-421.