Extraction of Chitosan from Shrimp Shell Waste and its Application in Waste Water Purification

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ABSTRACT

Crustaceans are known to have a large amount of chitosan. Types of crustaceans include shrimp, crab, krill, lobster, prawn, etc. In the present study, shrimp shells were used for chitosan extraction. Chitosan is the deacetylated form of chitin, which is a natural polymer. It is the second most abundant polysaccharide after cellulose. It is widely used in the pharmaceutical industry, the food industry as well as in biological treatments. For this study, we extracted chitosan from shrimp shell waste. The extraction of chitosan starts with demineralization followed by deproteinization and further deacetylated to obtain chitosan. The chitosan yield obtained was 32%. Antioxidant activity and scavenging ability on DPPH of the extracted chitosan were determined. Chitosan has antibacterial activity and the extracted chitosan was used for water treatment and showed an antibacterial effect against Gram-negative bacteria.

Keywords: Antioxidant activity, Chitosan, Scavenging ability on DPPH, Shrimp shell, Water treatment.

INTRODUCTION

Chitosan is a derived form of natural polymer chitin obtained from crustaceans. The main components of crustacean shells are chitin, proteins, calcium, and magnesium carbonate (Aung et.al., 2018). Crustaceans also contain other bioactive components such as carotenoprotein, minerals, lipids as by-products which can be utilized to produce bioactive compounds (Trunget.al., 2012). The chitin content of crustacean shells varies from species to species and it ranged from 7-40% (Tolaimateet.al., 2003). K. Mohan et.al., 2021 reported extraction of 20% of chitin from shrimp shell waste, 21.25% from crab shell waste, 23.75% from squilla shell waste, and 17.50% from lobster shell waste. Due to the presence of a high level of chitin in the shell waste of crustaceans, it has been used widely for manufacturing commercial chitin (Yadav et.al., 2019). Shrimp waste contains diverse high-value products such as carotenoids, chiton and proteins (Ghorbelet.al., 2012). Shrimp and crab shells are considered natural and principal sources of chitin (Daniel et.al., 2016). Shrimp shells contain a large amount of chitin which is used as an essential ingredient in many foods, cosmetic and pharmaceutical industries (Islam et.al., 2016). Chitosan contains a rigid crystalline structure and therefore it is insoluble in water and other common organic solvents (Vanithaet al., 2018). The solubility of the chitosan is due to the presence of free amine groups in the chitosan chain, which gets dissolved in the diluted aqueous acidic solvents (Sarbonet.al., 2015). Chitosan along with its antioxidant properties is a biopolymer of glucosamine derived from chitin (Shiekhet.al., 2018). It is used in water purification also as antibacterial and antiviral in the field of biotechnology, agriculture because it contains biological properties (Vanithaet.al. 2018). It is a natural water coagulant that can decrease turbidity, color and it is also effective for the extraction of organic pollutants, heavy metals, and bacteria from water (Chopra et.al., 2015). It can scavenge free radicals by donating hydrogen ions and it contains two key functional groups like hydroxyl group (OH) and amino group (NH_2) for antioxidant activity (Rajalakshmiet.al. 2013).

Material:

Shrimp shells were collected from Bhiwandi-Thane, Maharashtra, India. The shrimp shells were cleaned by removal of waste from it and further washed with distilled water and kept in the polyethene bags and preserved at 4℃ for further process.

Methods:

A) Extraction of Chitosan

Demineralization: The preserved shrimp shells were kept at room temperature and washed with normal tap water. The shells were placed inside the oven at 60℃ for 24 hours, ground, and further demineralized by treating with 15% of hydrochloric acid (HCl) at 60℃ for 2 hours. The shell sample was filtered using filter paper and the filtrate was washed with water and the demineralized shells were dried in the oven at 60℃ for 24 hours **(***Sarbonet al., 2015***)**.

Deproteinization: The demineralized shrimp shells were treated with 15% sodium hydroxide (NaOH) at 60℃ for 5 hours for the process of deproteinization. The sample was filtered using filter paper and the filtrate was washed with water and the deproteinized shells were dried in the oven at 60℃ for 24 hours (*Sarbonet al., 2015*).

Decolourization: The deproteinized shrimp shells were further decolourised by treating with acetone at room temperature for 2 hours. The sample was filtered using filter paper and the filtrate was washed with water and the decolourised shells were dried in the oven at 60℃ for 24 hours to obtain shrimp chitin (*Sarbonet al., 2015*).

Deacetylation of Chitin: The chitin obtained by the process of decolourization was treated with 60% sodium hydroxide (NaOH) at 100℃for 2 hours. The chitin was filtered using filter paper and the filtrate was washed with distilled water to recover chitosan. The recovered chitosan was dried at 60℃ for 24 hours in the oven **(***Sarbonet al., 2015).*

B) Characterization of Chitosan

Antioxidant activity of Chitosan: Different concentrations of 2.5 mL of chitosan samples were prepared in the range of 2-10 mg/mL and mixed with 2.5 mL of 200 mM sodium phosphate buffer and 2.5 mL of 1% potassium ferricyanide in 0.2% acetic acid solution. The mixture was incubated for 20 minutes at 50℃. Further 2.5 mL of 10% trichloroacetic acid was added and the mixture was centrifuged at 3500 rpm for 20 minutes. After centrifugation, 5 mL of supernatant was mixed with an equal amount of distilled water and 1 mL of 0.1% ferric chloride. The absorbance was measured at 700nm. Higher absorbance shows higher reducing power ***(****Yen et.al., 2008).*
Scavenging ability on 1, 1-diphenyl-2-picrylhydrazyl radicals: Different concentrations of 4 mL chitosan sample was prepared in the range of 2-10 mg/mL) and mixed with 1 mL of 10 mM methanolic acid solution containing DPPH radicals in 0.2% acetic acid solution. The mixture was mixed and kept in the dark condition for 30 minutes. The absorbance was measured at 517 nm *(Yen et.al., 2008).*

C) Collection of Water Sample

Water samples was collected from Bhadwad, Biotechnology laboratory (B. N. N. College) and Varaladevi lake (Kamatghar) and was named as sample A, sample B, and sample C respectively.

D) Determination of Microorganisms in wastewater

Various culture media like Nutrient Agar, Mac Conkey's Agar, Thiosulphate Citrate Bile (TCBS) Agar, Salt Mannitol Agar, and Salmonella Shigella Agar were ordered from Hi-media, prepared and sterilized as per the manufacturer's instruction. A loopful of wastewater samples was streaked on each medium and plates were incubated at 37℃ for 24 hours to observe the growth of various organisms. Isolated organisms were determined biochemically.

E) Effect of Chitosan on Water Treatment

About 1 gm of extracted chitosan powder was weighed and mixed with 5 mL of 1% acetic acid solution and left to stand for about 30 minutes to dissolve. Further, it was diluted with 100 mL distilled water and stirred for 1 hour at 25℃. Three samples of 100 mL of raw water were taken and treated with extracted chitosan *(Al-Manhelet.al., 2018).*

RESULT AND DISCUSSION

Determination of yield of chitosan

Chitosan content varies in different species of Crustaceans. The crustaceans like sea snails, crabs, woodlice, and barnacles have strong and hard exteriors with a high amount of minerals that lead to low chitin yield compared to other crustaceans

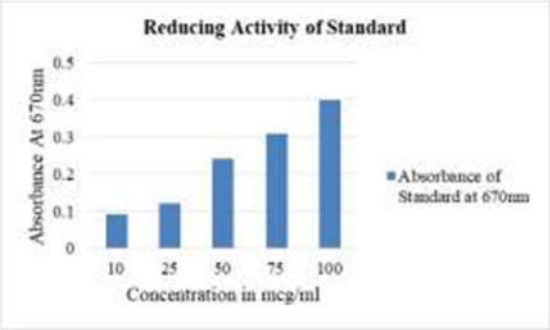
like mollusks, shrimp, and aquatic invertebrates (*Abdouet.al., 2007; Tolaimateet.al., 2003*). The yield of the extracted chitosan from shrimp shell waste was found to be 32%. Vanithaet.al., 2018 reported that a 38% yield of chitosan from the crab shells and this decrease in the yield can be due to extraction methods, amount of removal of acetyl groups from the polymer during the deacetylation process. The yield of chitosan determined by Sarbonet.al., 2014 had 44.57 ± 3.44% yield from the mud crab shells which have been used as an economic source for the production of chitosan on an industrial scale due to the availability and low cost of the source. Al-Manhelet.al., 2018 had obtained a 12.93% yield of chitosan from the shrimp shells.

Reducing Activity of chitosan

By restraining oxidative chain reactions, limitation or inhibition of nutrient oxidation occurs which is called antioxidant activity. Different concentrations of chitosan were checked for their antioxidant activity. The table 1 and graph 1 show the antioxidant activity of standard at different concentrations (10-100 mcg/mL) at the absorbance of 670 nm. The table 2 and graph 2 show the antioxidant activity of Chitosan extracted from Shrimp Shell waste. The reducing power of the extracted chitosan from the shrimp shell waste was found to be 0.40 OD, at a concentration of 10 mg/mL colorimetrically at 670 nm. Sarbonet al., 2014 had obtained reducing the power of chitosan from mud crab shells was 0.23 OD at a concentration of 10 mg/mL at the absorbance of 700 nm. Yen et.al., 2008 extracted chitosan from snow crab shells which found a reducing power of 0.32 OD at a concentration of 10 mg/mL. Thus, chitosan used as an antioxidant, which has a reducing power, can help in the reduction of Fe^{3+} to Fe^{2+} because the amine group (NH_2) present in the composition of chitosan can give hydrogen to the free radical in the reaction. Hydrogen combines with the free radical and thus it breaks the free radical to become stable and less readily available for propagation. This results in Chitosan exhibiting a potential primary antioxidant activity (Sarbonet.al., 2014).

Table 1: Reducing Activity of Standard.

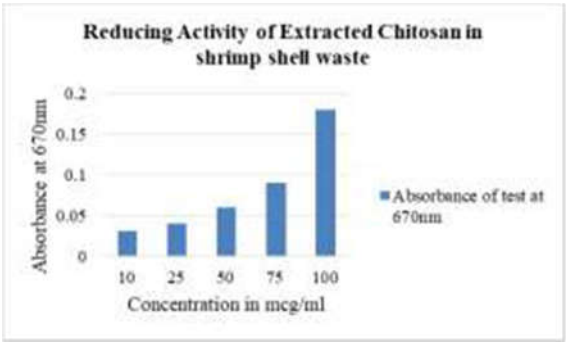
Concentration in mcg/ml	Absorbance of standard at 670nm
10	0.03
25	0.04
50	0.06
75	0.09
100	0.18



Graph 1: Reducing Activity of Standardat different concentrations

Table 2: Reducing Activity of Extracted Chitosan in Shrimp Shell Waste

Concentration in mcg/ml	Absorbance of Test at 670nm
10	0.03
25	0.04
50	0.06
75	0.09
100	0.19



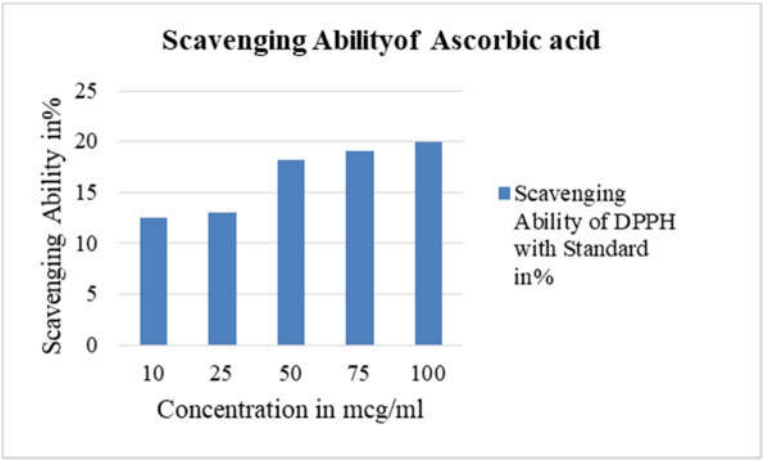
Graph 2: Reducing Activity of Chitosan Extracted in Shrimp Shell at Different Concentration

Scavenging Ability of chitosan on DPPH

Antioxidants can scavenge the free radicals by donating the some of their electrons; hence they inhibit the oxidative chain. DPPH is a common abbreviation for the organic chemical compound 2,2-diphenyl-1-picrylhydrazyl. It is a dark-colored crystalline powder composed of stable free radical molecules. DPPH has two major applications, both in laboratory research one is a monitor of chemical reactions involving radicals, most notably it is a common antioxidant assay. The scavenging ability of extracted chitosan was determined colorimetrically at 517 nm. Graph 2 shows the scavenging ability of DPPH of the extracted chitosan from the shrimp shell waste at different concentrations (10- 100mcg/mL) at the absorbance of 517 nm. The scavenging ability on DPPH of the extracted chitosan from the shrimp shell waste was found to be 7.5% at a concentration of 10 mg/mL at the absorbance of 517 nm. Sarbonet.al., 2014 had obtained scavenging activity on DPPH which was 30% at a concentration of 10 mg/mL at the absorbance of 517 nm from the mud crab shell. Yen et.al., **2008** had 46.4% scavenging ability on DPPH from the extracted chitosan of snow crab shells. Hence, an amine group present in the chitosan reacted partially with DPPH to form stable molecules. High scavenging ability shows a reduction of most of the DPPH radical molecules. The DPPH radical scavenging activity was calculated as: DPPH scavenging effect (%) $=A_0-A_1$.
When, A_0 and A_1 are absorbance of a control mixture without antioxidant and a mixture containing antioxidant, respectively.

Table 3: Scavenging Ability of Ascorbic acid.

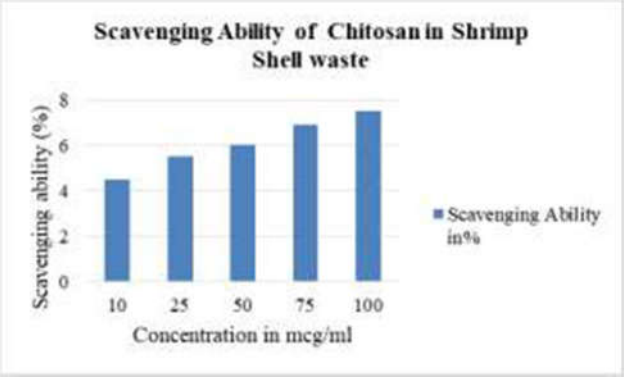
Concentration in mcg/ml	Scavenging Ability in%
10	12.50
25	13.04
50	18.18
75	19.04
100	20.00



Graph 3: Scavenging Ability of Ascorbic acid at different Concentrations

Table 4: Scavenging Ability of Chitosan in Shrimp Shell waste

Concentration in mcg /ml	Scavenging Ability (%)
10	4.5
25	5.5
50	6.0
75	6.9
100	7.5



Graph 4:Scavenging Ability on DPPH of the extracted Chitosan from shrimp shell waste at different Concentrations

Effect of chitosan on water treatment

The three raw water samples was tested prior before being treated with extracted chitosan and Table no.1 represents the growth of the organisms in the water sample.

Table 1 Growth of the organisms in the water sample

Sr. No A	gar Medium	Sample A Sa	ple B San	ple C
1.	Thiosulphate Citrate Bile Salt Agar	-	+	+
2.	Mac Conkey's Agar	-	+	-
3.	Salmonella Shigella Agar	-	-	-
4.	Salt Mannitol Agar	-	+	+
5.	Nutrient Agar	+	+	+

Key: (+) Growth; (-) No Growth

The raw water sample before treatment with chitosan showed the growth of microbes on Thiosulphate Citrate Bile Salt (TCBS) agar, Salt Mannitol agar, Mac Conkey's agar, and Nutrient agar. There was no growth observed on Salmonella Shigella agar. The identification of the isolated organisms was done by various biochemical tests. Based on morphological characteristics and biochemical results, organisms were concluded as E.coli, Vibrio species, and Staphylococcusaureus. After using chitosan no growth was observed on any agar and these show the antibacterial activity of chitosan on water treatment. **Al-Manhelet al., 2018** observed similar and concluded that chitosan has antibacterial activity against Gram-negative bacteria as compared to the Gram-positive bacteria. Chitosan inhibits the bacteria between the amine group (NH^+) of the chitosan and phosphoryl group of phospholipid found in bacteria.

Statistical Analysis: The data are presented at the mean +/- SD, Followed by Unpaired t-test were performed. The two tailed p-value equals to 0.228, by conventional criteria this difference is considered to statistically significant.

CONCLUSION

Chitosan is derived from chitin obtained from crustaceans like shrimp, crab, lobsters, etc. Chitin is the second most abundant natural polymer after cellulose obtained from crustaceans. For this study chitosan was extracted from dried

shrimp shells. About 20 grams of dried shrimp shells yield 32% of chitosan. The results of this work demonstrate that the extracted chitosan exhibits potent antioxidant activity whereas scavenging ability is found less. Thus, it can be concluded that extracted chitosan from shrimp shell waste is a good antioxidant. Chitosan inhibits Gram-negative bacteria present in wastewater as Chitosan has an amine group present in it. Hence, it can be used for wastewater treatment for the removal of dispersed particulates as well as dissolved pollutants. Chitosan can also be used as a biological treatment as a biological dressing of wounds and also for the large-scale purification of water reservoirs.

ACKNOWLEDGMENT

We wish to thank our institute of B.N.N. college of Arts, Science and Commerce and also thankful to the Head of the Department of Biotechnology for providing the lab facilities and all the other necessary equipment throughout the research work.

Conflicts of interest: The authors declare no conflict of interest.

Authors' contribution: The corresponding author, Malika Ahuja initiated the project idea and guided the project followed by reviewing the manuscript. Areeba Ansari and AkshayAnumanla carried out the research work, concluded the results, and developed the manuscript.

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