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Study on the Extraction and Characterization of Polymer (Chitosan) Obtained from Scales of *Channa striatus*, Exoskeleton of *Barytelphusa guerini*, *Macrobrachium rosenbergii* and Fresh Water Mussel from Fresh Water Bodies of Madhya pardesh

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ABSTRACT

Background: The most prevalent naturally occurring amino polysaccharide is chitosan. Chitosan's biocompatibility biodegradability and non-toxicity have led to its wide spread use in the pharmaceutical industry according to research. The biopolymer chitosan is typically extracted from the crustacean shells such as, Crab, Prawn, Lobster, fish scales *etc*.

Methods: The research was accomplished during 2021-2022 to examine the extraction and characterization of chitosan (polymer) from four different organism of fresh water *i.e.* fish scales, bivalve, prawn and crab shells. Extracted chitosan has been produced by using various parameters *i.e.* demineralization deproteinization and deacetylation as chemical methods.

Result: Results showed that chitosan was found maximum in the crab (*Bareytelphusa guerini*) 87.29% followed by Prawn (*Macrobrachium rosenbergii*) and scales of fish (*Channa striatus*) which have an average yield (38.55% and 32.56%) respectively and (18.14%)which is lowest yield among all obtained from fresh water mussel (bivalve). The chitosan extracted from the samples were characterized by Fourier transform infrared spectroscopy (FTIR) and existence of chitosan was confirmed and best yield of chitosan was obtained from *Bareytelphusa guerini*.

Key words: Bivalve, Chitosan characterization, Crustacean shells, Fish scales, FTIR.

INTRODUCTION

The animal origin polysaccharide chitin are found abundantly in nature and characterized by a fibrous structure. The main constituent of chitin involved the outer skeleton of insects and crustacean such as prawn, crabs and lobster. Chitin, also well-known as poly 2-acetamido2-deoxy-β-d-glucose firstly was acknowledged in 1884 as pure polysaccharides and available in large amount organic biopolymer material found in the physical world (Rinaudo 2006) Chitin is found next to cellulose and the chemical structure of chitin is similar to cellulose, consisting one hydroxyl group on each monomer substituted with an acetylamine group. This biopolymer demonstrate outstanding properties such as biodegradability, non-toxicity, ability to form film, biocompatibility and adsorption, which make it an striking biopolymer to pharmaceutical, biochemical applications and in the industrial zone . Physiochemical factors such as the degree of acetylation, solubility, viscosity and molecular weight have made known excellent outcome in the purification of biopolymer chitin and when Chitin is subject to deacetylation and the repeating units in the polymer are mostly without the acetyl functional group, such as β-1,4-D-glucosamine, the polymer is known as chitosan. Chitosan is weak base so it is insoluble in water but soluble in aqueous acidic solutions and it is mainly considered by its molecular weight (MW) and the degree of acetylation (DA) (Younes and Rinaudo, 2015). Chitosan film is regarded as bio functional material, well tolerated against pathogen, particularly applicable as edible coating to prolong shelf life and preserve quality of foods. It also has been tested in pharmaceutical industries and bone engineering (Kalut, 2008). Chitosan has potential use in food and nutrition, biotechnology, material science, drugs and pharmaceuticals, agriculture and environmental protection and recently in gene therapy (Cheung *et al.* 2015).

Fig 1 Shows the structure of Chitosan which is a linear polysaccharide made up of N-Acetyle-D-glucose amine

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(acetylated unit) and randomly arranged β-(1-4)-linked D-glucoseamine (deacetylated unit). It is made by soaking shrimp and other crustacean's chitin shells in an alkaline solution such as potassium or sodium hydroxide.

MATERIALS AND METHODS

The research work was carried during 2021-2022 in the department of Zoology, Govt. MVM Bhopal. The sample of crab (*Barytelphusa guerini*) and prawn **(***Macrobrachium rosenbergii*) were brought from the Bhopal's local market, while fish (*Channa striatus*) and fresh water mussel sample were gathered from the upper lake of Bhopal. The shells were removed from the organism with the help of forceps and were gutted with valve water to do away with soluble organic matters and others filths. Attained gutted shell wastes were dried in an oven at 35°C (molluscs and fish shell) and 70°C (crustaceans shell) for 12-24 h. dried shell were pulverized into fine powder with the the help of pestle and mortar and grinder. Dried shells were used for chitin analysis. The shells from four different organisms' wastes were shown (Fig 2.)

Image 2 shows the exoskeleton of four different aquatic organisms. A) Sample of dry, stiff exoskeleton of *Channa striatus* (fish), B) Sample of the processed dry shell of *Macrobrachium rosenbergii* (Prawn) C) Sample of the processed dry shell of Fresh water mussel (Bivalve),D) Sample of the processed dry shell of *Barytelphusa guerinii* (crab).

Chitin extraction by chemical method

In the process of isolating chitin for the natural raw materials, we considered two steps: Demineralization and deproteinization (Abdulkarim *et al*. 2013).

Demineralization

The predicated shells were carried out by stirring in dilute HCl solution to remove acid and calcium chloride, calcium phosphate and water-soluble impurities. All species were treated with 2.5% HCl solution (1:20 w/v) (mollusks and crustacean) and 1% HCl (fish scales) (Abdulkarim *et al*. 2013) at ambient temperature (approximately 37°C). The treatments with HCl and their durations depend on the nature of species after action of the performing admixture was irrigated using distilled water 2-3 times until neutral pH was attained and the product was dried to constant weight 35°C to 60°C for 24 h (Majekodunmi, 2016).

De-proteinization

The deproteinization of the Demineralised shell was ended using 2% KOH (1:20v/w) for (crustacean and molluscs shell), 1% KOH (1:1 v/w) (fish scales) at ambient temperature (approximately 30°C), to dispose of residual acid and calcium chloride. The treatments with KOH and their durations 18-24 h depend on the nature of species. The tintless solution specify the absence of proteins. The distilled water was used to wash the solution 2 to 3 times to make it neutral and the resulting mixture obtained was dried at 35°C to 60°C for 24 h (Zamri *et al*., 2020).

Extraction of chitosan

Alkaline hydrolysis (Bader *et al*., 1997).

Deacetylation

The deacetylation process was carried out by adding 40% of NaOH solution, with a ratio of 1:20 and boiled at 70-80°C for 2 hours with continue stirring in magnetic stirrer. After cooling the sample was washed with distilled water with whattmann filter paper to filtered in order to retain the solid matter and dried in hot air oven (60°C) the obtained powder is chitosan (Paul *et al*., 2014).

Percentage yield

Yield (%) of chitosan was calculated as the total weight of chitosan powder extracted to the total weight of dry shells used for chitin chemical modification (Zamri *et al*., 2020).

$$\text{Yield (\%)} = \frac{\text{Total weight of chitosan powder extracted}}{\text{Total weight of dry shell}}$$

Characterization of chitosan

Ash content

The ash content of the chitosan samples was calculated using a high temperature burning method. 2 g of chitosan sample (triplicate) was weighed after being fired, cooled and weighed in a quartz crucible. The samples were first carbonized at 300°C in a muffle furnace and then heated to 600°C for 3 hours. The crucible was placed in desicators after cooling to less than 200°C in the furnace. After cooling the samples, the ash content and empty crucible were weighed separately (Huang *et al,* 2020). The heating and cooling operations were repeated until the weight remained consistent. The following equation was used to compute then ash content.

$$\text{Ash content \%} = \frac{\text{Weight of the ash sample}}{\text{Weight of the sample}} \times 100$$

pH

A microprocessor pH meter was used to determine the pH of the chitosan solution.

Solubility

Chitosan powder was dissolved in 1 per cent acetic acid solution and suction filtered, with the filter membrane dried and weighed. The undissolved residue was filtered and dried in an oven before being weighed (Hung *et al*, 2020). Triplicate measurement were taken. The solubility y was calculated using the following formula:

$$\text{Insoluble content} = \frac{Wf - Wi}{W} \times 100$$

Fourier transform infrared spectroscopy (FTIR)

An infrared spectrometer was used to characterize the chitosan samples (Perkin Spectrum BX).To acquire a transmittance infrared spectrogram, Chitosan sample were converted into KBr Pellets and scanned in the range of 400-4000 cm^{-1}. (Osman and Arof 2003).

RESULTS AND DISCUSSION

Extraction of chitosan

Chitosan was obtained from four different aquatic organisms such as *Channa striatus* (Fish) *Barytelphusa guerini* (crab), *Macrobrachium rosenbergii* (prawn) and fresh water mussel (bivalve) by the process of demineralization, deproteinization and deacetylation. Extracted chitosan shows colour variations from light pink of *Channa striatus* to dark brown of *Barytelphusa guerini*. Fig 3.

Table 1 shows Physiochemical characteristic of chitosan from four different organism under study. The chitosan yield (by percentage) of *Channa striatus, Macrobrachium rosenbergii, freshwater mussel and Barytelphusa guerini* is 32.56, 38.55, 18.14 and 87.29 respectively. The ash value observed was 1.26, 3.56, 3.87 and 1.58 respectively. Whereas the pH found was 7, 6, 6 and 6.5 respectively. Also all the four samples under investigation were soluble in acetic acid.

Fig 4 show comparative graphical representation of percentage chitosan yield, ash value and pH. The results clearly shows that maximum chitosan yield *i.e.* 87.29 % was found in *Barytelphusa guerini* and minimum percentage yield *i.e.* 18.14 % was found in fresh water Mussel. The maximum and minimum ash value was found in fresh water Mussel (3.87) and *Channa striatus* (1.26).

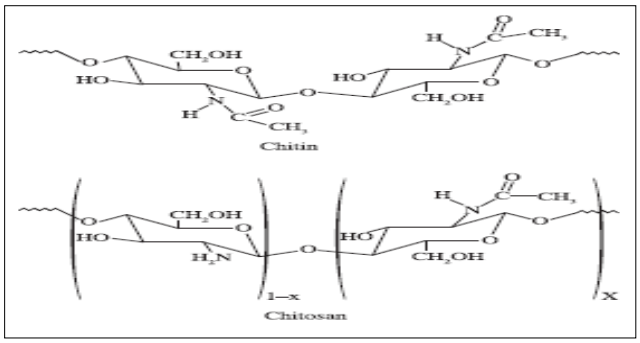

Fig 1: Schematic representations of the chemical structures of the chitin and chitosan.

Extraction of chitosan

Chitosan was obtained from four different aquatic organisms, that is *Channa striatus* (Fish), *Barytelphusa guerini* (crab), *Macrobrachium rosenbergii* (prawn) and fresh water mussel (bivalve) by demineralization and deproteinization.

FTIR interpretation

Fig A: IR spectra of fish chitosan were detected in the range of 3649 -3958 cm^{-1}allied to accompanying in N-H bond showed peak of primary amines, 2922 cm^{-1} - 3450 cm^{-1} was allied with C=O of carboxylic acid, 1789-2535 cm^{-1} was allied with C=N, C≡N of aliphatic amine and 437 cm^{-1}- 866 cm^{-1} C - N Aromatic (Bending) (Jang *et al*. 2004). Fig 5.

Fig B: IR spectra of prawn chitosan showed the peaks at 3930.26-3635.08 (alcohol group), 3205.82 (carboxylic acid O-H stretching), 3007.93 (amine N-H stretching), 2797.14 (Alkane C-H stretching), 1761.94 (Amide C=O group) and 1484.02 (Alkane C-H bending). So IR spectra of chitosan indicate the presence of functional group like alcohol, carboxylic acid and amide.

Fig C: The FTIR spectra in fresh water mussel gave characteristics bands of - NH2 at 3457cm^{-1} and carbonyl group band at 1655 cm^{-1}. It exhibited that the frequency ranges for the different classes of carbonyl compound overlap and the carbonyl frequency alone is not sufficient to characterize the functional group (Coates, 2000).

Fig D: IR spectra of crab chitosan showed peaks at 3945.85-3604.13 (Alcohol group), 3130.97 (primary amine group), 2885.16 (alkane group), 1756-1512.37 (carbonyl group), 1381.23 (amine stretching) and 568.21 (Out of the plane N-H bending) (Osman Z, Arof AK).

The FTIR analysis has confirmed the successful extraction of chitosan from all four different organism's waste

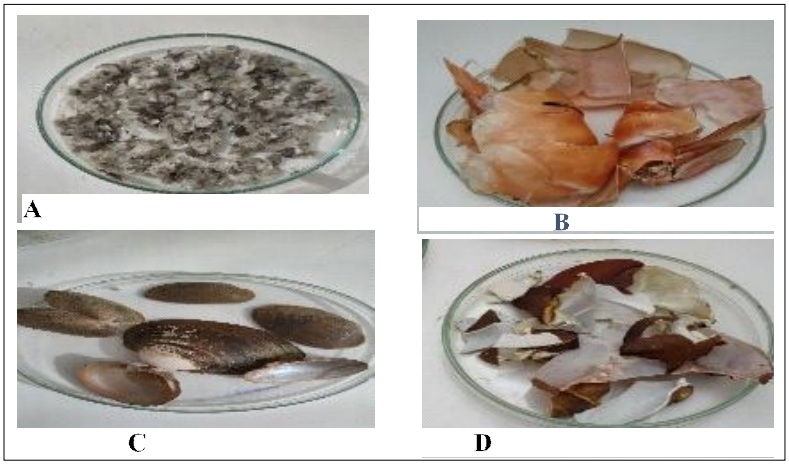

Fig 2: Exoskeleton of four different aquatic organisms. A) Sample of dry, stiff exoskeleton of Channastriatus (fish), B) Sample of the processed dry shell of macrobrachiumrosenbergii (Prawn); C) Sample of the processed dry shell of Fresh water mussel (Bivalve), D) Sample of the processed dry shell of barytelphusaguerinii (crab).

such as fish, prawn, bivalve and crab and extraction result showed that the maximum yield of chitosan obtained from *Barytelphusa guerini*. (Table 1) as also reported by Panchakshari *et al.* 2016, Vani and Shaleesha 2013. The FTIR band observed are in close match with commercial available chitosan. Chitosan produced from different sample organism has a spectrum that is extremely similar to commercial chitosan.

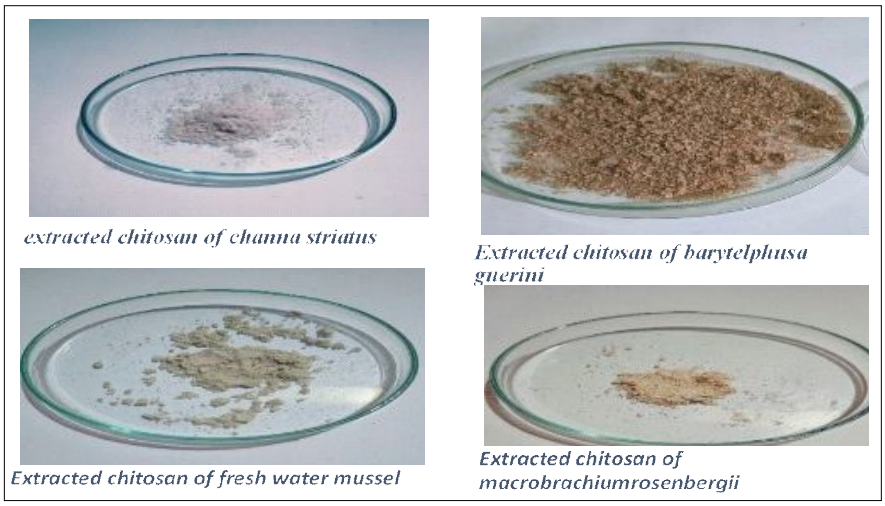

Fig 3: Extraction of chitosan.

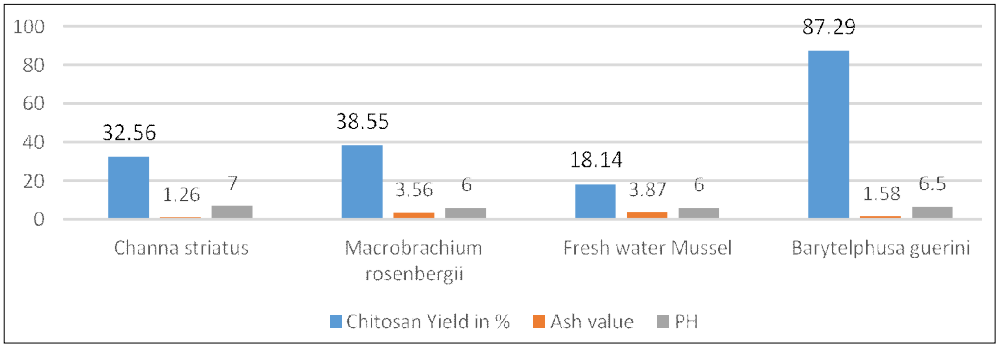

Fig 4: Comperative percentage chitosan yield, ash value and pH.

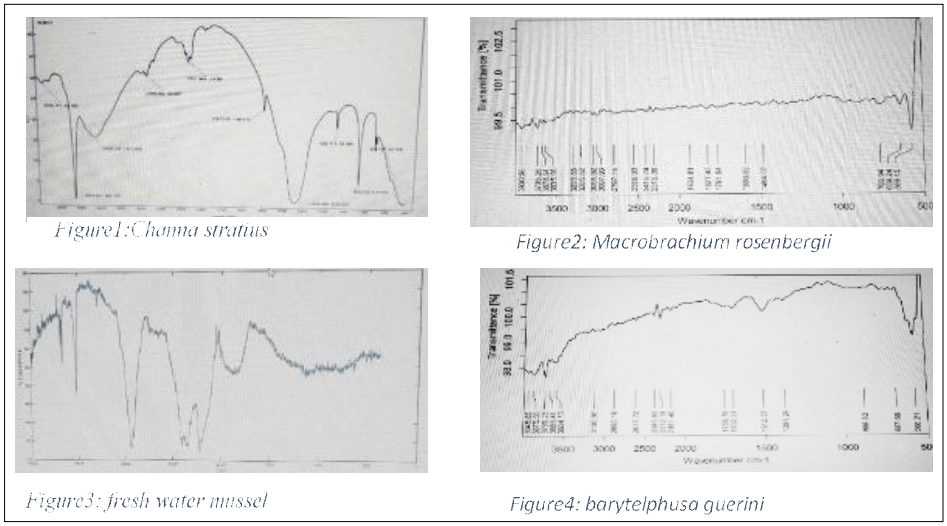

Fig 5: Fourier transform infrared spectroscopy (FTIR).

Table 1: Physiochemical charcterstic of chitosan from four different organismi.e *Channa striatus, Macrobrachium rosenbergii, freshwater mussel* and *Barytelphusa guerini*.

Sample organism	Chitosan yield (In %)	Ash value	Solubility	pH
Channa striatus	32.56	1.26	Acetic acid	7
Macrobrachium rosenbergii	38.55	3.56	Acetic acid	6
Fresh water mussel	18.14	3.87	Acetic acid	6
Barytelphusa guerini	87.29	1.58	Acetic acid	6.5

CONCLUSION

From the present study it was concluded that the shell waste of crustacean (Crab and prawn) mollusks (Fresh water mussel) and fish scales contain chitosan which is natural polymer in which the removal of proteins and mineral was carried out successfully during preparation and was successfully analyzed using the several physiochemical parameters of the chitosan products. The crustacean such as crab and prawn show an effective yield of chitosan fish scale giving good average and fresh water mussel report a low yield of chitosan.

Conflict of interest: None.

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