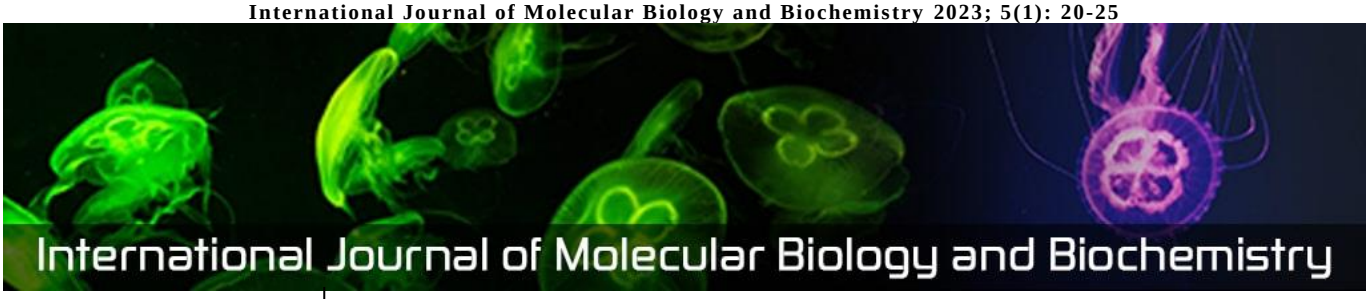

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Study on the extraction and characterization of polymer obtained from scales of *Channa striatus*, exoskeleton of *Barytelphusa guerini*, *Macrobrachium rosenbergii* and fresh water mussel from fresh water bodies of Bhopal, Madhya Pradesh

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Abstract
A research was accomplished 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 DE acetylation as chemical methods. Results showed that chitosan was found at 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.

Keywords: Chitosan, *Barytelphusa guerini*, demineralization, Deproteinization, deactylation, 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 M, 2006) [3] 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. Demineralization and Deproteinization method which is used For the extraction of chitin in which removal of calcium carbonate generally by hot reaction with HCL,HNO3 etc. and removal of protein followed by proteinization through the process of alkaline treatment e.g. with NaOH (Kumar ABV, Varadaraj MC, *et al.* 2005) [15]. 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) [4]. Chitosan film is regarded as biofunctional material, well tolerated against pathogen, particularly applicable as edible coating to prolong shelf life and preserve quality of foods. (Assis OBG & Pessoa JDC 2004) [16]. It also has been tested in pharmaceutical industries and bone engineering.

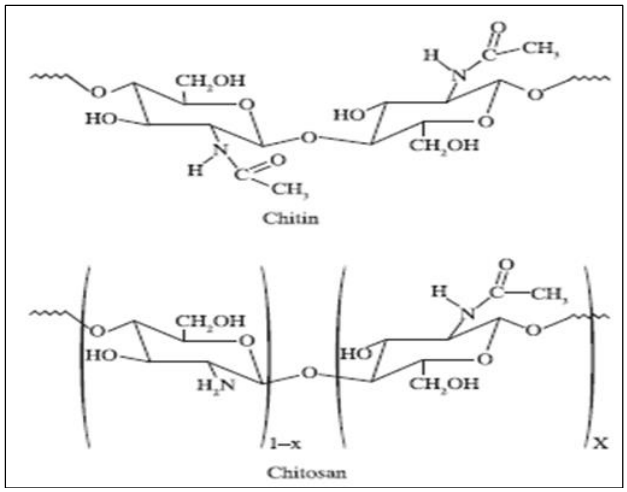
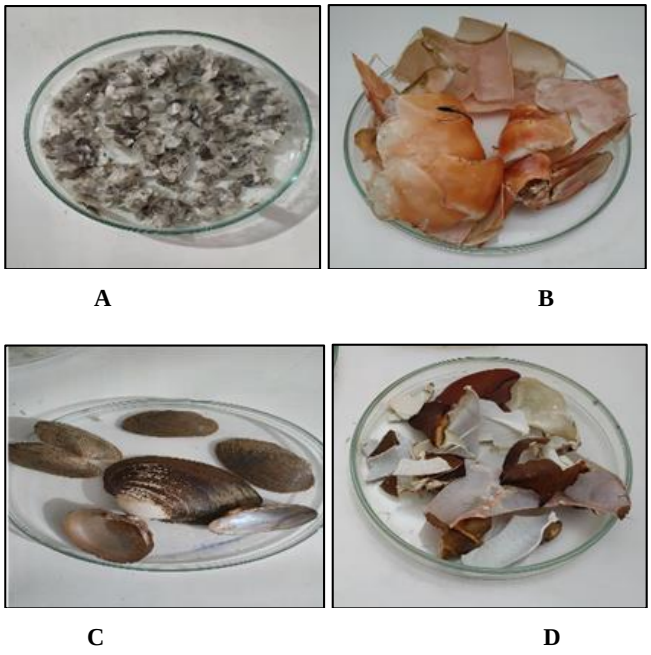


Fig: Schematic representations of the chemical structures of the chitin and chitosan

Methodology

Materials and Methods

The sample of crab (*Barytelphusa guerini*,) and prawn **(***Macrobrachium rosenbergii*) were purchased from the local market of Bhopal and the sample of fish (*Channa striatus*) and fresh water mussel were collected from the upper lake of Bhopal. The shell was removed from the organism by the help of forceps were cleaned with tap water to do away with soluble organic matters and others impurities. Obtained cleaned 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 1).



A **B**

C **D**

Fig 1: 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 A, Isa AMT *et al.* 2013) [2].

Demineralization

The grounded shells was 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 A, Isa AMT *et al.* 2013) [2] at ambient temperature (approximately 37 °C). The treatments with HCl and their durations depend on the nature of species after action of the resulting mixture was rinsed using distilled water 2-3 times until neutral pH was obtained and the product was dried to constant weight 35 °C to 60 °C for 24 h. (Majekodunmi, 2016) [17].

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 colorless specified the absence of proteins. Then the solution was washed 2-3 times with distilled water to neutrality and the resulting mixture was dried to constant weight 35 °C to 60 °C for 24 h (Zamri *et al*., 2020) [14]

Extraction of Chitosan

Alkaline hydrolysis (Bader *et al*., 1997) [18].

Samples

Fish (*Channa striatus*) Crab (*Barytelphusa guerini*) prawn (*Macrobrachium rosenbergii*), fresh water mussel (Bivalve).

Deacetylation

The deacetylation process was carried out by adding 40% of NaOH solution, with a ratio of 1:20 and boiled at 70-80oC for 2 hours with continue stirring in magnetic stirrer. After cooling the sample was washed with distilled water with whattmann filter paper to filter in order to retain the solid matter and dried in hot air oven (60 ºC) 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) [14].

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

Characterization of chitosan

Ash content

The ash content of the chitosan samples were calculated according to the method of burning of high temperature. 2 g of chitosan sample (triplicate) was placed into quartz crucible previously ignited, cooled and weighed. The samples were carbonized at 300 ℃ in a muffle furnace in the first place and heated to 600 ºC for 3h. The crucible were left to cool to less than 200 ºC in the furnace and

placed into desiccators. The weight of the ash content and empty crucible was weighed separately after samples being cooled (Huang *et al*., 2020) [19]. The operation of heating and cooling were repeated until the weight was constant. The ash content was calculated using the following equation:

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

PH

The pH measurements of the chitosan solutions was carried out using a microprocessor pH meter.

Solubility

Chitosan powder was dissolved in 1% acetic acid solution and subjected to suction filtration that the filter membrane had been dried and weighed. The undissolved residual was filtered and dried in the oven and weighed eventually (Huang *et al*., 2020) [19]. Measurement was performed in triplicate. The solubility was got using the following expression:

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

Fourier transform infrared spectroscopy (FTIR)

The chitosan samples were characterized by infrared spectrometer (Perkin Spectrum BX). Chitosan samples were made into KBr Pellets to obtained transmittance infrared spectrogram which is scanned in the range of 400-4000cm$^{-1.}$ (Osman Z, Arof AK 2003) [6].

Result and Discussion

Extracted chitosan of channa striatus

Extracted chitosan of fresh water mussel

Extracted chitosan of barytelphusa guerini

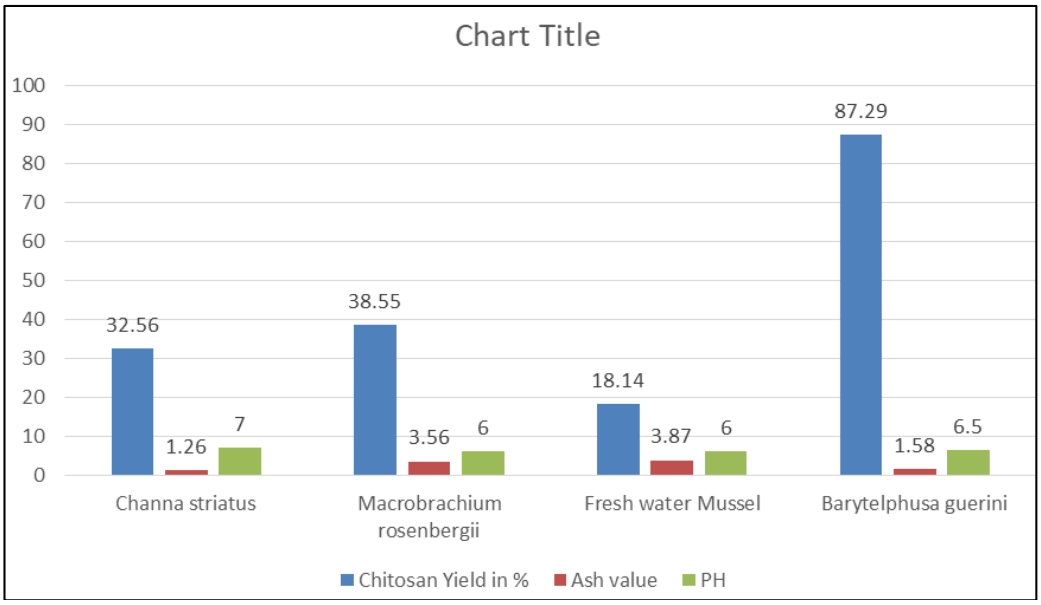
Extracted chitosan of macrobrachium rosenbergii

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 demineralization and Deproteinization.

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

S. No.	Sample Organism	Chitosan yield (In %)	Ash value	Solubility	pH
1.	*Channa striatus*	32.56	1.26	Acetic acid	7
2.	*Macrobrachium rosenbergii*	38.55	3.56	Acetic acid	6
3.	*Fresh water mussel*	18.14	3.87	Acetic acid	6
4.	*Barytelphusa guerini*	87.29	1.58	Acetic acid	6.5



Fourier transform infrared spectroscopy (FTIR)

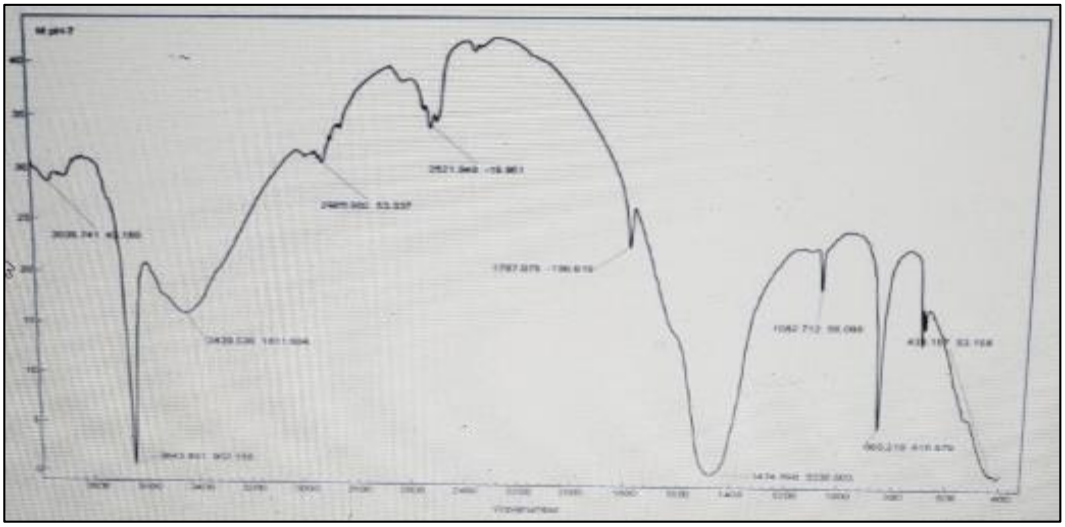


Fig 1: *Channa stratius*

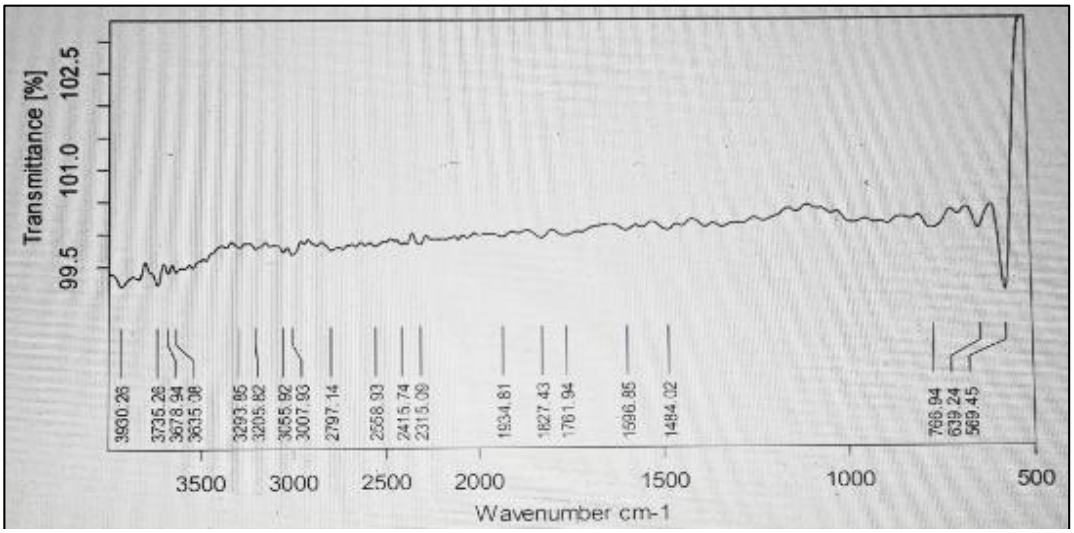


Fig 2: *Macrobrachium rosenbergii*

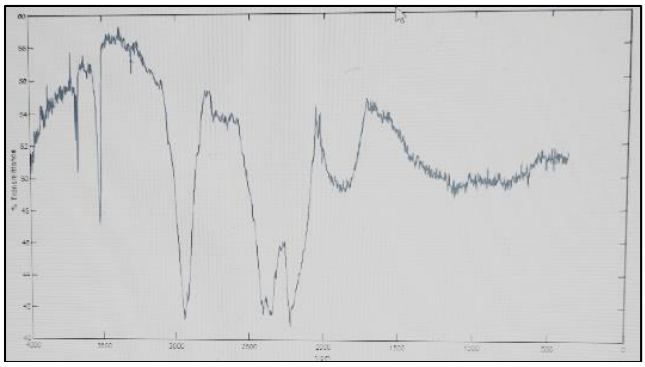


Fig 3: Fresh water mussel

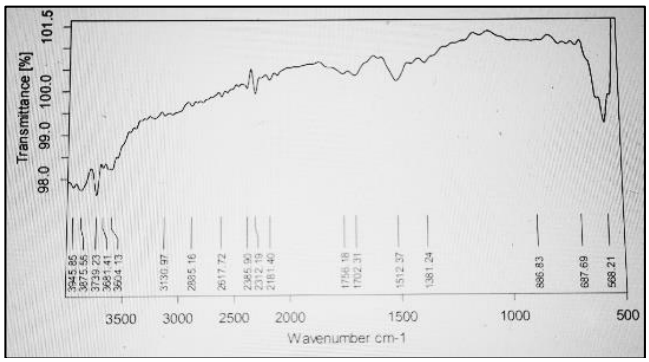


Fig 4: *Barytelphusa guerini*

FTIR Interpretation

Figure 1: 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 MK, Kong BG *et al.* 2004) [8].

Figure 2: 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.

Figure 3: the FTIR spectra in fresh water mussel gave characteristics bands of –NH2 at 3457cm^{-1} and carbonyl group band at 1655cm^{-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) [9].

Figure 4: 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).

The FTIR analysis has confirmed the successful extraction of chitosan from all four different organism's waste such as fish, prawn, bivalve and crab and extraction result showed that the maximum yield of chitosan obtained from *Barytelphusa guerini.* (Table 1).

Conclusion

It is then 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.

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