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Research article

Extraction and Estimation of Antimicrobial Efficiency of Chitosan from Locally available Species Macrobrachium lamarrei lamarrei of Bhopal

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ABSTRACT

Chitosan, a handy hydrophilic polysaccharide resulting from chitin, has a comprehensive antimicrobial spectrum to which gram-negative, gram-positive bacteria and fungi are highly vulnerable. In present study, the anti-bacterial activity of chitosan extracted from local species of Macrobrachium lamarrei lamarrei (prawn) of Bhopal city, against gram positive and gram negative bacteria was investigated. The exoskeleton, taken from outer body of prawn, was demineralized with 2.5% of HCL solution for 2 hours and then, deproteinized with 2% KOH solution for 2 hours at room temperature. Then deacetylized with 40% NaOH for 2 hours at room temperature. After deacetylization of chitosan, it was further analyzed by FTIR spectroscopy and anti-bacterial activity of chitosan was evaluated against Proteus vulgaris (Gram-negative) and Streptococcus mutans (Gram-positive) bacteria by well diffusion assay. The results of antibacterial activity of chitosan was found efficient at higher concentrationin 75 µg/ml with maximum zone of inhibition (31.67± 1.5 mm) and (35± 2 mm) against Proteus vulgaris and Streptococcus mutans. Thus, it was concluded that the chitosan extracted from Macrobrachium lamarrei species of prawn possess the potential antibacterial activity against Proteus vulgaris and Streptococcus mutans which may be used as n better antimicrobial agent in various industrial applications.

Keywords: Macrobrachium lamarrei lamarrei, Deproteinization and demineralization, deacetylation, Antimicrobial activity

Introduction

For the past years, Environmental concerns and population pressure have been forcing us to look for ways to turn waste into energy and other usable product (**FAO,1998**) The concept of REDUCE, REUSE and RECYCLE has become way of life for municipalities the world over. The sea food industry has seen an unprecedented shift in the way it disposes and utilizes the waste in usable form. Chitin is bio-polymer extracted from the crustacean shells that has handy industrial application (**Muzzareli, 1977: Kramer *et al*, 1995**). The shell of prawn has low economic value and is treated as biowaste. Thus, this Bio-waste can be used to produce treasured product such

as chitosan. Chitosan is the second most abundant polysaccharides found on earth after the cellulose. It is a polycationic polymer and the N-deacetylated derivative of the natural polymer chitin (**Alipour *et.al*. 2009**). Chitosan prepared from chitin white to light red powder. The antimicrobial activity of chitosan against a wide range of food-borne bacteria, fungi and yeast has made it potential food preservative. Chitosan is widely used as a biomaterial because it has a biological property of biocompatibility, biodegradability and non-toxic. Its antibacterial and antifungal properties making it remarkable for agriculture, medicine, environment, cosmetics and textile industries (**Li *et al* 2008 and Lu *et al*.2012**) Chitosan comprised of three reactive functional group, namely amino group, primary and secondary hydroxyl groups. The amino (NH2) and hydroxyl (OH) group in chitosan are functional groups of antioxidant activity. Chitosan is insoluble in water but soluble in acidic aqueous solution and indigestible by human digestive enzyme (**No *et al* 2007**). Chitosan is prepared in many ways from the shell of crustacean reliant on its intentional use and quality preferred (**Wang *et al*., 2004**).The extraction of chitosan consists of three major steps: Demineralization, Deproteinization and Deacetylation.

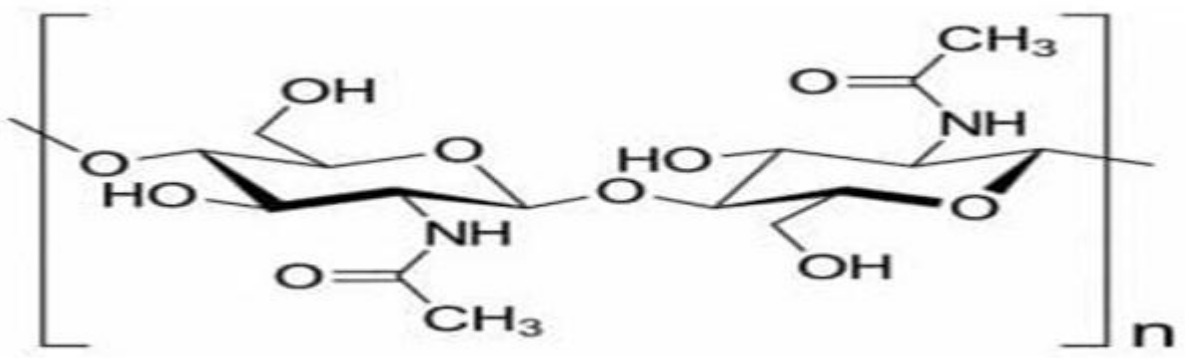


Figure 1: Structure of Chitosan

Literature Review

Collection of samples

sample were collected from the local market of Bhopal and were placed in zipped polythene bag to evade further contamination and cleaned several times with tap water and to detached from flesh with the help of forceps. Collected shell kept in the hot air oven (**Scientech SE-127**) at 70°C for 4 hrs or until the moisture content (%) reached between 8-10%. Dried shell was crushed by using grinder into coarse (800 micrometer). Then, the dried powder was mixed with cord liver oil and makes slurry by continuously stirring. Then, further dry the sample in hot air oven at 60-70oC until the moisture was removed **(Ali et al., 2019).**

Macrobrachium lamareii lamareii | *Extracted Raw shells* | *Grinded shells*

In this research five different chemical method were used for chitosan extraction such as Demineralization, Deproteinization, Decolouration, Deacytylation and precipitation.

Demineralization

The grounded shell in the form of powder which is obtained from the above process was soaked in 2.5 % HCl for 2 hours at the ratio of 1:20 of shell powder to the acid solution at room temperature (37 ^{0}C). This step was repeated twice. Then, sample was kept in Magnetic stirrer (REMI) for 2 hours. The mixture was cooled at room temperature (~26°C) and rinsed using distilled water 2-3 times until neutral pH was obtained **(Majekodunmi, 2016).**

Deproteinization

Demineralised prawn powder obtained was then immersed in 2% KOH solution at the ratio of 1:20 of sample to the solution at 70-80°C in Magnetic stirrer (REMI) for 2 hours. The mixture was cooled at room temperature (~26°C) and sample were then filtered using whatman filter paper and filtrate were washed rinsed using distilled water 2-3 times until neutral pH was obtained **(Zamri et al., 2020).**

Decolorization

The deproteinized powder was treated with acetone. The powder was washed with acetone for 2-3 times. After washing with acetone the powder was further dry in hot air oven at 40-50 ^{0}C.

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 and filtered in order to retain the solid matter, which is chitosan **(Paul *et al.*, 2014).**

Precipitation

Chitosan was purified by precipitation method. The chitosan obtained was dissolved in 2% glacial acetic acid solution with 1:100, for 4hour with constant stirring. The solution was obtained in a separate flask and 0.5mol/dm3 NaOH was added. The solution was kept for 5-10 minutes so that precipitation occurs. The precipitates were filtered, washed and dried at 110°C in hot air oven for 1 hour **(Hussain *et al.*, 2013**). Here we got the chitosan powder. Now this processed chitosan is suitable for further industrial application.

CHARACTERIZATION OF PREPARED CHITOSAN

pH

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

By Fourier transform infrared spectra (FTIR) studies of chitosan:

The FTIR spectra were used to identify the active fictional group in the compound. A small amount of the chitosan of *Macrobrachium lamarreilamarrei* (prawn) in the form of powder was incorporated into the selenium bromide crystal. The vertical rod is pulled down in a drug sample placed over the crystal. FTIR spectrum was run. The detected IR spectrum was smoothed. Lastly, functional groups were identified by relating the obtained IR ranges through a reference ranges available.

Percentage yield

The shell of Macrobrachium*lamarrei lamarrei* **(**prawn**)** sample was extracted by chemical modification extraction method and the percentage yield calculated by the following formula: -

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

Invitro antimicrobial activity

Anti-bacterial efficiency (Well diffusion assay):

- The Chitosan was tested for their antibacterial activity through agar well diffusion method and the Minimum inhibitory Concentration was calculated by the lowest concentration of chitosan which inhibits the growth of bacteria was considered as the minimum inhibitory concentration(MIC) (**E.gokulalakshmi *et al.* 2017**). The bacterial strains proteus vulgaris (Gram -ve) and streptococcus mutans (Gram +ve) were used for this study. This culture suspension was scattered in agar plates by the pour plate technique. Four wells of 6mm were made using a cork borer at equal distance and were filled with different concentration (75,100,150,200µg/ml) of sample and another plate well was filled with 50µl of standard drug respectively.

- The chitosan solution is then incubated at room temperature for 24 hrs. The formation of a clear zone (inhibitory zone) around the cavity is an indication of antimicrobial activity. . The zone of inhibition (ZOI) was observed and measured in mm. Zones were measured to a nearest millimeter using a ruler, which was held on the back of the inverted Petri plate. The background of the petri plate should be black and non-reflecting.
- The diameters of the zone of complete inhibition (as judge by unaided eye) were measured, including the diameter of the well **(Manandhar *et al.*, 2019).**

Results and Discussions

Extraction of chitosan: Chitosan extracted from prawn through a various process (Fig.2)

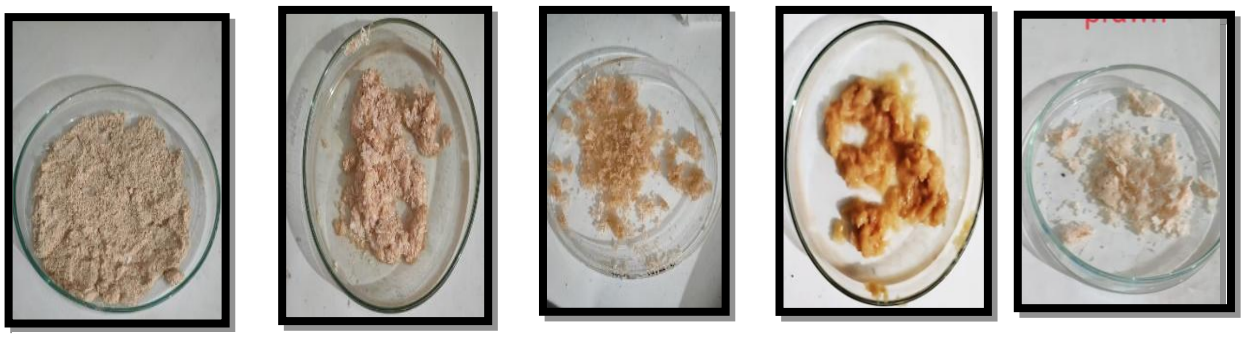


Figure.2: Demineralization Deproteinization Decolourization Deacetylation Precipitation

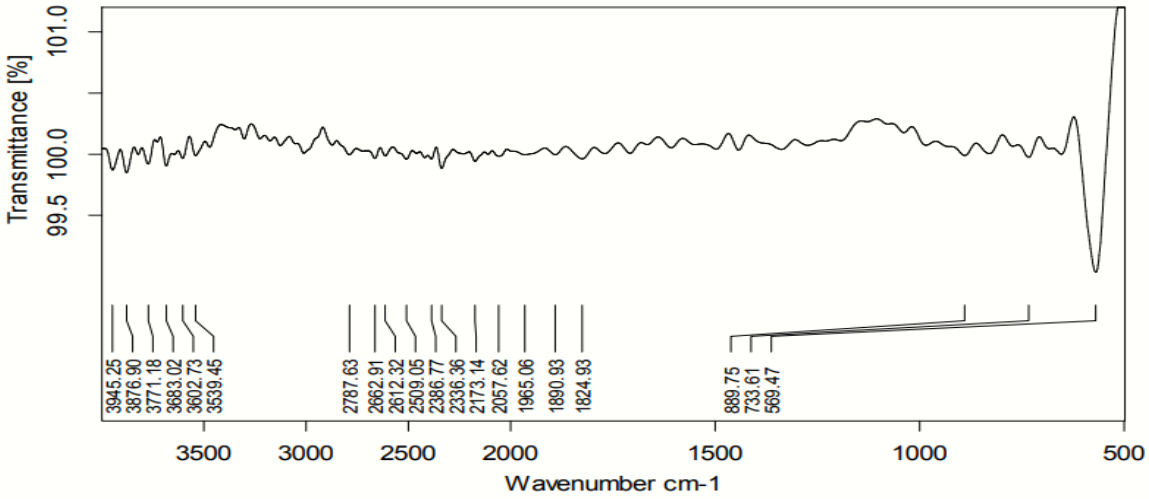
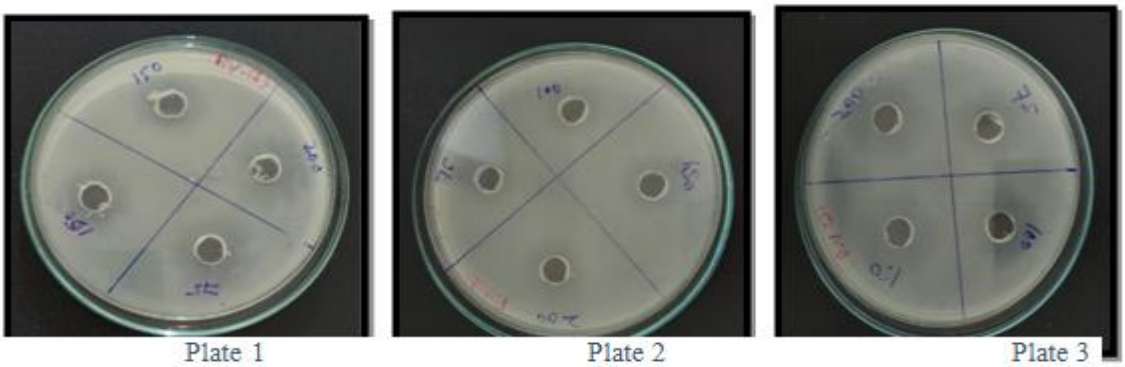


Figure 3: IR spectra of prawn chitosan showed the peaks at 3945.24-3602.73 (Alcohol group), 3539.45 (intermolecular bonded alcohol group), 2787.62-2612 (aldehyde group), 2509.05 (carboxylic acid O-H stretching), and 2336.35 (Carboxylic acid). IR spectra of prawn chitosan indicate the functional group present such as alcohol, aldehyde, and carboxylic acid.

S. No.	Name of sample	Colour of extracted powder	Weight of raw shell (gm)	Weight of extracted chitosan (gm.)	% yield
1.	*Macrobrachium lamarrei lamarrei*	Pale white	3.12 gram	0.31 gram	9.93%

Antibacterial activity of chitosan derived from prawn shell against S.mutans

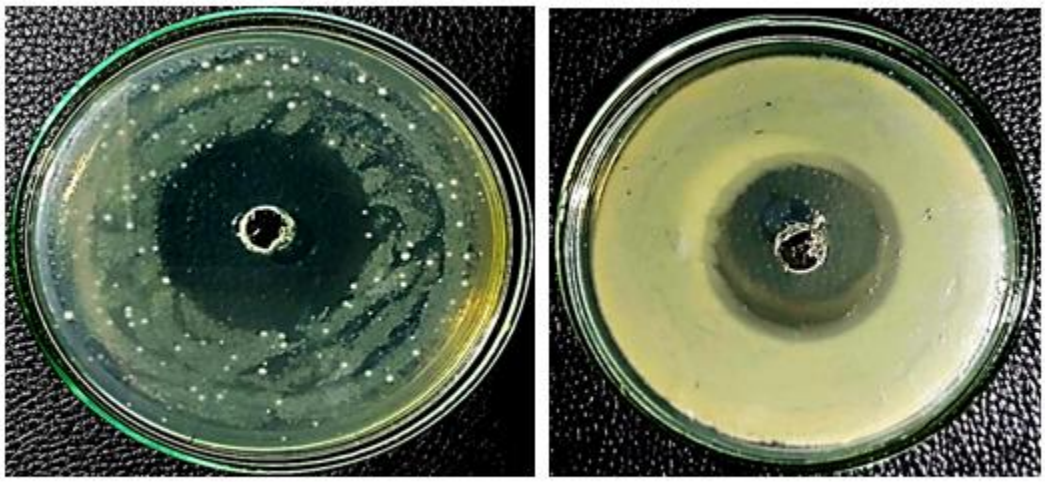


Antibacterial activity of chitosan derived from prawn shell against P.vulgaris

Table 1: Antimicrobial activity of Macrobrachium lamareii lamareii against S mutans (Gram positive bacteria) and P vulgaris (Gram negative bacteria)

s.no	Test organisms	Concentration in µg/ml	Zone of inhibition (in mm)			Mean± SD
			Plate 1	Plate 2	Plate 3	
1	*S mutans (Gram positive)*	75	37	33	35	35± 2
		100	34	36	30	33.33± 3
		150	33	33	35	33.67± 1
		200	35	32	36	34.33± 2
2	*P vulgaris (Gram negative)*	75	32	33	30	31.67± 1.5
		100	29	22	31	27.33± 4.5
		150	30	28	32	30± 2
		200	31	27	34	30.67± 3.5

Antimicrobial activity of Standard against Gram positive bacteria and Gram negative bacteria



Antimicrobial activity of Standard (Amoxycillin) against S. mutans

Antimicrobial activity of Standard (Ofloxacin) against *P. vulgaris*

Table 2: Antimicrobial activity of Standard against Gram positive bacteria and Gram negative bacteria

Microorganism	Zone of inhibition (mm)
P. vulgaris	30 mm
S. mutans	34 mm

Conclusion

The physiochemical properties of the extracted chitosan from *Macrobrachium lamarrei lamarrei*were characterized by FTIR. The antibacterial activity of chitosan against s.mutans and p.vulgaris was evaluated by calculation of minimum inhibitory concentration (MIC). This shows that the antibacterial efficiency of prawn chitosan is quite good and high antibacterial activity against pathogen. Chitosan can be widely used in food preservations, making of laceration dressing, and in pharmaceutical industry.

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