EXTRACTION, CHARACTERIZATION AND APPLICATIONS OF CHITOSAN FROM FISH SCALES

Sumathi.N1, Vignesh.R2 and Madhusudhanan.J3
1Department of Biotechnology, Shri Andal Alagar College of Engineering
2,3Department of Biotechnology, SAACE

Abstract—Chitosan was extracted from fish scales by a sequence of chemical processes involving Demineralization, Deproteinization and Deacetylation. The chitosan extracted had been characterized using FTIR analysis. The absorption band reveals the characteristic feature of chitosan polysaccharide and the degree of deacetylation. The anti-oxidant activity was studied by DPPH scavenging assay. The Anti-microbial activity was carried out by agar well diffusion method for the organisms viz. Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Aspergillus niger and Aspergillus terreus. The extracted chitosan had been analysed for its effects on seed germination. The chitosan extracted showed good anti-oxidant activity and also promoted seed germination.

Keywords- Chitosan, Demineralization, Deproteinization, Deacetylation, FTIR, Anti-microbial, Anti-oxidant, Seed germination.

I. INTRODUCTION

Three parts of India is surrounded by ocean and its inner land is also very much rich with ponds, lakes, and lagoons. The proper utilization of those water resources in terms of research in chitin and chitosan can bring the economic and academic prosperity of the nation [4]. Crustacean shell wastes contains about 30-40% of protein, 30-50% of calcium carbonate and 20-30% of chitin [7]. Chitin is the most important natural polysaccharide after cellulose found in crustaceous shell or in cell wall of fungi [6]. It is a naturally occurring nitrogen containing polysaccharide [2]. This biopolymer consists of 2-acetamido-2-deoxy-β-D-glucose through a β(1→4) linkage and that has intra and inter-molecular hydrogen bonds [1]. Chitin was extracted from shells by using hydrochloric acid and sodium hydroxide for deproteinizing and demineralizing the shells [3]. Chitosan was obtained by deacetylating the chitin obtained by using 50% sodium hydroxide [7]. Infrared (IR) characterization of chitin and chitosan was performed with spectrum frequency range of 400 - 4000cm⁻¹ [1]. Chitosan and chitooligosaccharides possess various biological activities including hypocholesterolemic, antimicrobial, immunostimulating, antitumor and anticancer effects, accelerating calcium and iron absorption, anti-inflammatory, antioxidant and Angiotensin-I-converting enzyme (ACE) inhibitory activities and so on, which are all correlated with their structures and physicochemical properties [8].

II. MATERIALS AND METHODS

A. Materials required

Fish scales, Conical flasks, 1% Potassium Permanganate, 1% oxalic acid, 40% NaOH, 1M HCl, 0.5% NaOH, Deionized water, Tap water, 50mM phosphate buffer, linolenic acid, ethanol, distilled water, ammonium thiocyanate, ferrous chloride, α-tocopherol, Muller Hinton Agar, Potato dextrose agar, sterile petriplates, sterile conical flasks, sterile cotton swabs, sterile discs, Microorganisms, Micropipette, Microtips, Autoclave, Hot plate, laminar air flow chamber, Weighing machine, Refrigerator, Hot air oven, UV-visible spectrometer.

B. Methodology

Collection of samples

Fish scales are collected from the local fish market at Chengalpet, Tamilnadu.
Extraction of chitosan

The fish scales were washed with tap water and sun dried. The dried scales were demineralized with 1M HCl (1:5 w/v) for 24 hours (hrs) at 30°C. The demineralized scales were then rinsed several times with deionized water and deproteinized using 0.5% NaOH (1:1 m/v) overnight at room temperature. After deproteinization, the chitin was rinsed several times with deionized water and then dried for 4hrs at temperature of 80°C. The deacetylation process was carried out by adding 100ml of 50% NaOH to each sample and then boiled at 120°C for 120 minutes (mins) on a hot plate. Then the samples were washed continuously with deionized water till clear solution was obtained and filtered in order to retain the solid matter, which is chitosan. The samples were left uncovered and oven dried at 120°C for 24hrs.

Anti-oxidant activity

The free radical scavenging activity of methanol extract was measured by using 1,1-diphenyl-2-picryl-hydrazyl (DPPH). 0.1mM solution of DPPH in methanol was prepared and 1ml of this solution is added to 3ml of various concentrations of methanol extract and reference compound (500 and 1000μg/ml). After 30min, absorbance was measured at 517nm. Ascorbic acid was used as reference material. The percentage of inhibition was calculated by comparing the absorbance values of reference compound (control) and samples.

\[
\text{Inhibition (\%)} = \left[1 - \frac{\text{Absorbance of sample}}{\text{Absorbance of control}}\right] \times 100
\]

Anti-bacterial activity

The antibacterial activity was determined by well diffusion method. About 25ml of molten Mueller Hinton agar was poured into a sterile Petri plate. The plates were allowed to solidify, after which 18hrs grown (OD adjusted to 0.6) 100μl of pathogenic bacteria were transferred onto plate and made culture lawn by using sterile L-rod spreader. After five mins setting of the pathogenic microbes, a sterile cork borer was used to make 5mm well on the agar. The test samples were dissolved in sterile saline and loaded in to wells with various concentrations such as 25μg/well, 50μg/well, 75μg/well and 100μg/well. The solvent saline loaded well served as negative control and Azithromycin (30μg/ml) well served as positive control. The plates were incubated at 37°C in a 40W florescent light source (~ 400nm) for 24hrs. The antibacterial activity was determined by measuring the diameter of the zone of inhibition around the well using antibiotic zone scale.

Anti-fungal activity

The antifungal activity was determined by well diffusion method. About 25ml of potato dextrose agar was poured into a sterile Petri plate. The plates were allowed to solidify, after which two days grown fungal disc (5mm) of mycelial fungal pathogens were placed separately on to the mid of the agar plate and wells were made. The test sample was dissolved in sterile water and loaded in to wells with various concentrations such as 25μg/well, 50μg/well, 75μg/well and 100μg/well. The clotrimazole added well served as positive control. All the drug loaded plates were kept for 72hr. The antifungal activity was determined by measuring the diameter of the zone of inhibition around the well using antibiotic zone scale.

Effect of chitosan on seed germination

20 mature green gram seeds were carefully selected and 10 seeds were placed in each test tube where first test tube received water alone, 2nd test tube received 0.1g of fish chitosan. Then incubated for 6hr thereafter, samples were put in the individual blotting paper then rolled as pole and placed inside the plastic bottle. After sun light and night incubation, the result of plant growth promotion on samples were studies after 4 days incubation. The shoot and root length was scored using ruler.
III. RESULTS

A. Extraction of chitosan

a) Fish Scales

b) Demineralized Scales

c) Deproteinized Scales
d) Deacetylated Scales

e) Fish Chitosan

Fig 1: Extraction of chitosan

Fig 1(a) shows the fresh fish scales collected from the local fish market. Fig 1(b), 1(c), 1(d) shows the demineralized scales, deproteinized scales and deacetylated scales obtained as the result of demineralization, deproteinization and deacetylation process respectively. Fig 1(e) shows the chitosan obtained from fish scales. On chemical treatment of 20g dried fish scales, 1.78g of chitosan was obtained.

B. FTIR results for fish chitosan

Fig 2: FTIR spectra
The fig 2 represents the spectra of fish chitosan. The absorption band in the region of 3429.50cm\(^{-1}\) and 3339.24cm\(^{-1}\) corresponds to OH stretching of water and NH\(_2\) stretching of free amino acids. The intensive peak at 1313.10cm\(^{-1}\) and 1607.56cm\(^{-1}\) corresponds to the vibration of NH\(_2\) which is a characteristic feature of chito polysaccharides indicate the occurrence of deacetylation.

C. Anti-oxidant activity

*Table 1: DPPH scavenging assay*

<table>
<thead>
<tr>
<th>Name of the sample</th>
<th>DPPH free radical scavenging ability (OD)</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fish</td>
<td>500μg</td>
<td>0.392</td>
</tr>
<tr>
<td></td>
<td>1000μg</td>
<td>0.321</td>
</tr>
<tr>
<td>CONTROL</td>
<td></td>
<td>0.624</td>
</tr>
<tr>
<td>ASCORBIC ACID</td>
<td>500μg</td>
<td>0.142</td>
</tr>
<tr>
<td></td>
<td>1000μg</td>
<td>0.012</td>
</tr>
</tbody>
</table>

Table 2 shows the DPPH scavenging ability of fish chitosan. It showed a maximum scavenging activity of 49.05% at concentration of 1000μg.

D. Anti-microbial activity

*Table 2: Anti-microbial activity of fish chitosan*

<table>
<thead>
<tr>
<th>SAMPLE NAME</th>
<th>Zone of Inhibition (mm)</th>
<th>Fish Chitosan</th>
<th>Standard</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>25 μg/ml</td>
<td>50 μg/ml</td>
</tr>
<tr>
<td>E.coli</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>K.pneumoniae</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>P.aeruginosa</td>
<td>-</td>
<td>-</td>
<td>9</td>
</tr>
<tr>
<td>A.niger</td>
<td>-</td>
<td>-</td>
<td>9</td>
</tr>
<tr>
<td>A.terreus</td>
<td>-</td>
<td>-</td>
<td>8</td>
</tr>
</tbody>
</table>

Table 2 shows the zone of inhibition of fish chitosan against various microbes. No significant zone of inhibition was obtained even at higher concentration (i.e 100μg/ml).

E. Effect of chitosan on seed germination

*Table 3: Seed germination percentage using roll towel model*

<table>
<thead>
<tr>
<th>Sample</th>
<th>Germination %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>90</td>
</tr>
<tr>
<td>Fish</td>
<td>100</td>
</tr>
</tbody>
</table>

Table 3 shows that all seeds treated with fish chitosan germinated while only 90% of the seeds served as control germinated.

*Table 4: Shoot and root length of germinated seeds*

<table>
<thead>
<tr>
<th>Sample</th>
<th>Shoot length (cm)</th>
<th>Root length (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4.1</td>
<td>16.2</td>
</tr>
<tr>
<td>Fish</td>
<td>5.2</td>
<td>17.6</td>
</tr>
</tbody>
</table>
Table 4 shows that the seeds treated with fish chitosan had greater shoot and root length compared to the control. This shows that the fish chitosan has effects on promoting seed germination.

**Fig 3: Effect of fish chitosan on seed germination**

Figure 3 shows the germinated seeds served as control and seeds treated with fish chitosan.

### IV. DISCUSSION

Chitosan was extracted from fish scales by chemical process including demineralization, deproteinization and deacetylation. The extracted chitosan was characterized using FTIR analysis. The anti-oxidant assay was studied using DPPH scavenging assay. The chitosan extracted from fish showed higher anti-oxidant activity. The anti-microbial assay was carried out by agar well diffusion method against *E.coli, K.pneumoniae, P.aeruginosa, A.niger* and *A.terreus*. The chitosan extracted had also been studied for its effect on seed germination. The seeds treated with fish chitosan were found to be effective on seed germination.

### REFERENCES


