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# Preliminary Study on Physicochemical and Biological Properties of Chitosan Extracted from Mud Crab (*Scylla olivacea*) Shells

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Chitosan, a non-toxic and biodegradable biopolymer, has been immensely exploited due to its inherent features, such as its ability to inhibit bacterial growth and free radical scavenging. This biopolymer can be extracted from crustaceans, molluscs, insects, or fungi chitin. Interestingly, different sources of this biopolymer affect the physicochemical and biological activities. Thus, this study aims to characterise, compare the physicochemical and biological properties of extracted chitosan (E.ch) from mud crabs, *Scylla olivacea* (*S. olivacea)*, and commercial chitosan (C.ch). The E.ch was extracted via the chemical method, and their physicochemical and biological properties, such as degree of deacetylation (DD), viscosity, antibacterial, antifungal, as well as antioxidant activity, were characterised. The results revealed that E.ch with high DD and low viscosity showed better antibacterial activity against *Pseudomonas aeruginosa* (*P. aeruginosa*), better antifungal activity against *Aspergillus flavus* (*A. flavus*) and high antioxidant activity compared to C.ch. The higher DD of E.ch contributed to better antibacterial, antifungal, and antioxidant activity. In conclusion, the findings of this study on the physicochemical and biological properties of E.ch from mud crabs could be utilised in various fields such as biomedical, pharmaceutical, and agriculture applications.

**Keywords**: Antibacterial; antifungal; antioxidant; biopolymer; chitosan; mud crab

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The mud crab, scientifically known as *Scylla olivacea* (*S. olivacea*), is a crab species that commonly inhabits burrows within densely populated mangrove areas in the Indo-West Pacific region [1]. Mud crab is one of the most delicious seafood delicacies owing to its tender meat and flavourful taste. However, hard exoskeletons are waste because they are not consumable. Improper seafood waste management can lead to environmental pollution. The current study used mud crab shell waste to extract chitosan and produce functional materials. Chitosan is a linear polysaccharide consisting of 2-amino-2-deoxy-(1-4)-β-D-glucopyranose residues (D-glucosamine units) [2]. Several methods have been used to extract chitosan, including chemical and biological extraction [2]. The chemical extraction method used in this study involved four main steps: demineralization, deproteinization, discolouration, and deacetylation. Acid treatment is applied in demineralization to remove calcium carbonate, whereas deproteinization involves alkali treatment to remove proteins from mud crab shells [3]. The discolouration step is applied to remove the colour pigment and obtain a white appearance on the sample [4]. Deacetylation uses alkali treatment to remove the acetyl group from the chitin backbone to produce chitosan [5]. Chitosan has many applications in various fields, such as agriculture, pharmaceuticals, cosmetics, and food processing [6].

Their physicochemical and biological properties were characterised to determine whether mud crab chitosan could be used in future applications. The physicochemical and biological properties tested in this study were chitosan yield percentage, degree of deacetylation (DD), viscosity, morphology, antibacterial, antifungal, and antioxidant properties. Determining DD and viscosity is crucial because they influence chitosan's biological properties and applications [6]. The higher DD of chitosan indicates its high purity and a high cationic effect in biological applications [7]. Chitosan extracted from crab (*Carcinus mediterraneus*) shells can inhibit *Escherichia coli* (*E. coli*) and *Staphylococcus aureus* (*S. aureus*) but more effectively on *E. coli* [8]. In addition, the antifungal activity of crab chitosan extracted by Hajji et al. [8] was able to inhibit *Aspergillus niger* (*A. niger*) and *Fusarium solani* (*F. solani*) with the same zone of inhibition (17 mm).

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*Aspergillus flavus* (*A. flavus*) is a pathogenic fungus that causes crop spoilage due to aflatoxin production [9]. Meanwhile, *Marasmius cladophyllus* (*M. cladophyllus*) can be a pathogenic fungus once changes occur with the tree or plant host [10]. Besides, a previous study reported that *Marasmius palmivorus* (*M. palmivorus*), which is in the same genus as *M. cladophyllus*, can be applied as a bioherbicide against pathogen fungi, but *M. palmivorus* also can cause palm bunch and stem rot disease of coconut palms [11,12]. Thus, *M. cladopyhllus* can become pathogenic depending on the environmental conditions, its interaction with the host plant, or the presence of endobacteria and mycoviruses that can influence fungal behaviour [13]. There are still few reports on the antifungal activity of chitosan extracted from mud crab shells (*S. olivacea*) (E.ch) against *A. flavus* and *M. cladophyllus*, so it is essential to investigate the potential antifungal activity against these fungi for future application in agriculture. In this study, a commercial chitosan (C.ch) was used as a reference to confirm that chitosan extracted from mud crab shells was successful. E.ch can have different physicochemical properties than C.ch, which can cause variations in its biological properties. Thus, the current study aims to characterise and compare the physicochemical and biological properties of E.ch with C.ch.

## EXPERIMENTAL

### Chemicals and Materials

Commercial chitosan from crabs with medium molecular weight (Mw) and 2,2-diphenylpicrylhydrazyl (DPPH) were purchased from Sigma Aldrich Corporation. Mud crab shells were obtained from a wet market in Kota Samarahan, Sarawak, Malaysia, to extract chitosan. *E. coli*, *S. aureus*, *P. aeruginosa*, *A. flavus*, and *M. cladophyllus* strains were subcultured and provided by Molecular Genetic Lab of the Faculty of Resource Science and Technology, Universiti Malaysia Sarawak (UNIMAS). All chemical reagents used in this study were used directly without further purification.

### Sample Collection and Preparation

Mud crab shells were washed under running water to remove contaminants and adhering proteins. The mud crab shells were dried in an oven at 70°C until thoroughly dried [14]. The dried shells were crushed and ground into powder using a mortar and pestle. The mud crab shell was stored in a freezer at 4°C for long-term storage and dried in an oven at 70°C before chitosan extraction.

### Chitosan Extraction

Mud crab shells undergo four steps: demineralization, deproteinization, discolouration, and deacetylation. In the demineralization steps, mud crab shells were treated with 7 % (v/v) hydrochloric acid (HCl) at a ratio of 1:10 (w/v) for 3 hours at 60°C [15]. The demineralized sample was washed with distilled water until it reached a pH of 7. Next, 2 % (w/v) potassium hydroxide (KOH) was used in the deproteinization steps at 90°C for 2 hours with a 1:20 (w/v) ratio [16]. The extracted chitin was washed with distilled water until it reached pH 7. Then, in the discolouration steps, the sample was treated with acetone repeatedly to obtain a white colour and dried in an oven overnight at 50°C after air-drying for two hours. Finally, deacetylation was conducted using sodium hydroxide (NaOH) at a concentration of 45 % (w/v) in a 1:20 (w/v) ratio for two hours at 110°C. The extracted chitosan was washed with distilled water until it reached pH 7. The final product was dried overnight in an oven at 50°C.

### Percentage Yield (%)

The percentage yield of E.ch was calculated according to Equation (1) [17].

$$\text{Yield (\%)} = \frac{\text{Dry weight of chitosan (g)}}{\text{Dry weight of raw crab shell (g)}} \times 100 \quad (1)$$

### Degree of Deacetylation (DD)

The DD of E.ch and C.ch was estimated using Attenuated total reflectance-Fourier transform infrared (ATR-FTIR) spectroscopy (Agilent Technologies, Cary 630 FTIR spectrometer). The sample was analysed with an attenuated reflectance accessory from 650-4000 $cm^{-1}$. The degree of acetylation (DA) and DD of the samples were estimated using Equation (2) and Equation (3) [18,19]. $A_{1320}$ and $A_{1420}$ are the absorbances of the peaks at wavenumbers 1320 and 1420 $cm^{-1}$, respectively.

$$\text{DA (\%)} = \frac{\left(\frac{A_{1320}}{A_{1420}}\right) - 0.3822}{0.03133} \quad (2)$$

$$\text{DD (\%)} = 100\ \% - \text{DA (\%)} \quad (3)$$

### Viscosity

E.ch and C.ch were diluted in 1 % acetic acid (Acac). The viscosity was determined using a Brookfield viscometer equipped with spindle number two at a speed of 50 rotations per minute (rpm) and a temperature of 25°C [16]. The value was expressed in millipascal-second (mPa.S).

### Morphology

The morphologies of E.ch and C.ch were determined using a JSM-IT200 InTouchScope™ Scanning Electron