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# Extraction and characterization of Chitin from *Lethrinus ornatus* (Ornate Emperor) fish scales

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**ABSTRACT**

*Chitin, an amino polysaccharide, is widely found in the exoskeletons of insects, crustacean shells, and fungal cell walls. However, the potential of fish scales—commonly discarded as industrial waste—as an alternative chitin source remains largely unexplored. This present study aimed to extract and characterize chitin from the scales of Lethrinus ornatus (Ornate Emperor). The yield of chitin extraction from L. ornatus scales was 34.4% (±1.5) on a dry weight basis. Structural elucidation through UV-Vis and FTIR spectroscopic analyses revealed the presence of characteristic bands with spectral patterns closely aligned with the literature descriptions. Additionally, SEM studies displayed that the extracted chitin has an amorphous and granular structure. These results indicated that the L. ornatus scales could be used as a novel and alternative source of chitin.*

## 1. INTRODUCTION

Chitin ranks as the second most abundant structural amino polysaccharide in nature, second only to cellulose (Abdou et al., 2008; Kumari & Rath, 2014; Elieh-Ali-Komi et al., 2016; John Kasongo et al., 2020). It comprises 2-acetamido-2-deoxy-d-glucopyranose (GlcNAc) connected through β-(1→4) linkages. Chitin shares a structurally similarity with cellulose but features an acetamide group at the $C_2$ position instead (Elieh-Ali-Komi et al., 2016). Chitin is a white, hard, and inelastic nitrogenous polysaccharide that is known to be non-toxic, biodegradable, and biocompatible and can be converted into another derivative, chitosan (Kurita, 2006). These biopolymers have been utilized in biomedical applications, including controlled drug delivery, wound healing, molecular imaging, diagnosis, and tissue engineering (Song et al., 1992; Ahmad et al., 2020). They are also used in food and textile industries (Rathke & Hudson, 1994), cosmetics (Aranaz et al., 2009; Ito et al., 2014), and wastewater treatment for heavy metal removal, as well as the emerging pollutants like antibiotics (Nechita & Nechita, 2017; da Silva Alves et al., 2021).

Although insects, exoskeletons of marine invertebrates such as crustaceans, and fungi's cell walls are considered the most abundant sources of chitin, fish scales can also be an alternative. Fish scales mainly comprise collagen and underutilized components, such as chitin, which can be transformed into chitosan through deacetylation (Kumari & Rath, 2014; Molina-Ramírez et al., 2021). The fishing industry has been rapidly growing in the past few years, with the overall volume of fisheries production in the Philippines from January to December 2023 reaching 4.26 million metric tons (Philippine Statistics Authority,

2024). Consequently, fish scale waste has dramatically increased (Coppola et al., 2021). Approximately 8 million tons of shell and scale waste are generated worldwide each year, with 1.5 million tons coming from Southeast Asia (Cadano et al., 2021). These waste products are inedible, lack nutritional value, and represent approximately 1% of the total weight of fish (Molina-Ramírez et al., 2021). They often end up in landfills with other solid wastes, causing odor and damage to the environment and human health.

Recent studies have managed to extract and characterize chitin and chitosan from the scales of *Cyprinus carpio* (Zaku et al., 2011), Nile tilapia (Boarin-Alcalde & Graciano-Fonseca, 2016), *Labeo rohita* (Kumari & Rath, 2014; Srivastav et al., 2018), *Prochilodus magdalenae* (Molina-Ramírez et al., 2021), *Catla catla* (Durairaj et al., 2018), *Chlorurus sordidus* and *Lutjanus argentimaculatus* (Rumengan et al., 2017), *Sardina pilchardus* (Aboudamia et al., 2020), pang and silver fish scales (Alabaraoye et al., 2018), with varying chitin and chitosan yields ranging from 10 to 45%. Although there have been significant studies on chitin extraction from various sources, there is still a need to explore new and nonconventional sources to meet the emerging demand and new applications for chitin. *Lethrinus ornatus*, commonly known as "*kiros*" or "*katambak*", is a species of fish that is mainly found in the Atlantic, Indian, and Pacific Ocean, including the waters in the Philippines (Carpenter et al., 2016). It is highly abundant in the Visayas region and commercialized informally by street vendors, as it serves as a vital food source for communities living in the coastal areas. In this study, we have presented the extraction and characterization of chitin from *L. ornatus* scales as a novel and alternative source of chitin.

## 2. MATERIALS AND METHOD

### 2.1. Materials

*L. ornatus* scales were collected in the Bagsakan at the South Road Properties (SRP), Cebu City, Philippines, with the help of the local artisanal fishermen. Before use, the fish scales were carefully rinsed with distilled water to eliminate soluble organic matter, impurities, and adhesive proteins. Cleaned fish scales were air-dried in a well-ventilated closed space for six (6) days. The dried samples were later crushed and pulverized using a laboratory blender at 3000 rpm for 3 minutes and sieved to a fine powder. The pulverized samples were weighed, placed in a sealed plastic container, and desiccated at room temperature. All reagents and solvents were used without prior purification or modification. Hydrochloric acid (37%), sodium hydroxide pellets (98%), and other laboratory reagents and materials were purchased from local chemical suppliers and distributors.


**Figure 1. Photographs of *L. ornatus* fish samples: (a) before the descaling process, (b) the collected fish scales**

### 2.2. Extraction of chitin from *L. ornatus* fish scales

The method described by Molina-Ramírez et al. (2021), with modifications from Boarin-Alcalde and Graciano-Fonseca (2016), was used to extract chitin from fish scales. The extraction process is summarized in three main steps: demineralization, deproteinization, and decolorization.

#### *2.2.1. Demineralization*

Fish scales were demineralized with a 1% hydrochloric (HCl) solution at room temperature and a sample-to-solvent ratio of 1:10 (w/v) under constant stirring at 300 rpm for 16 hrs. The resulting mixture was filtered using Whatman No. 1 (125 mm) filter paper and washed repeatedly with distilled water for neutrality. The resulting residue was dried to constant weight for 12 hrs. at 30°C.

#### *2.2.2. Deproteination*

Demineralized scales were deproteinized with a 1% sodium hydroxide (NaOH) solution at room temperature and a sample-to-solvent ratio of 1:10 (w/v) for 16 hrs. Subsequently, the resulting mixture was filtered, and the residue was rinsed with distilled water until neutrality. Additional heating using distilled water for 2 hrs. under a temperature of 80°C was done to fully separate the collagen and other proteins from the sample. The residue was filtered and dried in the laboratory oven for 12 hrs. at 30°C.

*2.2.3. Decolorization*

The deproteinized product was treated with a 1% sodium hypochlorite (NaClO) solution at room temperature under constant stirring at 300 rpm for 2 hrs. The residue was filtered, washed with distilled water until neutrality, and dried for 12 hrs at 30°C. The chitin thus obtained was desiccated at room temperature before characterization.

### 2.3. Structural characterization procedures

*2.3.1. Ultraviolet-visible (UV-Vis) spectroscopy*

A 0.1 gram of chitin was heated in 20 millimeters of 85% phosphoric acid ($H_3PO_4$) at 60°C for 40 minutes while continuously stirred. Once the chitin was fully dissolved, a 1-milliliter solution was pipetted and diluted with 100 milliliters of distilled water. The UV-Vis absorption spectrum of the chitin was measured using a UV-Vis spectrophotometer (Model: Mettler Toledo UV5Nano) across a wavelength range of 190 to 800 nm.

*2.3.2. Fourier transform infrared (FTIR) spectroscopy*

The FTIR spectrum of the extracted chitin was recorded using an FTIR spectrometer (Model: Perkin Elmer Spectrum Two) equipped with a lithium tantalate ($LiTaO_3$) detector with a universal attenuated total reflectance (ATR) accessory. The chitin was scanned at a resolution of 4 $cm^{-1}$, and the transmittance values (T%) were evaluated in the wavelength range of 450 to 4000 $cm^{-1}$. The resulting spectrum was processed using Perkin Elmer Spectrum 10 software.

*2.3.3. Scanning electron microscopy (SEM)*

The chitin samples were characterized using a field emission scanning electron microscope (Model: JEOL JSM IT500HR/LA) operating at an accelerated voltage of 30 kV in high vacuum mode. The samples were directly mounted on stubs using double-sided carbon tape, then coated with a thin layer of platinum using an automated sputter coater (Model: JEOL JEC-3000 FC) for 40 seconds. They were subsequently scanned under SEM at 10,000x magnification.

## 3. RESULTS AND DISCUSSION

### 3.1. Yield of Chitin extraction

The yield of the chitin extraction from *L. ornatus* scales was 34.4% (±1.5) on a dry weight basis, which was highly comparable to the values reported by Alabaraoye et al. (2018) with 33 and 36% yield from pang and silver fish scales, respectively, and Rumengan et al. (2017) with 33% yield from *Lutjanus argentimaculatus*. Conversely, the amount of chitin extracted from *L. ornatus* scales exceeds the chitin yield obtained from *Sardina pilchardus* (Zahra Aboudamia et al., 2020), *Chanos chanos* scales (Cadano et al., 2021), *Oreochromis niloticus* (Boarin-Alcalde & Graciano-Fonseca, 2016), and *Labeo rohita* (Muslim et al., 2013).

### 3.2. UV-Vis analysis

The UV-Vis spectrum of chitin from *L. ornatus* recorded in dilute phosphoric acid was presented in Figure 2. The absorption spectrum displayed a prominent absorption peak ($\lambda_{max}$) around 197 nm, which can be attributed to the $\pi \rightarrow \pi^*$ transition of the carbonyl group present in the *N*-acetylglucosamine units of the chitin structure (Negrea et al., 2015). This absorption peak is a defining characteristic of chitin and closely aligns with the values previously reported in earlier studies (Liu et al., 2006; Wu & Zivanovic, 2008; Kumirska et al., 2010; Negrea et al., 2015).

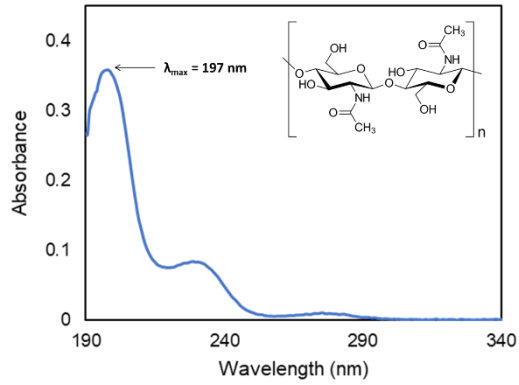


**Figure 2. UV-Vis Spectrum of chitin from *L. ornatus* scales**

A small absorption peak was also observed approximately at 230 nm, which can be ascribed to the $n \rightarrow \pi^*$ transition of the secondary amide fragment and dehydration products (Wu & Zivanovic, 2008; Negrea et al., 2015; Amelia et al., 2021). Moreover, this UV-Vis spectrum can also serve as an indicator to determine the purity of the chitin samples. The absence of a prominent peak around 280 nm in the spectrum indicates minimal contamination from proteins, amino acids and other organic compounds that absorb in this particular region.

### 3.3. FTIR analysis

The FTIR spectrum of the chitin obtained from *L. ornatus* is illustrated in Figure 3. The characteristic medium band of N-H stretching is at 3279 $cm^{-1}$, while the stretching of the O-H group may exist around 3000-3500 $cm^{-1}$. The strong band at 1643 $cm^{-1}$ is associated with the carbonyl in the acetamide group (amide I). This band also indicated that the amorphous state of chitin is very likely to be in β-allomorphic form by observing its undivided appearance (Sagheer et al., 2009; Kaya et al., 2017; Delezuk et al., 2019; Zahra Aboudamia et al., 2020). Furthermore, the sample spectrum shows that the N-H (amide II) bending and C-N stretching vibrations from weak bands confirm the acetamide group at 1517 $cm^{-1}$. The peak at 1444 $cm^{-1}$ can be attributed to $CH_2$ ending and $CH_3$ deformation, while the one at 1024 $cm^{-1}$ alludes to the presence of C-O stretching in the glucosamine ring. Significant peaks at 601 and 561 $cm^{-1}$ are due to N-H and C-O bending vibrations. The spectral pattern observed in the FTIR spectrum reveals the presence of distinct functional groups, including alcohol, carbonyl, ether, and amide groups.

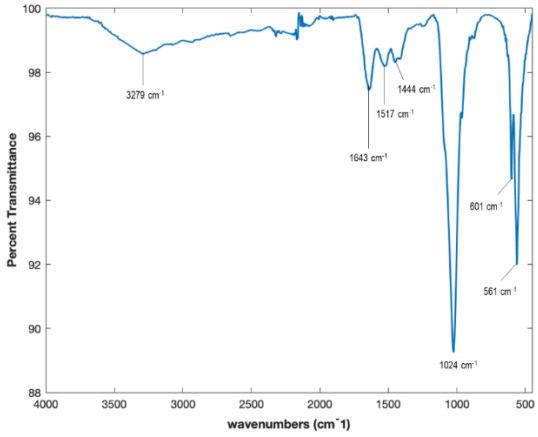


**Figure 3. FTIR spectrum of the extracted chitin from *L. ornatus* scales**

Moreover, the relevant peaks identified in the FTIR spectrum were also compared to earlier studies (Boarin-Alcalde & Graciano-Fonseca, 2016; Demir et al., 2016; Mohan et al., 2021). FTIR studies of chitin from Nile tilapia presented a characteristic large band at 3500 $cm^{-1}$ regions corresponding to axial O-H stretching and a significant peak at 1655 $cm^{-1}$ associated to the axial strain of C═O present in chitin (Boarin-Alcalde & Graciano-Fonseca, 2016). Demir et al. (2016) have observed major absorption bands at 1651, 1034, and 895 $cm^{-1}$ relating to C═O stretching in the acetamide group, C–O stretching, and a ring stretching corresponding to the characteristic bond for β-(1→4) glycosidic linkage, respectively, in blue crab chitin. In the chitin spectrum of several crustacean shell wastes, two distinct peaks at 1650 and 1620 $cm^{-1}$ confirmed the presence of the amide I group, and the absorption band at 3445-3442 $cm^{-1}$ is associated with O-H stretching, while those at 1075-1074 $cm^{-1}$ were corresponding to the asymmetric C-O-C stretching in phase ring (Mohan et al., 2021). Furthermore, the FTIR bands of the chitin obtained from *L. ornatus* closely resemble those of chitin extracted from various marine sources.

### 3.4. SEM analysis

The surface morphology of chitin extracted from *L. ornatus* was observed using a scanning electron microscope and illustrated in Figure 4. The chitin samples exhibited an amorphous and granular structure and displayed an apparent aggregation at 10000x magnification. It was also observed that there were no nanofibers and nanopores on the surface of the extracted chitin. Similar results can be seen in the SEM image of chitin extracted from millipede, which may be due to the intrasheet and intersheet hydrogen bonding (Sriram et al., 2018).

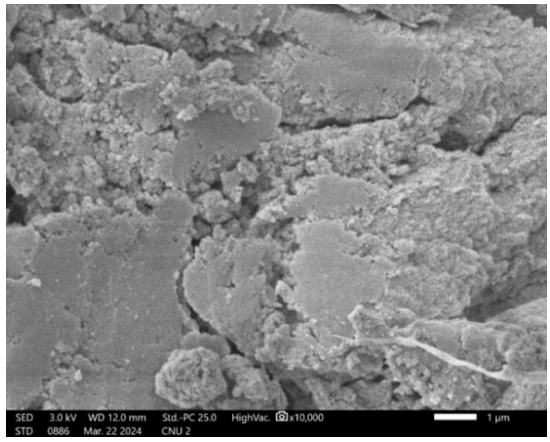


**Figure 4. SEM image of the chitin extracted from fish scales of *L. ornatus***

## 4. CONCLUSIONS

This present study is basically focused on the extraction of chitin from the scales of *L. ornatus*. These fish species are mainly found in the Atlantic, Indian, and Pacific Oceans. The yield of chitin extraction from *L. ornatus* was comparatively the same as other fish scale sources. Spectroscopic and microscopic analyses confirmed distinct characteristic bands with spectral patterns and

surface morphology aligned closely with literature descriptions. Hence, *L. ornatus* fish scale represents a novel and promising source of chitin. Further research on the conversion to chitosan, characterization, and different applications of the chitin derived from this source is needed.

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