Extraction of Chitosan Waste Nanoparticles from Prawn Shell Waste and Evaluation of Anti-Inflammatory Activity

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***Abstract*—Inflammation is a protective biological response against tissue injury and infection however, prolonged or uncontrolled inflammation is associated with various chronic diseases such as arthritis, cardiovascular disorders, and cancer.Chitosan, a biopolymer obtained from crustacean shell waste, has gained considerable attention due to its biodegradability, biocompatibility, and notable biological properties, including antiinflammatory activity. The present study aimed to extract chitosan from prawn shell waste, synthesize chitosan-based nanoparticles, and evaluate their in-vitro anti-inflammatory potential. Prawn shell waste was processed and subjected to alkaline deacetylation to obtain purified chitosan. Chitosan nanoparticles were synthesized using the ionic gelation method with sodium tripolyphosphate as a cross-linking agent, a widely reported and efficient technique for nanoparticle preparation. The formation of nanoparticles was confirmed by UV–Visible spectrophotometric analysis. The in-vitro anti-inflammatory activity of the synthesized chitosan nanoparticles was evaluated using the lipoxygenase (LOX) inhibition assay, as lipoxygenase plays a key role in inflammatory mediator synthesis. The study concludes that prawn shell–derived chitosan nanoparticles can serve as a promising, eco-friendly, and biocompatible natural anti-inflammatory agent, while also promoting the sustainable utilization of marine .**

***Index Terms*—Prawn shell, Chitosan, Lipoxygenase, Nanoparticles .**

I. INTRODUCTION

Inflammation is a fundamental biological response that plays a critical role in host defense against infection, tissue injury, and foreign substances. However, prolonged or uncontrolled inflammation contributes to the pathogenesis of several chronic disorders, including arthritis, cardiovascular diseases, inflammatory bowel disease, neurodegenerative disorders, and cancer (Medzhitov, 2008). Conventional anti-inflammatory drugs such as non-steroidal antiinflammatory drugs (NSAIDs) and corticosteroids are effective in controlling inflammation but are often associated with adverse side effects during long-term use.

Biopolymers obtained from natural sources have emerged as promising candidates for biomedical applications due to their biodegradability, biocompatibility, and low toxicity. Among them, chitosan, a deacetylated derivative of chitin, has gained significant attention for its pharmaceutical and therapeutic potential (Rinaudo, 2006). Chitosan is widely distributed in nature and is abundantly obtained from crustacean shell waste such as shrimp, crab, and prawn shells. The utilization of prawn shell waste not only provides a cost-effective source of chitosan but also addresses environmental concerns associated with seafood processing by-products.

Chitosan exhibits a wide range of biological activities, including antimicrobial, antioxidant, wound-healing, and anti-inflammatory properties. However, its practical application is often limited by poor solubility, low stability, and reduced bioavailability under physiological conditions (Dash et al., 2011). The development of chitosan-based nanoparticles has proven to be an effective strategy for improving solubility, increasing surface area, and enhancing cellular uptake.

Nanoparticles derived from chitosan possess unique physicochemical properties that make them suitable for biomedical applications, particularly in

inflammation-related studies. Their small size and positive surface charge facilitate interaction with negatively charged cell membranes, enabling efficient cellular internalization (Mohanraj and Chen, 2006). Furthermore, chitosan nanoparticles can modulate inflammatory pathways by inhibiting the production of pro-inflammatory mediators such as nitric oxide (NO), prostaglandins, and cytokines. In-vitro anti-inflammatory evaluation using macrophage cell lines has become a widely accepted approach to study inflammation at the cellular and molecular levels. Activated macrophages play a central role in inflammatory responses by releasing inflammatory mediators, including nitric oxide, tumor necrosis factor-α (TNF-α), and interleukins (IL-6, IL1β) (Nathan, 2002). Therefore, assessing the ability of chitosan-based nanoparticles to suppress these mediators provides valuable insight into their anti-inflammatory potential. The present study focuses on the development of chitosan-based nanoparticles derived from prawn shell waste and the evaluation of their in-vitro anti-inflammatory activity.

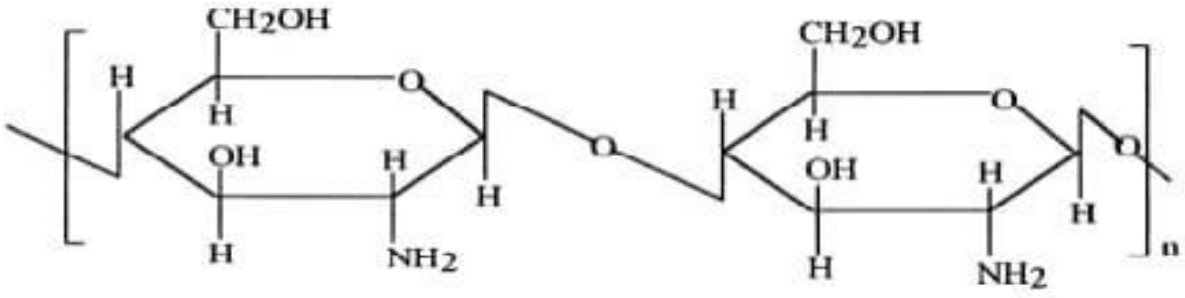


Figure-1: Chemical structure of chitosan

Collection and Preparation of Prawn Shell

Prawn shell waste was collected from a local seafood processing unit. The shells were washed thoroughly with running tap water followed by distilled water to remove adhering tissue and impurities. The cleaned shells were sun-dried and then oven-dried at 60 °C until constant weight was achieved. The dried shells were ground into a fine powder using a mechanical grinder and stored in airtight containers for further processing.

Figure-2: Storing of prawn shell

Extraction of Chitosan from Prawn Shell

Chitosan was prepared by deacetylation of chitin. The chitin obtained was treated with 50% (w/v) sodium hydroxide solution at 90–100 °C for 4 hours. After cooling, the mixture was filtered and washed repeatedly with distilled water until neutral pH was reached. The final product was oven-dried and stored as prawn shell-derived chitosan. After repeated washing with distilled water and confirmation of neutral pH, the purified chitosan was dried in a hot air oven at 60 °C until constant weight was obtained. The dried chitosan was allowed to cool to room temperature and then dissolved in distilled water (or 1% acetic acid, if required for complete solubilization) under continuous magnetic stirring to obtain a homogeneous solution.

Characterization of chitosan nanoparticle

The formation of chitosan nanoparticles was preliminarily confirmed by UV–Visible spectrophotometric analysis. The synthesized chitosan nanoparticles were dispersed in distilled water by mild sonication to obtain a uniform suspension. The UV–Visible absorption spectrum of the nanoparticle suspension was recorded in the wavelength range of 200–800 nm using a UV–Visible spectrophotometer, with distilled water used as the blank.

II. ENZYME INHIBITION ASSAY

Assay for Screening of Lipoxygenase (LOX) Inhibition

The Lipoxygenase Inhibitor Screening Assay Kit detects the hydroperoxides produced in the lipoxygenation reaction using a purified LOX and is used to screen for inhibitors of LOX enzyme. The assay protocol requires the addition of plant extract to the enzyme prior to start of reaction followed by initiation of reaction with the addition of substrate. The 96-well plate was placed on a shaker for at least five minutes, followed by the addition of chromogen to stop enzyme catalysis. The plate was then covered and placed on a shaker for five minutes. The absorbance was measured at 490 nm using a plate reader after removing the plate cover. The percent inhibition for each inhibitor can be calculated using the following equation. Standard = zileuton (100µg) and control = no inhibitors

$$\text{Percentage Inhibition (\%)} = \frac{\text{Activity of control} - \text{Acitivity of test}}{\text{Activity of control}} \times 100$$

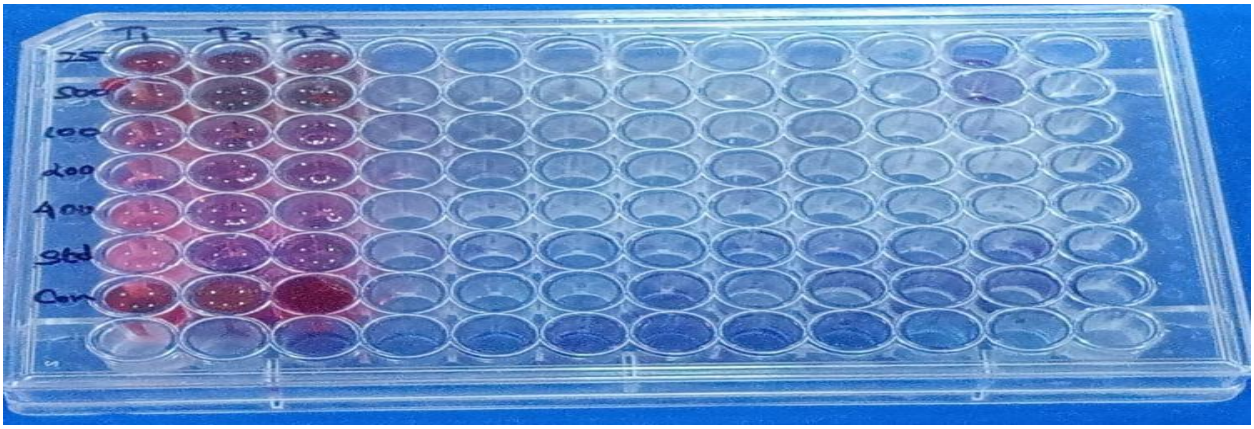


Figure-4: Minimal inhibition concentration (IC50) of the drug in microwell plate

III. RESULTS

UV–Visible Spectroscopic Characterization of Chitosan Nanoparticles

The nanoparticle suspension exhibited a characteristic absorption peak in the range of 220–230 nm when scanned between 200 and 800 nm. This absorption peak corresponds to electronic transitions associated with amino and hydroxyl functional groups of chitosan.

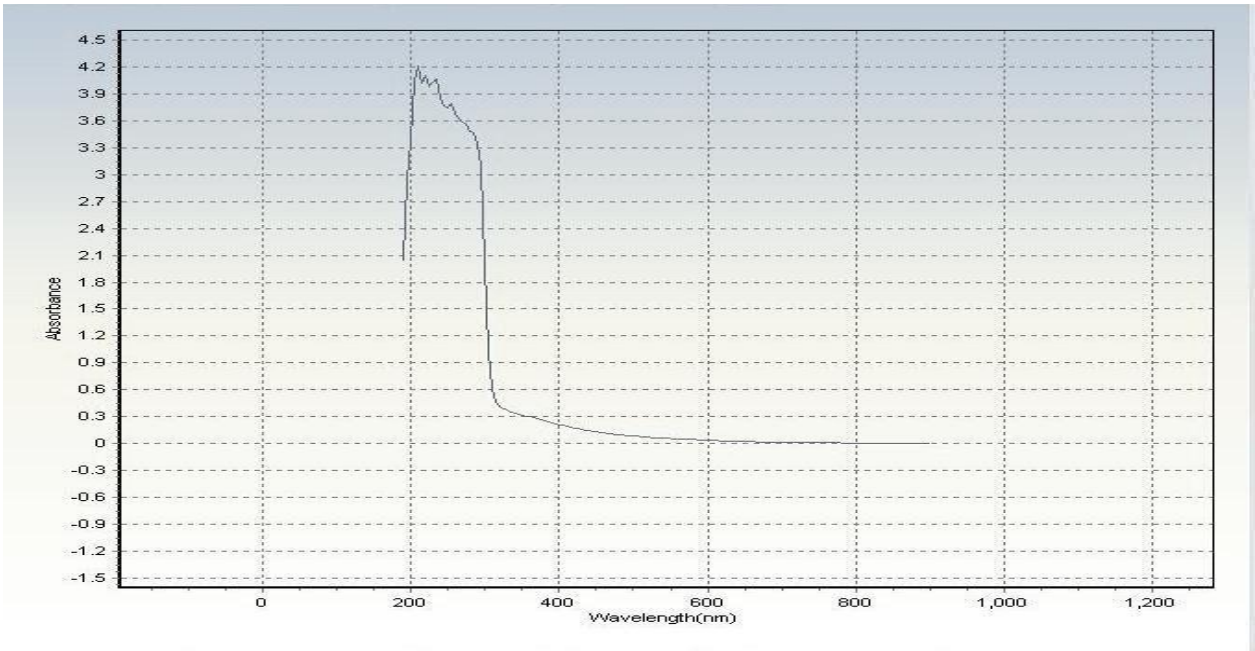
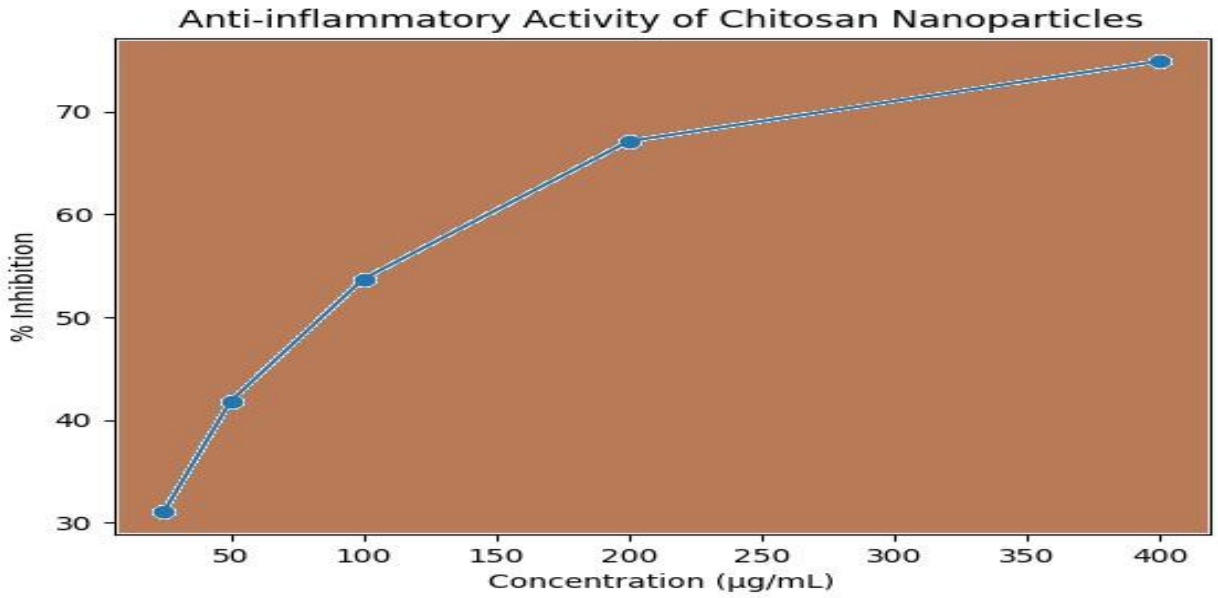


Figure-4: A graph was drawn with the Percent Inhibition as a function of the inhibitor concentration to determine the IC50 value (concentration at which there was 50% inhibition)

Compared to native chitosan, the nanoparticle formulation showed increased absorbance intensity and a slight shift in the absorption maximum, indicating structural reorganization during nanoparticle formation. The absence of additional peaks at higher wavelengths suggested uniform nanoparticle formation without aggregation or unwanted by-products.

LOX Inhibition assay

Sample	Concentration	OD1	OD2	OD3	Average	% inhibition
Chitosan	25	0.679	0.648	0.702	0.67633333	31.1%
Chitosan	50	0.567	0.589	0.56	0.572	41.8%
Chitosan	100	0.438	0.464	0.462	0.45466667	53.7%
Chitosan	200	0.316	0.326	0.328	0.32333333	67.1%
Chitosan	400	0.235	0.249	0.254	0.246	74.9%
Control(Negative)	No inhibitor	0.975	0.983	0.992	0.98333333	0
Standard(Dexamethasone)	100	0.094	0.096	0.091	0.09366667	90.4%



IV. DISCUSSION

The present study demonstrates the successful utilization of prawn shell waste as a sustainable raw material for the extraction of chitosan, its conversion into nanoparticles, and the evaluation of in-vitro anti-inflammatory activity using the lipoxygenase (LOX) inhibition assay. Chitosan was successfully obtained through alkaline deacetylation of chitin using concentrated sodium hydroxide. Similar observations have been reported in earlier studies on crustacean shell–derived chitosan, highlighting the importance of pH control and washing steps in achieving high-quality chitosan (Rinaudo, 2006; Younes and Rinaudo, 2015).

The synthesis of chitosan nanoparticles using the ionic gelation method was successful, as evidenced by the formation of an opalescent suspension upon interaction of chitosan with sodium tripolyphosphate (TPP). The electrostatic interaction between positively charged amino groups of chitosan and negatively charged phosphate groups of TPP results in stable nanoparticle formation (Calvo et al., 1997).UV–Visible spectroscopic analysis provided preliminary confirmation of nanoparticle formation. Similar UV–Visible spectral features have been reported for chitosan nanoparticles synthesized via ionic gelation (Mohanraj and Chen, 2006). The in-vitro anti-inflammatory potential of chitosan nanoparticles was evaluated using the LOX inhibition assay, which is a reliable method for assessing inhibition of inflammatory pathways. Therefore, inhibition of LOX activity is considered an important indicator of anti-inflammatory potential (Samuelsson, 1983).

V. CONCLUSION

The present study successfully demonstrated the extraction of chitosan from prawn shell waste, its formulation into nanoparticles using the ionic gelation method, and the evaluation of its invitro anti-inflammatory activity. UV–Visible spectroscopic analysis confirmed the successful formation and stability of chitosan nanoparticles. The LOX inhibition assay revealed that chitosan nanoparticles exhibited significant concentration-dependent anti-inflammatory activity. The study also emphasizes the value of prawn shell waste as a sustainable source of functional biomaterials. Further in-vivo studies and molecular investigations are elucidated to explore the therapeutic applicability of chitosan nanoparticles in inflammatory disorders.

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