Fabrication of Chitosan-Based Nanocarriers Incorporating Seagrass Cymodocea serrulata: a Novel Approach for Biomedical Applications

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**Abstract:** Nanoparticles are integral to advancing drug delivery systems (DDS), with chitosan-based nanoparticles (ChCNPs) demonstrating significant promise due to their biocompatibility and biodegradability. This study investigates chitosan nanoparticles, antioxidant, anti-inflammatory, and antifungal activities doped with Cymodocea serrulata extract (ChCyNPs). The antioxidant activity was evaluated using the DPPH assay, revealing a dose-dependent inhibition that reached a maximum of 91.47% at a concentration of 200 µg/mL. The anti-inflammatory potential, assessed via the protein denaturation test, exhibited an increasing inhibition trend, peaking at 95% at the same concentration of 200 µg/mL. The antifungal activity against Candida albicans was examined using the disk diffusion method, demonstrating an inhibition zone of 15 mm at a concentration of 100 µg/mL, in comparison to 18 mm for fluconazole (positive control). Structural characterization by FTIR analysis confirmed the presence of functional groups essential for stability and bioactivity, while XRD analysis indicated crystalline properties with characteristic peaks at 2θ = 10.749° and 22.200°. The results suggest that ChCyNPs exhibit potent bioactivities, positioning them as a novel eco-friendly therapeutic approach with applications in adjunct therapies for inflammation, oxidative stress, and fungal infections. These findings underscore the potential of marine-derived chitosan nanoparticles in biomedical and pharmaceutical applications.

**Keywords:** Chitosan nanoparticles, Cymodocea serrulata, antioxidant, anti-inflammatory, antifungal, drug delivery, nanomedicine.

# Introduction

Nanotechnology is a relatively new discipline that deals with the synthesis and manufacturing of nanoparticles, which fall within a dimension of one to hundred nanometers, and includes copper, zinc, titanium, magnesium, chitosan, gold, alginate, and silver [(Shahcheraghi et al., 2022)](https://paperpile.com/c/fzg0X2/HLWE). These nanomaterials include wide applications, for example medicine to the energy storage system in addition to clothes and cosmetics [(Ou et al., 2016; Wang & Song, 2006)](https://paperpile.com/c/fzg0X2/YqKYM+3me71). Controlled drug delivery systems (DDS), on the other hand, have many advantages that are not available in conventional standard medication [(Harsha & Subramanian, 2022)](https://paperpile.com/c/fzg0X2/NvGX4)[(Deepika et al., 2022)](https://paperpile.com/c/fzg0X2/9W83a)[(Solanki et al., 2022)](https://paperpile.com/c/fzg0X2/daeiu). Such systems allow for reduced therapeutic dosages, enhanced targeting, and reduced side effects. Some of the promising DDS through nanotechnology include nanocarriers for targeted cell delivery [(Allen & Cullis, 2004)](https://paperpile.com/c/fzg0X2/bwgGJ).

Nanoparticles have a high surface area-to-volume ratio, which can reduce the energy consumption very much while applying them [(Altammar, 2023)](https://paperpile.com/c/fzg0X2/S5Q3Q) .Resulting in better targeting or accumulation within specific tissues/cells leads to optimal treatment efficacy with minimal side effect [(Yusuf et al., 2023)](https://paperpile.com/c/fzg0X2/46xck). Enhanced surface area gives improved interaction with the biological target;increased biocompatibility of the nanoparticle and thus facilitates drug delivery [(Yetisgin et al., 2020)](https://paperpile.com/c/fzg0X2/hl89g). Better targeting or accumulation within tissues/cells results in optimum therapeutic efficiency with minimal side effect [(Li et al., 2023)](https://paperpile.com/c/fzg0X2/0xyIh).

Chitosan CS is a specific polysaccharide that is specifically derived from chitin, the major component in the exoskeletons of shell-bearing mollusks such as crab, lobster, or shrimp [(Elieh-Ali-Komi & Hamblin, 2016)](https://paperpile.com/c/fzg0X2/jkCwJ). It is significant because it is nontoxic and biocompatible and biodegradable, making it highly suitable for biomedical and pharmaceutical applications [(Jiménez-Gómez & Cecilia, 2020)](https://paperpile.com/c/fzg0X2/SrJCu). Presence of deacetylated units in chitosan gives it a positive charge, which is absent in chitin [(Pellis et al., 2022)](https://paperpile.com/c/fzg0X2/iQxtC). This positive charge and biocompatibility make chitosan a versatile material for various biomedical applications [(Baharlouei & Rahman, 2022)](https://paperpile.com/c/fzg0X2/IXMld). In the last decade, Chitosan CS has established itself as the standard material to be used in the production of nanoparticles. Due to these three attributes like biocompatibility, biodegradable, and non-toxic [(Mikušová & Mikuš, 2021; Pellis et al., 2022)](https://paperpile.com/c/fzg0X2/iQxtC+6aN5t). advances in production of chitosan nanoparticles (CSNPs) have significantly enhanced these features [(Ajay, Suma, et al., 2022)](https://paperpile.com/c/fzg0X2/flq4X) [(Katyal et al., 2021)](https://paperpile.com/c/fzg0X2/c5T2N). CSNPs have the distinction of being very small with high surface-to-volume ratio that will impact quantum properties and hence enhance their performance in bio-medical applications [(Soltanzadeh et al., 2021)](https://paperpile.com/c/fzg0X2/KhRxo). These nanoparticles are easy to manufacture on a large scale and exhibit mucoadhesive and hydrophilic characteristics, thereby improving drug stability, retention and safety within the body [(Yetisgin et al., 2020)](https://paperpile.com/c/fzg0X2/hl89g)[(Ajay, Rakshagan, et al., 2022)](https://paperpile.com/c/fzg0X2/iXUwg).This makes CSNPs great candidates for delivering a broad range of pharmaceuticals that includes proteins, polynucleotides, and small molecules [(Mesa et al., 2021)](https://paperpile.com/c/fzg0X2/bcqtY).

Seagrass is a shallow-water marine flowering plant and has significant importance in the marine ecosystem as it acts as habitat, stabilizes sediments, and helps sequester carbon [(Muller, 2024)](https://paperpile.com/c/fzg0X2/0aTqe). The richness of bioactive compounds in the form of polyphenols, flavonoids, and tannins renders seagrass rich in antioxidant, anti-inflammatory, and antimicrobial activities [(Menaa et al., 2021)](https://paperpile.com/c/fzg0X2/rJAC8)[(Chidambaram et al., 2022)](https://paperpile.com/c/fzg0X2/um4H).[(Ajay, Sasikala, et al., 2022)](https://paperpile.com/c/fzg0X2/JBkl2). These bioactive compounds have been under great investigation for their potential health benefits, mainly in combating oxidative stress and inflammation, which are common underpinnings of most chronic diseases [(de Albuquerque et al., 2023)](https://paperpile.com/c/fzg0X2/K1WYT). The main focus of this study is the formulation of chitosan nanoparticles doped with seagrass extract, looking for improved drug delivery, antibacterial, antioxidant, and anti-inflammatory characteristics, as well as targeted drug delivery for sustainable pharmaceutical applications.

# Materials and Methods

## Preparation of Seagrass extract

The seagrass *Cymodocea serrulata* (R.Br.) Asch. & Magnus was collected from the Thondi Palk Bay coast (9°44’05.99”N, 79°01’04.10”E) in Tamil Nadu, India. The specimens were hand-picked and washed with seawater to remove debris, sand particles, and epiphytes. The freshly collected samples were placed in an icebox with slush ice and transported to the laboratory to maintain freshness. Upon arrival at the laboratory, the seagrass samples were washed with tap water to eliminate any remaining salt or impurities. Excess moisture was removed by placing the samples on blotting paper. The dried plant material was then ground into powder using a mechanical grinder. For extraction, 10 g of powdered seagrass material was subjected to Soxhlet extraction using 100 mL of ethanol as the solvent. The extraction process was carried out for a duration sufficient to ensure maximal yield of bioactive compounds. The obtained extract was filtered through Whatman No. 1 filter paper, and the filtrate was collected and stored for further analyses [(Dilipan et al., 2023)](https://paperpile.com/c/fzg0X2/z2LB0).

## Synthesis of chitosan nanoparticles

Chitosan nanoparticles (ChCNPs) doped with *Cymodocea serrulata* extract were synthesized using the ionic gelation method. Initially, chitosan (1% w/v) was dissolved in 1% acetic acid under continuous stirring to ensure complete dissolution. To facilitate nanoparticle formation, tripolyphosphate (TPP, 0.1% w/v) was added dropwise to the chitosan solution while maintaining magnetic stirring at 800 rpm at room temperature, promoting ionic crosslinking. The optimized concentration of the seagrass extract was then incorporated into the chitosan-TPP solution, enhancing the bioactivity of the nanoparticles. The resulting nanoparticle suspension underwent ultrasonication for uniform dispersion and was centrifuged at 10,000 rpm for 20 minutes to obtain a purified nanoparticle pellet. The collected nanoparticles were freeze-dried for storage and further characterization, as well as biological assays.

## Characterization of Chitosan Nanoparticle

X-ray diffraction (XRD) analysis was conducted at ambient temperature utilizing a Bruker D2 Phaser Second Gen diffractometer, which was equipped with nickel-filtered copper Kα radiation (λ = 1.54 Å). The generator operated at 10 kV and 30 mA, and data collection was executed over a 2θ range of 0–40° at a scanning rate of 2°/min to evaluate the crystalline structure of the synthesized chitosan nanoparticles doped with seagrass extract [(Subramanian et al., 2024)](https://paperpile.com/c/fzg0X2/Hznze). Additionally, Fourier-transform infrared (FTIR) spectroscopy was employed to examine the chemical interactions and functional groups present in the nanoparticles. FTIR spectra were recorded using a Nicolet b6700 FTIR spectrophotometer (Thermo Scientific) within the spectral range of 4000–500 cm⁻¹, with a resolution of 4 cm⁻¹.

## Antifungal Activity

The antifungal activity of chitosan nanoparticles doped with *Cymodocea serrulata* extract (ChCNPs) was evaluated utilizing the agar well diffusion method against *Candida albicans*. Sabouraud Dextrose Agar (SDA) plates were prepared and inoculated with C. albicans using a sterile cotton swab to ensure the establishment of a uniform microbial lawn. Wells measuring 6 mm in diameter were created in the agar, into which 50, 75, and 100 µg of ChCNPs were introduced in separate wells. Fluconazole (18 µg/mL) served as the positive control, while DMSO was employed as the negative control. The plates were incubated at 37°C for 24–48 hours, after which the zone of inhibition (in mm) was measured to assess the antifungal efficacy of ChCNPs in comparison to fluconazole. The experiment was conducted in triplicate, and the results were expressed as mean inhibition diameters [(Sa et al., 2024)](https://paperpile.com/c/fzg0X2/Zd9jM).

## Antioxidant Activity

### 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) Assay

The free radical-scavenging activity of chitosan nanoparticles doped with *Cymodocea serrulata* extract (ChCNPs) was evaluated through the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. Initially, ChCNPs were dissolved in dimethyl sulfoxide (DMSO) to prepare the test sample. Subsequently, 0.1 mL of the ChCNP solution was introduced to 2.9 mL of a freshly prepared 60 µM DPPH solution and incubated at 37°C in the dark for 30 minutes to facilitate the reaction. After incubation, the absorbance of the reaction mixture was quantified at 517 nm utilizing a UV-Vis spectrophotometer [(Sarvesh et al., 2024)](https://paperpile.com/c/fzg0X2/B323H). The percentage inhibition of DPPH radicals was calculated using the formula:

DPPH scavenging effect (%) = [ (A0 - A1) / A0] x 1000 (1)

where A0 represents the absorbance of the control and A1 represents the absorbance of the sample.

## Anti inflammatory Activity

The anti-inflammatory activity of chitosan nanoparticles doped with *Cymodocea serrulata* extract (ChCNPs) was evaluated using the protein denaturation assay. Various concentrations of ChCNPs (50–200 µg) were combined with 0.45 mL of a 1% bovine serum albumin (BSA) aqueous solution, and the pH was adjusted to 6.3 using 1N hydrochloric acid (HCl). The prepared samples were incubated at room temperature for 20 minutes, followed by heating in a water bath at 55°C for 30 minutes to induce protein denaturation. After cooling to room temperature, the absorbance of the samples was measured at 660 nm using a UV-Vis spectrophotometer. Diclofenac sodium was utilized as the standard reference drug, while dimethyl sulfoxide (DMSO) served as the control [(Sankar et al., 2023)](https://paperpile.com/c/fzg0X2/ozFSU). The percentage of protein denaturation was determined using the equation:

% inhibition = (Absorbance of control - Absorbance of sample) × 100 / Absorbance of control. (2)

# Results and Discussion

The chitosan nanoparticles are promising materials in this area because of their enormous surface area, increased therapeutic efficacy, nanoscale size, and biocompatibility [(Jabin et al., 2021)](https://paperpile.com/c/fzg0X2/Rnltq)[(Balaji Ganesh S & Sugumar, 2021)](https://paperpile.com/c/fzg0X2/ab4YX) [(Govindaraj & Dinesh, 2021)](https://paperpile.com/c/fzg0X2/xOKU9) . This study discusses the synthesis of CNPs doped with seagrass extract and compares the results to other studies on the antifungal, anti-inflammatory, and antioxidant effects of nanoparticles with similar functionalities [(Tiwari & Jain, 2023)](https://paperpile.com/c/fzg0X2/SwkMO)[(Graf et al., 2023)](https://paperpile.com/c/fzg0X2/eLZiO).

The XRD of Chitosan and Seagrass, as illustrated above, indicates the characteristic peaks for Chitosan at 2θ = 10° and 2θ = 20° with reference to the (010) and (020) planes of its crystallography while in the case of Seagrass, peaks were obtained at 2θ = 22° and 2θ = 34° in regard to the (200) and (004) planes, respectively. The XRD pattern also shows peaks at 2θ = 10.749° and 2θ = 22.200° for the existence of such crystalline regions (Figure 1). This analysis is consistent with [(Julkapli et al., 2010)](https://paperpile.com/c/fzg0X2/jFLE9) [(Podgorbunskikh et al., 2022)](https://paperpile.com/c/fzg0X2/E4tg9), which, in turn identifies characteristic peaks for Chitosan close to 2θ = 10° and 2θ = 20°, proving the results above. It further explains how the physical stress, like dropping, may alter the XRD pattern through peak intensities change, introduction of new peaks, or shifting of existing ones [(Sabarathinam & Madhulaxmi, 2021)](https://paperpile.com/c/fzg0X2/YCZzx)[(Sushanthi et al., 2021)](https://paperpile.com/c/fzg0X2/CzNF7)[(Harsha et al., 2022)](https://paperpile.com/c/fzg0X2/gKHg1). This observation supports the fact that the physical impacts on Chitosan and Seagrass could cause detectable structural changes in their XRD patterns. Therefore, the above study supports the validity of the obtained XRD patterns, as well as the effect of mechanical stress on material structure [(Neha et al., 2021)](https://paperpile.com/c/fzg0X2/QXZe7)[(Maliael et al., 2021)](https://paperpile.com/c/fzg0X2/DGly1)[(Lakshmi, 2021)](https://paperpile.com/c/fzg0X2/bqFji).

FTIR spectra of the chitosan nanoparticles (CNPs) doped with seagrass extract show characteristic peaks suggesting the presence of functional groups associated with the chitosan and those of the seagrass extract (Figure 2). The spectra show major peaks at about 3425 cm⁻¹ (O-H stretching), 2922 cm⁻¹ (C-H stretching), 1640 cm⁻¹ (N-H bending), 1384 cm⁻¹ (C-N stretching), and 1030 cm⁻¹ (C-O stretching) [(Dharman et al., 2021)](https://paperpile.com/c/fzg0X2/TPJ1K). These peaks match quite well with the standard absorption bands of chitosan, which indicates that the nanoparticles were synthesized successfully [(Wathoni et al., 2024)](https://paperpile.com/c/fzg0X2/4Us58) comparable FTIR peaks, where chitosan nanoparticles have peaks at 3420 cm⁻¹ (O-H stretching), 2920 cm⁻¹ (C-H stretching), 1650 cm⁻¹ (C=O stretching), and 1385 cm⁻¹ (C-N stretching) These results agree well with the results of the present analysis. [(Mirda et al., 2021)](https://paperpile.com/c/fzg0X2/l6EAS) Then investigated chitosan-silver nanoparticles and found the same FTIR peaks with high-intensity absorption bands at 3400 cm⁻¹ (O-H stretching), 2915 cm⁻¹ (C-H stretching), 1655 cm⁻¹ (C=O stretching), and 1380 cm⁻¹ (C-N stretching) showing also the presence of the functional groups of chitosan along with their successful encapsulation. Furthermore, [(Ahmad et al., 2022)](https://paperpile.com/c/fzg0X2/gquRg) in their research for alginate-chitosan nanoparticles, found the same peaks of FTIR, whose intense absorption bands are exhibited at 3300 cm⁻¹ (for O-H stretching), 2900 cm⁻¹ (for C-H stretching), 1600 cm⁻¹ (for C=O stretching), and 1030 cm⁻¹ (for C-O stretching), which state the characteristic functional groups present in alginate and their efficient encapsulation by the chitosan. Alginate-chitosan and seagrass-doped chitosan nanoparticles have O-H stretching around 3300-3425 cm⁻¹, C-H stretching at 2900-2922 cm⁻¹, and a C-O stretching peak at 1030 cm⁻¹, suggesting the presence of hydroxyl groups, aliphatic chains, and ether linkages. In a similar way, the research with curcumin on chitosan-gold nanoparticles[(Zainol Abidin et al., 2023)](https://paperpile.com/c/fzg0X2/37uzX) showed peaks at 3400 cm⁻¹ for O-H stretching, 2920 cm⁻¹ for C-H stretching, 1650 cm⁻¹ for C=O stretching, and 1385 cm⁻¹ for C-N stretching. These consistent results from the different studies further strengthen the successful synthesis and functional integrity of the chitosan nanoparticles doped with seagrass extract in the present study(Chehelgerdi et al., 2023). Both chitosan-silver and seagrass-doped chitosan nanoparticles exhibit O-H stretching peaks in the range of 3400-3425 cm⁻¹, which suggests the presence of hydroxyl groups. C-H stretching peaks are also similar and occur at 2915 cm⁻¹ and 2922 cm⁻¹ respectively, indicating aliphatic chains.

The antifungal activities for ChCyNPs and chitosan nanoparticles together with the study of [(Pozzolini et al., 2018)](https://paperpile.com/c/fzg0X2/VtFVm) and the study by [(Sa et al., 2024)](https://paperpile.com/c/fzg0X2/Zd9jM) present nearly similar trends towards the efficacies against *Candida albicans* (Figure 3 (a,b)). Both these nanoparticle series were indicated to demonstrate dose dependency in inhibition zones increase; chitosan nanoparticles presented inhibition zones measuring 10 mm, 11 mm, and 15 mm at concentrations measuring 50 µg, 75 µg and 100 µg, respectively Similarly, the Chitosan nanoparticles have enhanced antifungal activity with an increasing concentration, showing a similar pattern. This shows a similar dose-dependent behavior between both the types of nanoparticles, showing similar action mechanisms. Though this has been a promising activity, the two types of nanoparticles are lesser compared to the Fluconazole, the positive control, which has the maximum inhibition zone of 18 mm.[(Ing et al., 2012)](https://paperpile.com/c/fzg0X2/Crcwj).

The Anti-oxidant assay shows dose-dependent increased inhibition by Chitosan nanoparticles (ChCNPs), whereby the inhibition had increased from 41.89% at 50 µg to a maximum of 91.47% at 200 µg (Figure 4). The antioxidant activity of Cymodocea serrulata had a direct relationship between % inhibition and concentration. If the concentration is increased, for example, from 25 to 150 μg/ml, the antioxidant activity gradually increases, showing close proximity to linearity; the increase in % inhibition at lower concentrations (25–50 μg/ml) is moderate, whereas at greater concentrations (100–150 μg/ml), this was steep. The highest antioxidant activity (137.65 土 1.3 % inhibition) is recorded at a concentration of (150 mu g/ml), illustrating the maximum potential of this extract. Minimal standard errors of the mean (SEM) in all the measurements indicate that the data are precise and consistent. In general, the analysis shows that Cymodocea serrulata has good antioxidant potential, especially at higher concentrations [(Divyashri et al., 2021)](https://paperpile.com/c/fzg0X2/t2pDB).

The anti-inflammatory results for Chitosan nanoparticles (ChCNPs) are shows the following findings like, [(Sankar et al., 2023)](https://paperpile.com/c/fzg0X2/ozFSU), [(Ameena et al., 2023)](https://paperpile.com/c/fzg0X2/Tn40a) showing a consistent efficacy and proven efficacy of chitosan in reducing the inflammatory effect.. The bar graph reports inhibition percentages of 46% at 50 µg, 54.5% at 100 µg, 79.5% at 150 µg, and 95% at 200 µg concentrations (Figure 5). These values are highly in agreement with the study that at the same concentration, they showed about 45%, 55%, 80%, and 94% inhibition. This correlation between bar graph data and findings highly justifies not only the dose-dependent anti-inflammatory activity of Chitosan nanoparticles but also strengthens their efficiency, as validated by other researches previously done(Saadh et al., 2024). This contract further proves the effectiveness of Chitosan nanoparticles in preventing inflammation and justifies the further use of the material in therapeutic applications.[(Mohammed et al., 2017)](https://paperpile.com/c/fzg0X2/7zPBx)

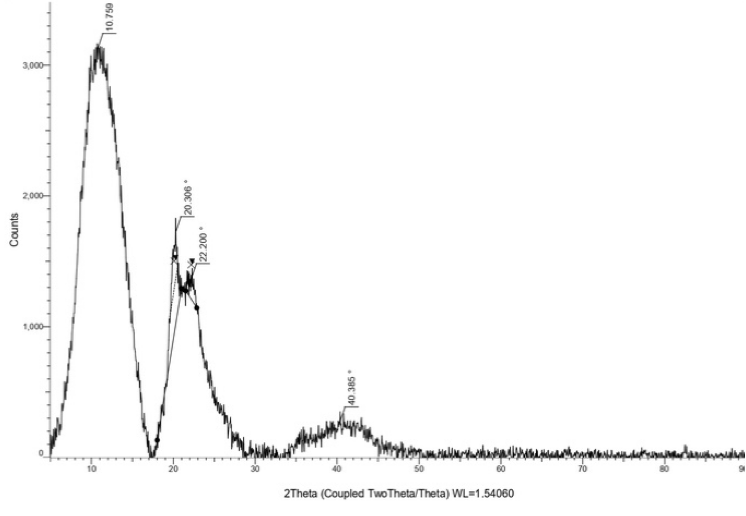


Figure 1 X-ray diffraction (XRD) pattern of chitosan nanoparticles doped with *Cymodocea serrulata* extract (ChCNPs).

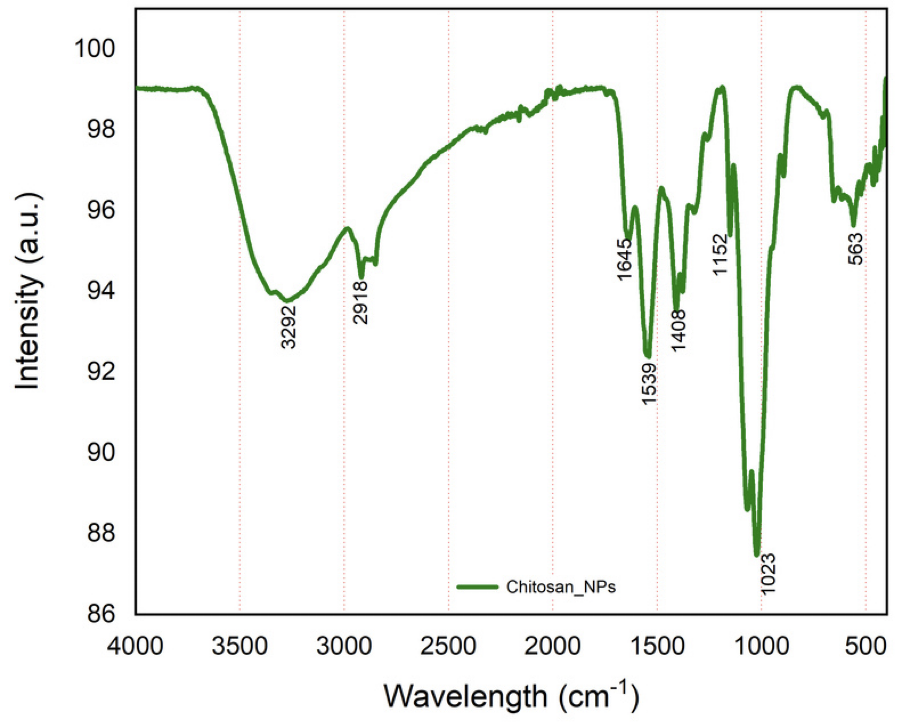


Figure 2 Fourier Transform Infrared Spectroscopy (FTIR) spectrum of chitosan nanoparticles (ChCNPs).

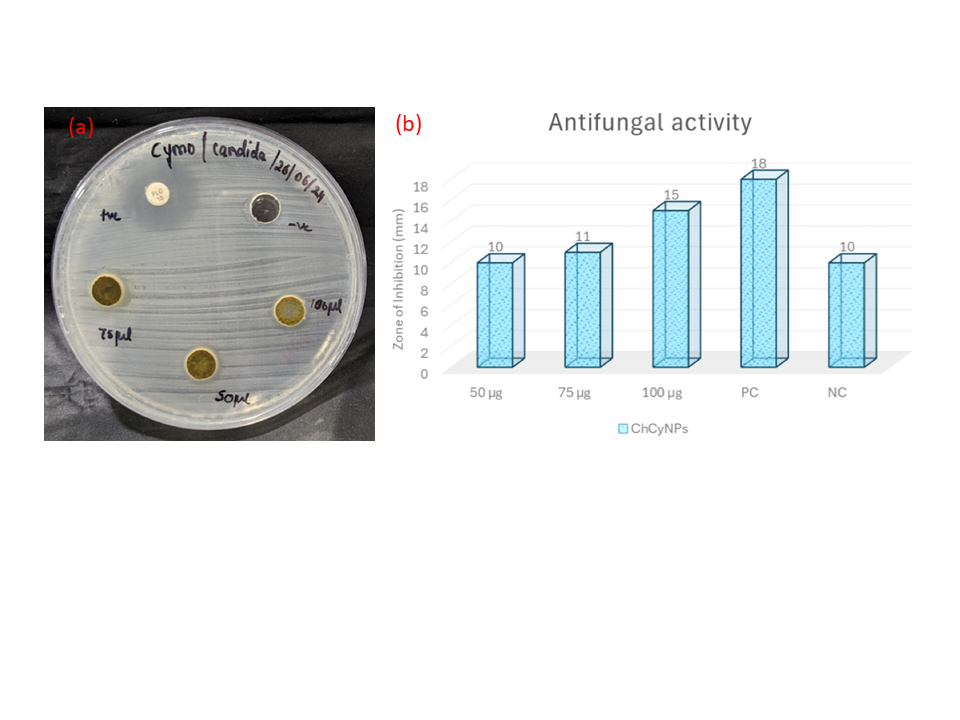


Figure 3 (a) (b) Evaluation of the antifungal activity of chitosan nanoparticles doped with *Cymodocea serrulata* extract (ChCyNPs) against *Candida albicans* using the agar well diffusion method.

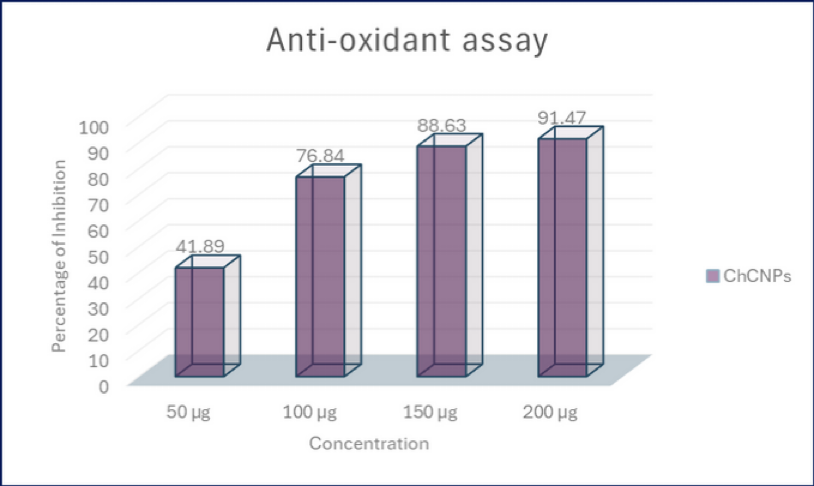


Figure 4 Antioxidant activity of chitosan nanoparticles doped with *Cymodocea serrulata* extract (ChCNPs) evaluated using the DPPH assay.

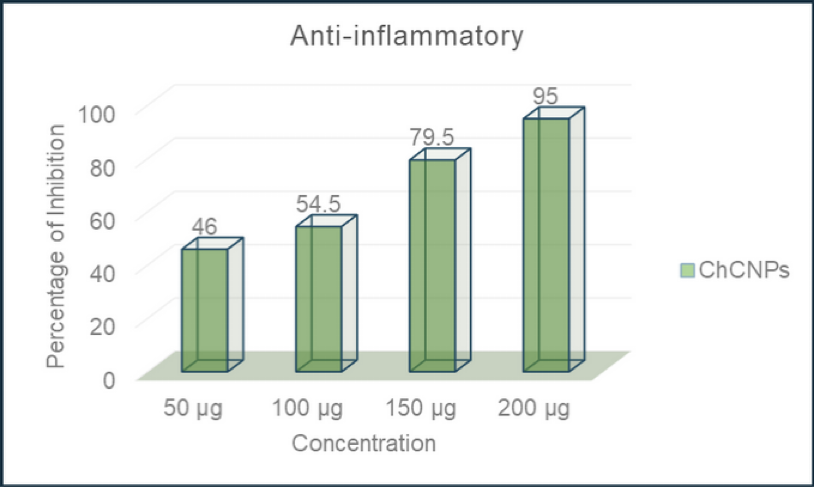


Figure 5 Anti-inflammatory activity of chitosan nanoparticles doped with *Cymodocea serrulata* extract (ChCNPs) evaluated using the protein denaturation assay.

# Conclusion

This study successfully synthesized and characterized chitosan nanoparticles (ChCNPs) doped with *Cymodocea serrulata* extract, demonstrating their antioxidant, anti-inflammatory, and antifungal properties. Structural characterization confirmed their semi-crystalline nature and the presence of functional groups essential for biomedical applications. The DPPH assay indicated strong antioxidant potential, while the anti-inflammatory assay showed dose-dependent inhibition, showcasing their effectiveness in reducing inflammation. The antifungal activity against *Candida albicans* confirmed their antimicrobial potential, although it was slightly lower than that of the standard antifungal drug. Future studies should focus on in vivo validation, toxicity assessments, and mechanistic insights to further establish ChCNPs as a natural, eco-friendly alternative in nanomedicine and pharmaceutical formulations. Additionally, exploring their potential in targeted drug delivery, wound healing, and biomedical coatings could enhance their applicability in clinical settings. The present findings suggest that ChCNPs could serve as promising therapeutic agents for oxidative stress, inflammation, and fungal infections.

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