

Original Research Article

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# Green-Synthesized Chitosan Nanoparticles Using *Citrus limon* for IAA Enhancement and Antimicrobial Activity: In Silico Molecular Docking

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## ABSTRACT

### Keywords

Chitosan nanoparticles; Indole-3-acetic acid; Plant growth-promoting rhizobacteria; Antimicrobial activity; Molecular docking; Sustainable agriculture

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This study reports an eco-friendly green synthesis of chitosan nanoparticles (CSNPs) using lemon (*Citrus limon*) juice as a natural reducing and stabilizing agent, and evaluates their dual functionality in enhancing microbial indole-3-acetic acid (IAA) production and inhibiting a Gram-negative pathogen. The synthesized CSNPs were characterized by UV–Vis spectroscopy, Fourier-transform infrared spectroscopy, scanning electron microscopy, and energy-dispersive X-ray analysis, confirming stable nanoparticle formation with spherical morphology and particle sizes in the range of 100- 200 nm. Functional group analysis indicated successful citric-acid-mediated cross-linking and incorporation of lemon-derived phytochemicals. Biological evaluation demonstrated that CSNP supplementation significantly enhanced IAA production in *Azotobacter sp.* and *Pseudomonas aeruginosa*, resulting in increases of 91.6% and 99.8%, respectively, compared to untreated controls. In addition, CSNPs exhibited antibacterial activity against *P. aeruginosa*. To gain mechanistic insight into the antimicrobial effect, in silico molecular docking was performed, revealing strong binding affinity of a chitosan oligomer toward outer membrane porins, particularly OprD, suggesting potential disruption of porin-mediated transport. Overall, the findings demonstrate that green-synthesized CSNPs possess multifunctional properties, simultaneously promoting beneficial microbial auxin production and suppressing pathogenic bacteria. These characteristics highlight their potential application as sustainable biofertilizer enhancers and eco-friendly antimicrobial agents in agricultural systems.

## Introduction

The rhizosphere is a biologically active zone surrounding plant roots where complex interactions among plants, soil, and microorganisms regulate nutrient cycling, plant development, and stress tolerance (Glick *et al.*, 2012).

Within this microenvironment, plant growth-promoting rhizobacteria (PGPR) play a critical role in enhancing plant productivity through mechanisms such as biological nitrogen fixation, phosphate solubilization, siderophore production, and phytohormone synthesis (Ahemad *et al.*, 2014). Among these mechanisms, microbial production

of indole-3-acetic acid (IAA) is particularly significant because IAA functions as a central auxin controlling cell elongation, lateral root initiation, and overall root system architecture (Maheshwari *et al.*, 2015).

Several rhizospheric bacteria, including species of *Azotobacter* and *Pseudomonas*, are well-recognized IAA producers and are widely exploited in biofertilizer formulations (Spaepen *et al.*, 2011). However, the efficiency of microbial IAA biosynthesis is often constrained by environmental stresses such as nutrient limitation, pH fluctuations, and oxidative stress, as well as by intrinsic metabolic limitations of the microorganisms (Fasusi *et al.*, 2021). These factors reduce the consistency and field performance of PGPR-based biofertilizers, highlighting the need for innovative strategies that enhance microbial activity while maintaining environmental sustainability.

Nanotechnology has emerged as a promising approach to improve microbial performance and agricultural inputs by modulating cellular processes at the nanoscale (Mittal *et al.*, 2020). Among various nanomaterials, chitosan nanoparticles (CSNPs) have attracted considerable attention due to their biodegradability, biocompatibility, low toxicity, and multifunctional biological properties (Divya *et al.*, 2017). Chitosan, a deacetylated derivative of chitin composed of  $\beta$ -(1,4)-linked D-glucosamine and N-acetyl-D-glucosamine units, exhibits intrinsic antimicrobial activity and the ability to interact with negatively charged microbial cell surfaces (Ahmed *et al.*, 2014). When formulated at the nanoscale, chitosan displays an increased surface area and enhanced reactivity, leading to improved biological efficacy compared to its bulk form (Younes *et al.*, 2015).

Conventional methods for synthesizing CSNPs often rely on chemical cross-linkers or energy-intensive processes that may generate toxic residues and environmental concerns (Singh *et al.*, 2016). In contrast, green synthesis approaches using plant-derived extracts offer an eco-friendly alternative by employing natural reducing and stabilizing agents (Hano *et al.*, 2021). Lemon juice obtained from *Citrus limon* is particularly suitable for this purpose due to its high content of citric acid, flavonoids, and ascorbic acid, which facilitate nanoparticle formation while simultaneously imparting antioxidant and antimicrobial properties (Alkhulaifi *et al.*, 2020). The incorporation of these phytochemicals into CSNPs may further enhance their biological functionality.

In addition to their potential role in enhancing microbial phytohormone production, CSNPs exhibit notable antimicrobial activity against a range of plant and human pathogens (El-Naggar *et al.*, 2022). *Pseudomonas aeruginosa*, a Gram-negative opportunistic pathogen, is of particular concern due to its intrinsic resistance mechanisms, including low outer membrane permeability, efflux pumps, and biofilm formation (Levy *et al.*, 2004). Chitosan-based nanomaterials are reported to disrupt bacterial membranes and interfere with membrane-associated proteins, offering an alternative strategy to conventional antibiotics (Timofeeva *et al.*, 2011).

In this context, the present study investigates the green synthesis of chitosan nanoparticles using lemon juice and evaluates their dual functionality: enhancement of microbial IAA production in PGPR and antimicrobial activity against *P. aeruginosa*. Furthermore, in silico molecular docking was employed to elucidate the interaction of chitosan oligomers with key outer membrane porins, providing mechanistic insights into their antibacterial action.

## Materials and Methods

### Materials

Chitosan (degree of deacetylation 80%) was purchased NR CHEM, Mumbai. Fresh lemons (*Citrus limon*) were obtained from a local market in Mumbai, India. Citric acid, acetic acid, L-tryptophan, ferric chloride, perchloric acid, Mueller–Hinton agar, and Luria–Bertani (LB) broth were procured from HiMedia Laboratories (India). Ciprofloxacin was used as a standard antibacterial agent. All reagents were of analytical grade.

### Green Synthesis of Chitosan Nanoparticles

Chitosan nanoparticles (CSNPs) were synthesized using a green method based on plant-derived organic acids, with minor modifications from previously reported protocols (Karamchandani *et al.*, 2024), (Santiago *et al.*, 2019). A 0.5% (w/v) chitosan solution was prepared by dissolving chitosan in 1% (v/v) acetic acid under continuous magnetic stirring at room temperature until complete dissolution (pH 6.3). Fresh lemon juice was filtered and centrifuged at 6000 rpm for 10 min to remove suspended solids. The clarified juice was added dropwise to the chitosan solution (20% v/v) and heated at

80 °C with stirring for 15 min. Citric acid (20% w/v) was then added slowly to induce ionic cross-linking. The resulting suspension was centrifuged at 10,000 rpm for 15 min, washed three times with distilled water, and oven-dried at 40 °C. The dried CSNPs were stored at 4° C in airtight containers for further analysis.

### Characterization of Chitosan Nanoparticles

The formation of CSNPs was initially confirmed by UV–Visible spectroscopy using a spectrophotometer (Thermo Scientific EVOLUTION 260 BIO) in the range of 200–800 nm (Sharma *et al.*, 2009). Surface morphology and particle size were examined by scanning electron microscopy (Carl Zeiss Model 55 Germany). Fourier-transform infrared (FTIR) spectroscopy (PerkinElmer Spectrum Two) was performed in the range of 4000–400 cm<sup>-1</sup> to identify functional groups and confirm chitosan cross-linking and incorporation of lemon-derived phytochemicals (Silverstein *et al.*, 1962).

### Bacterial Strains

Plant growth-promoting rhizobacteria *Azotobacter sp.* and *Pseudomonas aeruginosa* were obtained from the departmental culture collection. Bacterial strains were maintained on Ashby's and nutrient agar slants respectively at 4°C.

### Indole-3-Acetic Acid (IAA) Production Assay

IAA production was quantified using the colorimetric Salkowski method (Gordon *et al.*, 1951). Bacterial cultures were grown in Luria- Bertini supplemented with 0.5% (w/v) L-tryptophan. CSNP-treated cultures received 0.1 mL of CSNP suspension (0.5 g/L), while controls contained no nanoparticles. After incubation at RT for 24 h with shaking at 150 rpm, cultures were centrifuged at 8000 rpm for 10 min. One milliliter of supernatant was mixed with 2 mL of Salkowski reagent (0.5 M in 35%) and incubated in the dark for 30 min. Absorbance was measured at 530 nm. IAA concentration was calculated using a standard curve prepared with pure IAA.

### Antibacterial Activity Assay

The antibacterial activity of CSNPs against *Pseudomonas aeruginosa* was evaluated using the agar well diffusion method (Karamchandani *et al.*, 2024), (Landrum *et al.*,

2013). Mueller–Hinton agar plates were inoculated with a standardized bacterial suspension (0.5 McFarland). Wells (6 mm diameter) were filled with 100 L of CSNP suspension (25 mg/mL), ciprofloxacin (50 g/mL, positive control), or sterile distilled water (negative control). Plates were incubated at 37 °C for 24 h, and zones of inhibition were measured in millimeters. All experiments were performed in triplicate.

### In Silico Molecular Docking

Molecular docking was performed to investigate interactions between chitosan and bacterial outer membrane porins. A 5-unit chitosan oligomer was constructed and energy-minimized using RDKit with the MMFF94 force field (Trott *et al.*, 2010). Crystal structures of *P. aeruginosa* porins OprF (PDB ID: 4RLC) and OprD (PDB ID: 3SY7) were retrieved from the Protein Data Bank. Docking simulations were conducted using AutoDock Vina (DeLano *et al.*, 2002). Protein and ligand preparation was performed in UCSF Chimera, and docking poses were visualized using PyMOL and LigPlot+ to analyze hydrogen bonding and electrostatic interactions (Wallace *et al.*, 1995), (Malekshah *et al.*, 2021).

## Results and Discussion

### Characterization of Chitosan Nanoparticles

The formation and optical properties of green-synthesized chitosan nanoparticles (CSNPs) were evaluated using UV–Visible spectroscopy. The UV–Vis spectrum of the CSNP suspension synthesized with 20% (v/v) lemon juice exhibited a distinct absorption peak in the range of 230–335 nm, characteristic of chitosan-based nanoparticle formation (Fig. 1). The sharp and well-defined nature of the peak indicates uniform nanoparticle formation with limited aggregation. Notably, the absorption profile remained stable over storage periods up to 21 days at 4 °C, with no significant peak shift or broadening, confirming the colloidal stability of the synthesized CSNPs.

Morphological analysis by scanning electron microscopy (SEM) revealed that the CSNPs were predominantly spherical and well dispersed (Fig. 2 A). Particle size analysis from multiple SEM micrographs showed a size range of approximately 50–150 nm, with the majority of particles distributed between 80 and 120 nm. The

nanoscale dimensions and spherical morphology suggest a high surface-area-to-volume ratio, which is favorable for biological interactions and functional applications.

Elemental composition analysis using energy-dispersive X-ray spectroscopy (EDS) confirmed the presence of carbon (C), oxygen (O), nitrogen (N), and chlorine (Cl), consistent with the chemical structure of chitosan and citric acid-mediated stabilization (Fig. 3). Elemental mapping further demonstrated a homogeneous distribution of C, O, and N throughout the nanoparticles, supporting the uniformity of the synthesized CSNPs (Fig.2 B). The detected chlorine signal is likely associated with residual stabilization effects from lemon juice constituents or processing reagents.

Fourier-transform infrared (FTIR) spectroscopy was employed to investigate the chemical structure and functional group interactions within the CSNPs (Fig. 4). The spectrum displayed a broad absorption band around  $3400\text{ cm}^{-1}$ , corresponding to overlapping and stretching vibrations of chitosan. A prominent peak at approximately  $1710\text{ cm}^{-1}$  was attributed to stretching vibrations arising from citric acid-mediated cross-linking, while the band near  $1560\text{ cm}^{-1}$  corresponded to bending vibrations of chitosan amine groups. Additional absorption bands in the  $1000\text{--}1200\text{ cm}^{-1}$  region were assigned to and stretching vibrations, indicating the incorporation of lemon juice-derived phytochemicals into the nanoparticle matrix. These features collectively confirm successful cross-linking and functionalization of CSNPs.

## IAA Production

The effect of chitosan nanoparticles (CSNPs) on indole-3-acetic acid (IAA) production was evaluated in cultures of *Azotobacter sp.* and *Pseudomonas aeruginosa* using the Salkowski colorimetric assay. Following 24 h of incubation in Luria–Bertani broth supplemented with L-tryptophan, all cultures produced a characteristic cherry-red coloration upon reaction with Salkowski reagent, confirming IAA synthesis. Notably, CSNP-treated cultures exhibited a visibly more intense coloration than untreated controls, indicating enhanced auxin production.

Quantitative analysis based on absorbance measurements at 530 nm revealed a significant increase in IAA levels in the presence of CSNPs (Fig. 5). In *Azotobacter sp.*, untreated cultures showed an absorbance of  $0.334\pm 0.015$ , corresponding to an IAA concentration of

$33.4\pm 1.5\text{ }\mu\text{g mL}^{-1}$ . In contrast, CSNP-treated cultures exhibited an absorbance of  $0.640\pm 0.020$ , equivalent to  $64.0\pm 2.0\text{ }\mu\text{g mL}^{-1}$ , representing a 91.6% increase in IAA production. Similarly, *P. aeruginosa* cultures displayed an increase in absorbance from  $0.55\pm 0.018$  ( $55.3\pm 1.8\text{ }\mu\text{g mL}^{-1}$ ) in controls to  $1.105\pm 0.025$  ( $1105\pm 2.5\text{ }\mu\text{g mL}^{-1}$ ) following CSNP treatment, corresponding to a 99.8% enhancement.

IAA concentrations were calculated using a standard curve generated with pure IAA ( $0\text{--}100\text{ }\mu\text{g mL}^{-1}$ ), which demonstrated strong linearity ( $R^2=0.996$ ). All experiments were conducted in triplicate. Statistical analysis using one-way ANOVA followed by Tukey's post-hoc test confirmed that the increases in IAA production observed in CSNP-treated cultures were highly significant for both bacterial strains ( $p<0.01$ ). Overall, these results demonstrate that green-synthesized CSNPs markedly stimulate microbial auxin biosynthesis, with a slightly greater enhancement observed in *P. aeruginosa* compared to *Azotobacter sp.*

## Antimicrobial Activity and Molecular Docking Analysis

The antibacterial activity of chitosan nanoparticles (CSNPs) against the Gram-negative pathogen *Pseudomonas aeruginosa* was evaluated using the agar well diffusion assay. CSNPs produced a distinct zone of inhibition (ZOI) measuring 14 mm, indicating appreciable antibacterial activity (Fig. 6). In comparison, the positive control ciprofloxacin ( $50\text{ g mL}^{-1}$ ) exhibited a larger ZOI of 22 mm, while the negative control showed no detectable inhibition, confirming the validity of the assay.

The inhibitory performance of CSNPs was comparable to that reported for other nanoparticle-based antimicrobial agents against *P. aeruginosa*. Previous studies have documented ZOIs ranging from 10–16 mm for silver nanoparticles and 9–14 mm for zinc oxide nanoparticles, depending on synthesis conditions and particle size (Morones *et al.*, 2005), (Rai *et al.*, 2009), (Raghupathi *et al.*, 2011). Similarly, biosynthesized chitosan-based nanoparticles have been reported to produce inhibition zones between 11 and 15 mm (Qi *et al.*, 2004). These comparisons suggest that CSNPs exhibit antibacterial efficacy on par with other widely studied nanomaterials, although their activity remains lower than that of conventional antibiotics. The molecular basis underlying the observed antibacterial activity, in silico docking

studies were performed against the outer membrane porin OprD (PDB ID: 3SY7) of *P. aeruginosa*, a protein involved in nutrient uptake and antibiotic permeability. Docking simulations revealed that a 5-unit chitosan oligomer binds strongly to a secondary pocket of OprD, exhibiting a binding affinity of  $-8.5 \text{ kcal mol}^{-1}$ , which exceeded that of ciprofloxacin ( $-7.5 \text{ kcal mol}^{-1}$ ). Chitosan formed multiple stabilizing interactions, including a salt bridge with ASP232 and hydrogen bonds with ARG319, SER302, and LEU299 (Table 1).

Visualization of docking poses demonstrated substantial overlap between the chitosan and ciprofloxacin binding sites within OprD (Fig. 7), suggesting potential competitive inhibition. Two-dimensional interaction analysis further indicated that chitosan establishes a denser hydrogen-bonding and electrostatic interaction network with key porin residues compared to ciprofloxacin (Fig. 8). These interactions are likely to impede porin-mediated transport of nutrients and antibiotics, thereby contributing to membrane dysfunction and bacterial growth inhibition.

The in vitro antibacterial results and in silico docking analysis support a mechanism in which CSNPs exert antimicrobial activity through disruption of outer membrane porin function, complementing their surface-mediated antibacterial effects.

In this study, lemon juice-mediated green synthesis produced chitosan nanoparticles (CSNPs) with stable optical and structural signatures and clear bioactivity, supporting their potential as multifunctional nano-enabled agents. The UV-Vis response remained consistent over storage, and SEM confirmed predominantly spherical particles in the 100-200 nm range, indicating successful nanoscale formation with limited aggregation. FTIR features were consistent with the chitosan backbone and citric-acid-associated cross-linking, including a broad band attributed to overlapping and stretching vibrations and carbonyl-region signals consistent with interaction/cross-link formation in chitosan-based systems. Similar physicochemical outcomes have been reported for plant-assisted and organic-acid-mediated chitosan nanoparticle syntheses, where phytochemical components contribute to colloidal stability and functional surface chemistry (El-Naggar *et al.*, 2022), (Khezerlou *et al.*, 2018), (Vazquez-Munoz *et al.*, 2017).

CSNP supplementation substantially increased indole-3-acetic acid (IAA) production in both *Azotobacter sp.* and

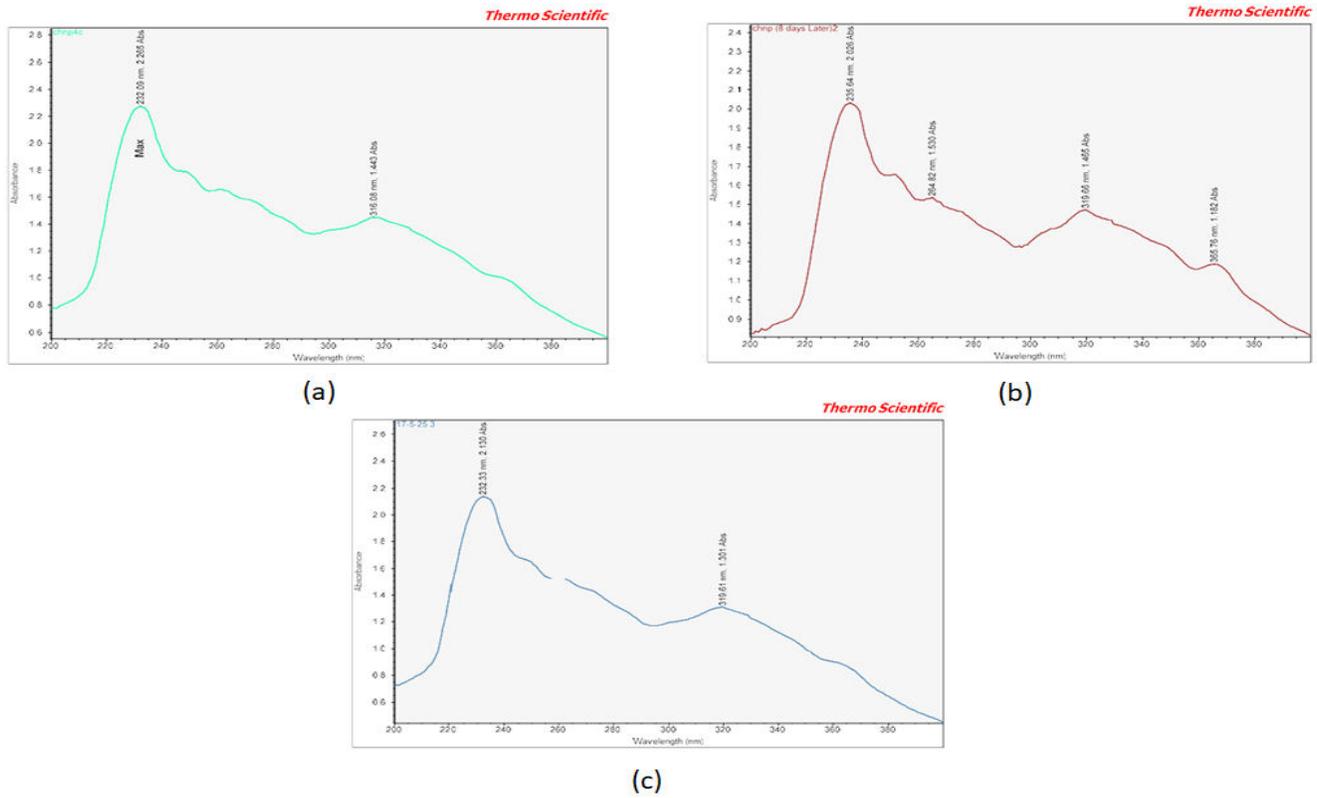
*Pseudomonas aeruginosa*, indicating that CSNPs can positively modulate PGPR functional output under the tested conditions. This observation is consistent with recent reports showing that chitosan nanoparticle exposure can enhance IAA biosynthesis in rhizospheric *Pseudomonas* strains and translate into improved plant growth responses (Panichikkal *et al.*, 2022), (Panichikkal *et al.*, 2022). Although the present study did not directly probe pathway-level mechanisms, several explanations are plausible and supported by current literature on PGPR-nanomaterial interactions, including altered membrane-associated transport of precursors (e.g., tryptophan), changes in redox homeostasis, and nanoparticle-driven shifts in metabolic allocation (dos Reis *et al.*, 2024), (Rajeshkumar *et al.*, 2023). In the present system, lemon-derived surface moieties may further contribute by reducing oxidative stress or stabilizing enzyme function involved in tryptophan-dependent IAA biosynthesis, although this requires targeted validation.

The CSNPs also displayed antibacterial activity against *P. aeruginosa*, producing a measurable inhibition zone at high concentration, which is notable given the intrinsic permeability barrier and resistance-associated traits of this organism. The inhibition magnitude is comparable to values reported for several nanoparticle systems and green-synthesized chitosan nanoparticles tested against Gram-negative pathogens (El-Naggar *et al.*, 2022), (Ke *et al.*, 2021).

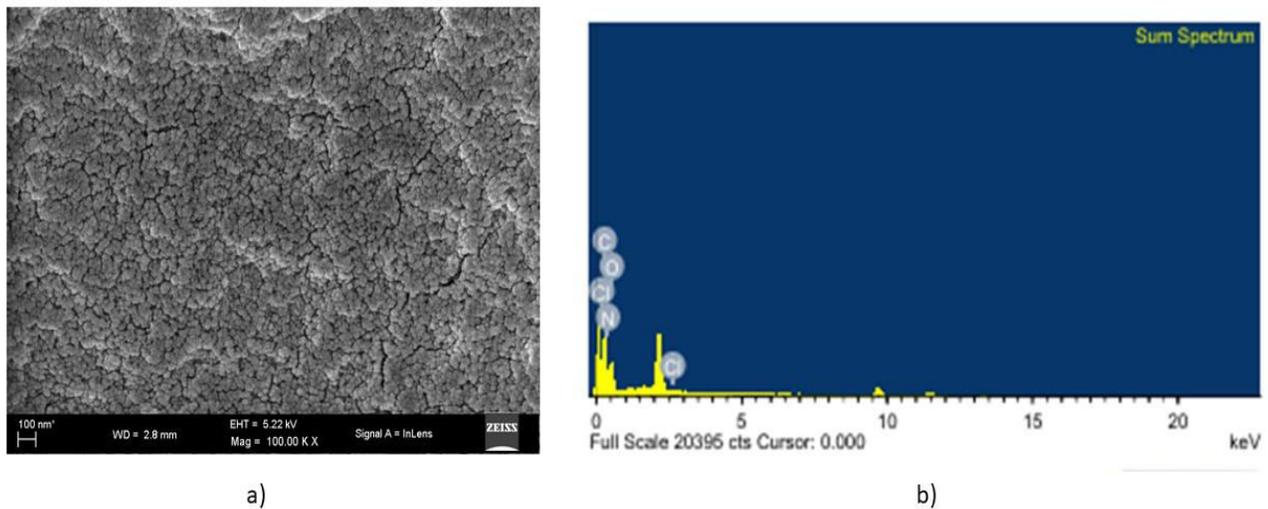
Mechanistically, chitosan nanoformulations are generally understood to act via multiple complementary routes, including electrostatic interaction with negatively charged envelope components, membrane perturbation, and disruption of essential ion balance or intracellular functions (Khezerlou *et al.*, 2018), (Maluin *et al.*, 2020), (An *et al.*, 2022). Such multimodal activity is relevant for applications where biodegradable and low-toxicity antimicrobials are preferred.

Docking analysis provided additional molecular-level support for outer membrane involvement by indicating favorable binding of a chitosan oligomer to porin proteins, particularly OprD, with predicted affinity comparable to or greater than the reference antibiotic ligand in the modeled pocket. While docking does not establish in vivo inhibition, the predicted dense hydrogen-bonding and electrostatic interaction network suggests that porin engagement could plausibly contribute to impaired transport across the outer membrane.

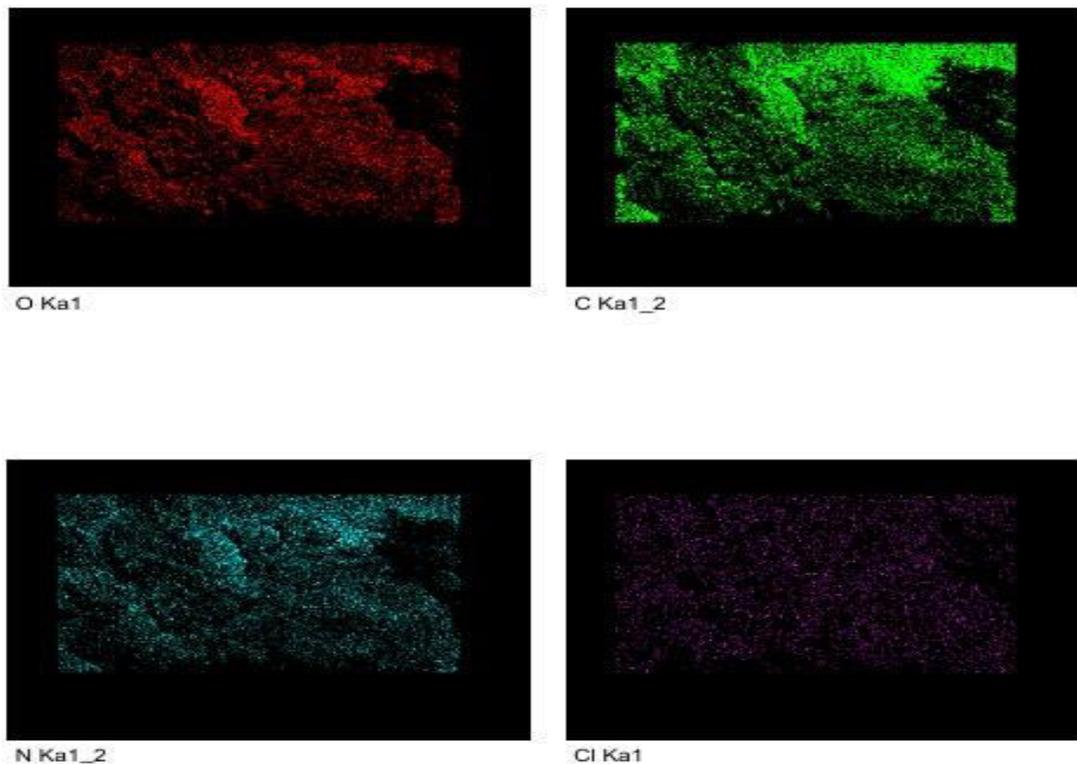
**Fig.1 UV–Vis absorption spectra of chitosan nanoparticles synthesized using lemon juice, recorded on Day 1, Day 8, and Day 21. The consistent absorption profile confirms nanoparticle formation and long-term colloidal stability.**



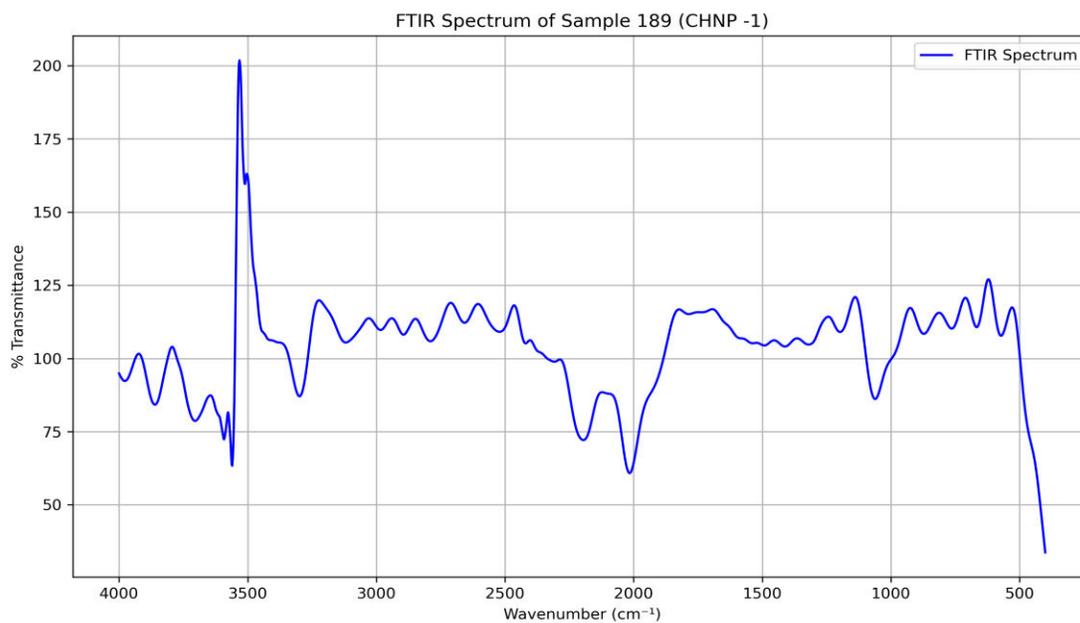
**Fig.2 SEM micrograph and EDS spectrum of biosynthesized chitosan nanoparticles, demonstrating spherical morphology and elemental composition consistent with chitosan-based nanostructures.**



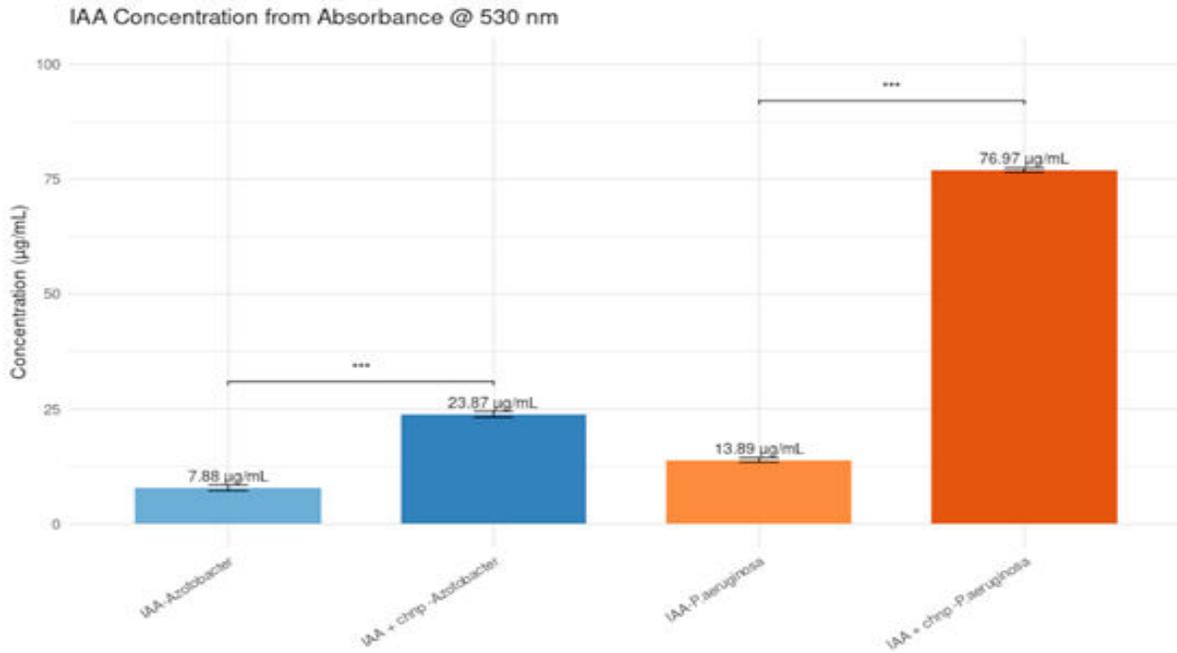
**Fig.3** EDS elemental mapping of chitosan nanoparticles showing uniform distribution of carbon (C), oxygen (O), nitrogen (N), and chlorine (Cl).



**Fig.4** FTIR spectrum of biosynthesized chitosan nanoparticles showing characteristic functional groups associated with chitosan, citric acid cross-linking, and lemon juice-derived phytochemicals.

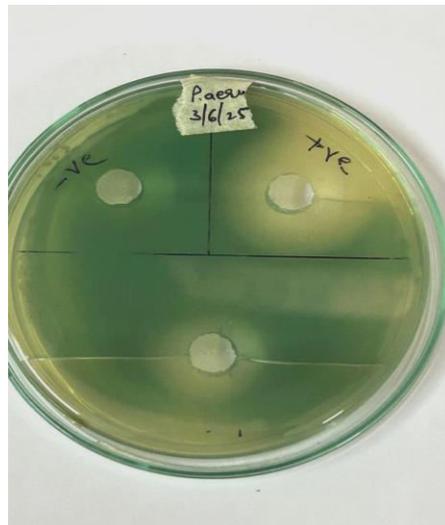


**Fig.5** Effect of chitosan nanoparticles (CSNPs) on indole-3-acetic acid (IAA) production in *Azotobacter* sp. and *Pseudomonas aeruginosa*. CSNP-treated cultures show enhanced color intensity in the Salkowski assay, a linear IAA standard curve, and significantly increased IAA concentrations compared to untreated controls.



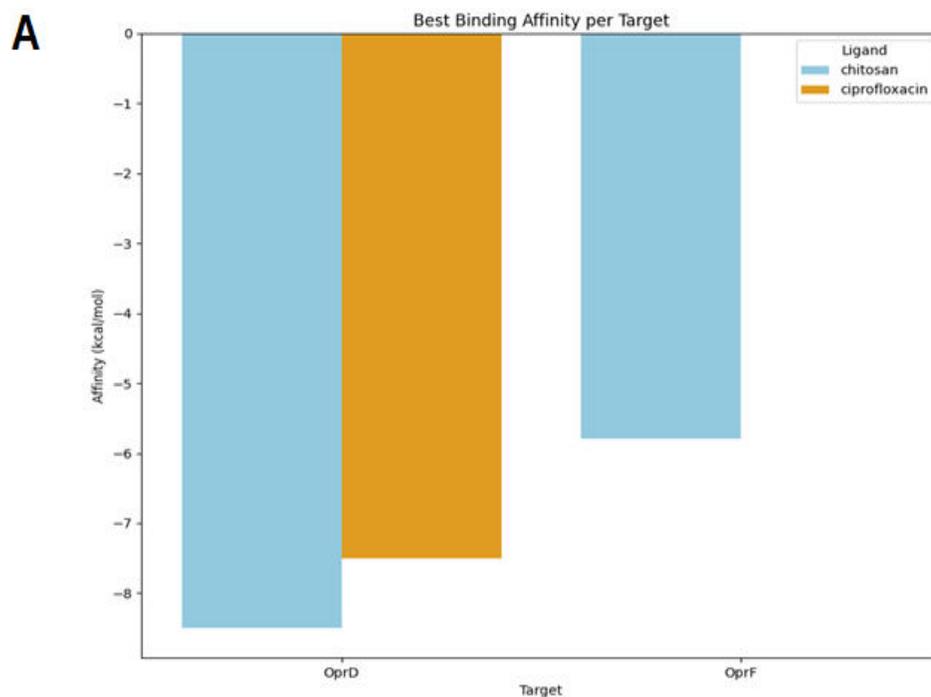
Data represent mean  $\pm$  SD (n = 3);  $p < 0.01$ .

**Fig.6** Antibacterial activity of chitosan nanoparticles (CSNPs) against *Pseudomonas aeruginosa* determined by agar well diffusion.



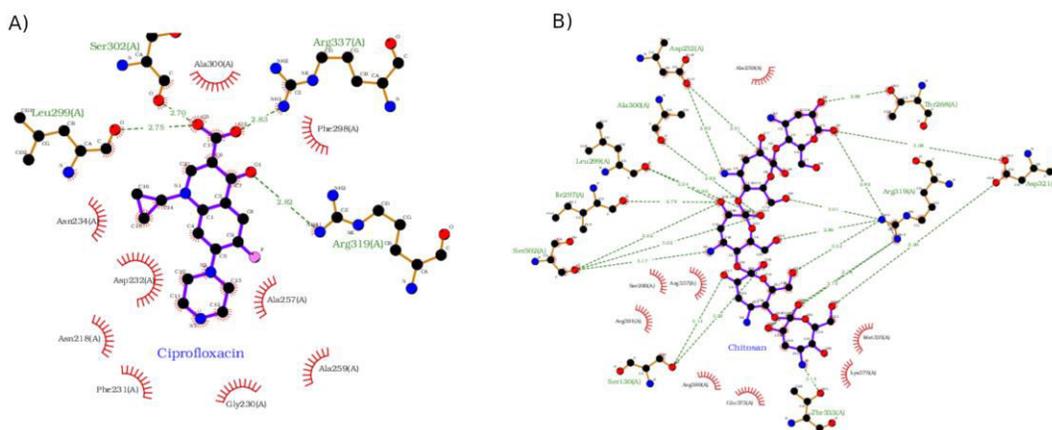
CSNPs produced a clear inhibition zone, while ciprofloxacin served as the positive control and distilled water as the negative control.

Fig.7 Molecular docking analysis of chitosan and ciprofloxacin with *Pseudomonas aeruginosa* porins



(A) Binding affinity comparison for OprD and OprF. (B) Three-dimensional visualization showing overlap of chitosan and ciprofloxacin binding sites within OprD, indicating potential competitive inhibition.

**Fig.8** Two-dimensional interaction maps of OprD showing (A) ciprofloxacin and (B) chitosan binding. Chitosan forms extensive hydrogen bonding and electrostatic interactions with key porin residues, indicating stronger binding stability.



**Table.1** Key interactions of chitosan and ciprofloxacin with common residues in the OprD porin (PDB ID: 3SY7)

Ligand	Residue	Distance (Å)	Interaction Type
Chitosan	ASP232	2.20–2.65	Hydrogen bond, electrostatic
Chitosan	LEU299	2.30–2.52	Hydrogen bond
Chitosan	ARG319	1.98–2.63	Hydrogen bond
Ciprofloxacin	ASP232	5.17	Electrostatic
Ciprofloxacin	LEU299	2.28	Hydrogen bond
Ciprofloxacin	ARG319	2.13	Hydrogen bond

Overall, these findings support the development of green-synthesized CSNPs as dual-function agents that can enhance beneficial microbial auxin production while suppressing a Gram-negative pathogen, aligning with recent interest in integrated PGPR-based biostimulants and nano-enabled agricultural inputs (dos Reis *et al.*, 2024), (Sun *et al.*, 2022).

Limitations include reliance on the Salkowski assay, which can be influenced by indolic intermediates, and the use of diffusion-based antibacterial testing, which does not directly yield MIC/MBC values. Future work should therefore include chromatographic confirmation of IAA (e.g., HPLC/LC–MS), broth microdilution and

biofilm assays for antimicrobial profiling, and validation in plant-soil systems to assess performance under realistic agronomic conditions.

In conclusion, this study establishes the successful green synthesis of chitosan nanoparticles (CSNPs) using Citrus limon juice as an eco-friendly reducing and stabilizing agent. The nanoparticles, characterized by UV-Vis, FTIR, SEM, and EDS analyses, exhibited spherical morphology (50–150 nm), structural stability, and the incorporation of lemon-derived bioactives, confirming their functionalization. Biologically, CSNPs significantly enhanced indole-3-acetic acid (IAA) production by plant growth-promoting rhizobacteria,

with increases of 91.6% in *Azotobacter sp.* and 99.8% in *Pseudomonas aeruginosa*. In parallel, CSNPs displayed notable antimicrobial activity against *P. aeruginosa*, producing a 14 mm inhibition zone, validating their dual role as both biofertilizer enhancers and biocontrol agents.

In silico docking reinforced these findings by demonstrating strong interactions of chitosan oligomers with bacterial outer membrane porins OprF and OprD, surpassing the binding affinity of ciprofloxacin for OprD. The observed hydrogen bonding and electrostatic interactions suggest that CSNPs can competitively inhibit porin-mediated transport, providing a mechanistic explanation for their antimicrobial activity.

Collectively, these results highlight the potential of green-synthesized CSNPs as a multifunctional platform in sustainable agriculture, capable of simultaneously promoting plant growth and mitigating bacterial threats. Future investigations should focus on dose–response analyses, field-level trials, and environmental impact assessments to support the translation of CSNP-based formulations into practical biofertilizers and biocontrol agents.

### Author Contributions

Shazia Ansari: Investigation, formal analysis, writing—original draft. Tushar Gaikwad: Validation, methodology, writing—reviewing. Rajendra Choure:—Formal analysis, writing—review and editing. Ulhas Patil: Investigation, writing—reviewing. Sangeeta Jangam: Resources, investigation writing—reviewing.

### Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

### Declarations

**Ethical Approval** Not applicable.

**Consent to Participate** Not applicable.

**Consent to Publish** Not applicable.

**Conflict of Interest** The authors declare no competing

interests.

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