PHYTOGENIC FABRICATION AND CHARACTERIZATION OF CHITOSAN NANOPARTICLES USING PLECTRANTHUS VETTIVEROIDES: ASSESSMENT OF BACTERICIDAL ACTIVITY

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ABSTRACT- *Chitosan nanoparticles (CNPs) have attracted significant interest owing to their excellent biocompatibility, biodegradability, and inherent antimicrobial properties. In this study, chitosan nanoparticles were biosynthesized using the ethanolic root extract of Plectra thus vettiveroides through the ionic gelation method. The formation and physicochemical characteristics of the synthesized nanoparticles were analysed using UV–Visible spectroscopy and Scanning Electron Microscopy (SEM). UV–Visible spectral analysis displayed a distinct absorption peak at 234 nm, indicating successful nanoparticle synthesis. SEM images demonstrated predominantly spherical nanoparticles with minor aggregation. The antibacterial efficacy of the biosynthesized chitosan nanoparticles was assessed against Staphylococcus aureus, Bacillus cereus, Escherichia coli, and Klebsiella pneumoniae using the agar well diffusion technique. Among the tested pathogens, Klebsiella pneumoniae showed the highest susceptibility to the nanoparticles. These findings highlight the potential of Plectranthus vettiveroides-mediated chitosan nanoparticles as effective antibacterial agents with prospective applications in biomedical and pharmaceutical fields.*

Keywords*:* Chitosan nanoparticles; *Plectranthus vettiveroides*; Ionic gelation; Antibacterial activity; SEM; UV–Visible spectroscopy.

1. INTRODUCTION

Chitosan is a naturally derived polyatomic biopolymer obtained through the DE acetylation of chitin and is widely valued for its biodegradability, biocompatibility, low toxicity, and inherent antimicrobial properties. Owing to the presence of reactive amino functional groups, chitosan can be readily fabricated into nanoparticle form, which has attracted considerable attention in biomedical and pharmaceutical fields, particularly for antimicrobial applications and drug delivery systems. However, many conventional nanoparticle synthesis techniques rely on hazardous chemicals and energy-intensive processes, thereby restricting their suitability for biological use.

In recent years, green synthesis approaches utilizing plant-derived materials have gained prominence as environmentally friendly and sustainable alternatives for nanoparticle production. *Plectranthus vettiveroides*, an aromatic medicinal plant belonging to the family Lamiaceae, is traditionally used for various therapeutic purposes and is known to possess a diverse range of bioactive phytochemicals with antimicrobial properties. The phytoconstituents present in its root extract can potentially function as natural cross-linking, reducing, and stabilizing agents during nanoparticle synthesis.

The present study aims to establish a simple, safe, and eco-friendly method for the synthesis of chitosan nanoparticles using *Plectranthus vettiveroides* root extract via the ionic gelation technique. It is hypothesized that the phytochemicals in the root extract play a crucial role in promoting nanoparticle formation and enhancing Antibacterial efficacy. This work is significant as it introduces a green strategy for producing biologically active chitosan nanoparticles with promising applications in pharmaceutical and biomedical domains.

Fig no: 1*Plectranthus vettiveroides*

Fig no: 2 Roots of *Plectranthus vettiveroides*

2. MATERIALS AND METHODS

Materials

Chitosan and sodium tripolyphosphate (TPP) were purchased from Sigma-Aldrich (USA). Acetic acid was obtained from HiMedia Laboratories, Chennai, India. All reagents and chemicals used in this study were of analytical grade and used without further purification.

Collection and Preparation of Plant Extract

The roots of *Plectranthus vettiveroides* were collected from the Kodumudi Dam region, Cuddalore District, Tamil Nadu, India, and taxonomically authenticated by a qualified botanist. Collected roots were thoroughly washed with distilled water to remove debris, shade-dried at room temperature, and pulverized into a fine powder. The powdered material was subjected to ethanol extraction, followed by filtration. The resulting extract was stored at 4 °C until further use.

Synthesis of Chitosan Nanoparticles

Chitosan nanoparticles were prepared using the ionic gelation technique. Chitosan was dissolved in 1% (v/v) acetic acid solution and the pH was adjusted to 5.5 using sodium hydroxide. A sodium tripolyphosphate (TPP) solution was then added dropwise to the chitosan solution under continuous magnetic stirring. The *Plectranthus vettiveroides* root extract was introduced into the chitosan solution prior to TPP addition. The formation of nanoparticles was indicated by the appearance of turbidity. The synthesized nanoparticles were collected by centrifugation, washed repeatedly with distilled water to remove unbound substances, and lyophilized for further characterization and analysis.

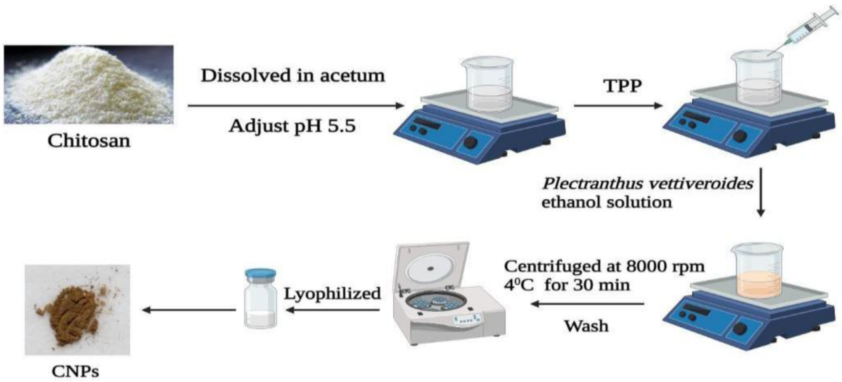

Fig no: 3 Syntheses of Chitosan Nanoparticles

Antibacterial Activity

TPP–chitosan nanoparticles (TCs) were synthesized using the ionic gelation approach involving electrostatic interaction between chitosan and TPP anions, with minor modifications to previously reported protocols. In brief, 20 mg of chitosan was dissolved in 10 mL of 1% (v/v) acetic acid under constant magnetic stirring at room temperature, and the pH was adjusted to 5.5. A TPP solution (2 mg/mL; 3.5 mL) was then added gradually to the chitosan solution with continuous stirring. The appearance of a clear opalescent suspension confirmed the formation of chitosan nanoparticles, which was further stabilized by short-duration sonication.

For the synthesis of *Plectranthus vettiveroides*–encapsulated TPP–chitosan nanoparticles, 1 mL of ethanolic root extract (3 mg/mL) was slowly introduced into the chitosan solution prior to the addition of TPP, followed by the same ionic gelation process. The nanoparticles formed were harvested by centrifugation at 8,000 rpm for 30 minutes at 4 °C. The supernatant was discarded, and the nanoparticle pellet was washed three times with double-distilled water to remove unbound constituents. The final purified nanoparticles were freeze-dried and stored for subsequent characterization and antibacterial evaluation.

3. RESULTS AND DISCUSSION

Analytical Characterization

UV-Vis spectroscopy

UV-Visible spectroscopy is basically used for the identification of chemical products and their qualitative analysis. The molecules or atoms absorb UV-Visible radiation and their electrons are excited from lower energy levels to higher energy levels. In the present study, we have obtained the broad peaks at 234 nm; this indicates the formation of chitosan nanoparticles at such wavelength (fig 4).

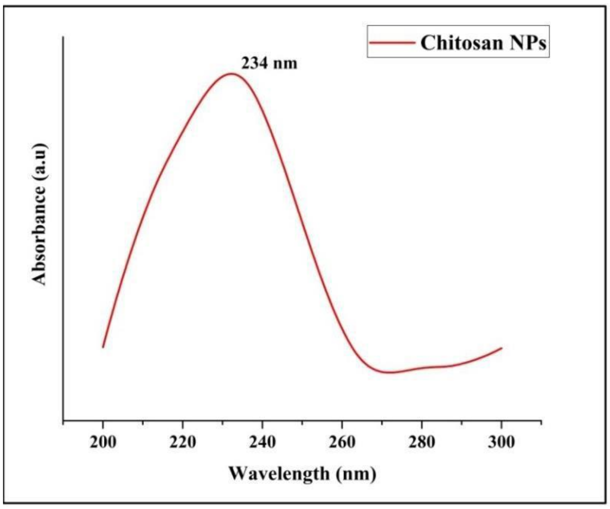

Fig no: 4 UV-Visible spectroscopy of CNPs synthesized using *Plectranthus vettiveroides* root extract.

Scanning Electron Microscopy (SEM)

The surface morphology of the CNPs synthesized using *Plectranthus vettiveroides* root extract was studied using SEM. The SEM image was shown in fig 5. The SEM results revealed the presence of agglomerates of nanoparticles and showed that the spherical morphology was in high resolution.

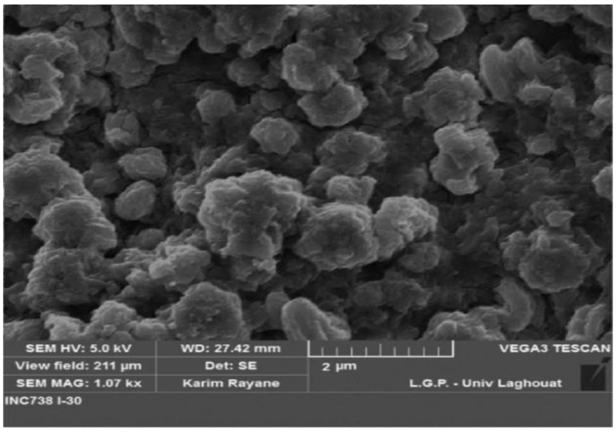

Fig no:5 SEM image of CNPs synthesized using *Plectranthus vettiveroides* root extract.

Antibacterial Activity

The antibacterial potential of chitosan nanoparticles (CNPs) was evaluated using the well diffusion method against four bacterial pathogens: *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli*, and *Klebsiella pneumoniae*. The biosynthesized chitosan nanoparticles, prepared using *Plectranthus vettiveroides* root extract, exhibited inhibitory effects against all tested strains. Notably, the antibacterial activity was more pronounced against Gram-negative bacteria compared to Gram-positive bacteria, likely due to differences in the structural composition of their cell walls. Among the pathogens tested, *Klebsiella pneumoniae* showed the largest zone of inhibition (24 mm), followed by *Escherichia coli* (20 mm), *Bacillus cereus* (18 mm), and *Staphylococcus aureus* (16 mm) at a nanoparticle concentration of 100 µL. These findings are consistent with previous reports demonstrating strong antibacterial activity of chitosan nanoparticles against *Klebsiella pneumoniae* and *Escherichia coli*.

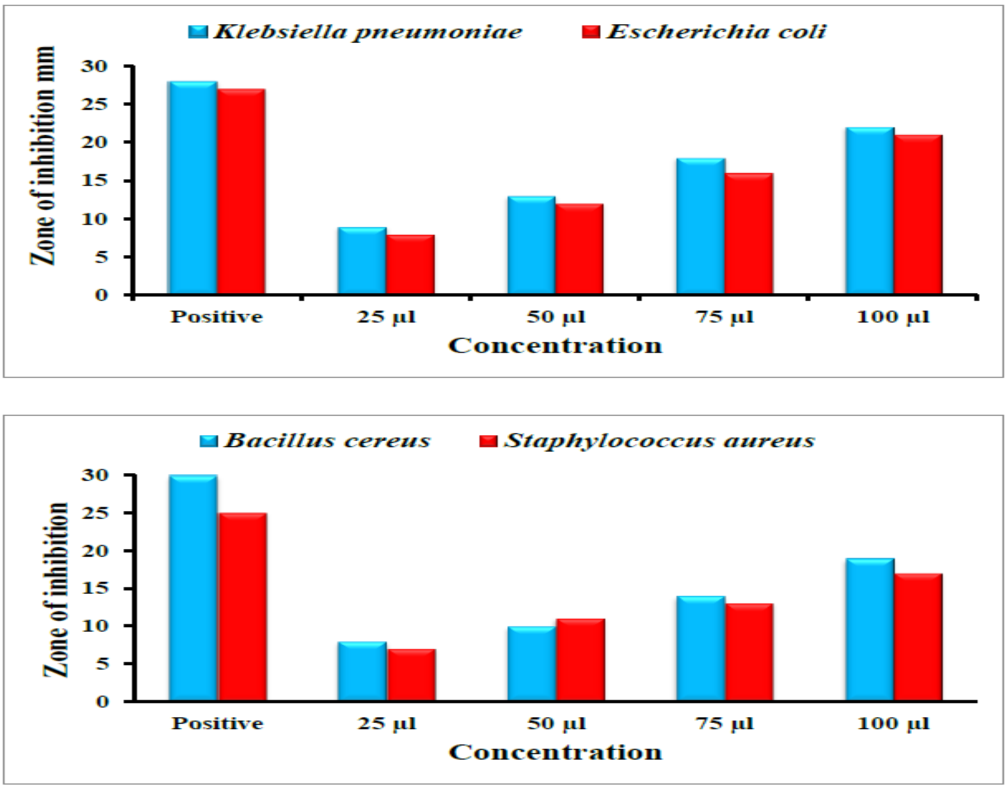

Fig no:6 Graphical representation of zone of inhibition of CNPs synthesized using *Plectranthus vettiveroides* root extract.

The mechanism of antibacterial activity of chitosan NPs may be attributed to the penetration and disintegration of the membrane by smaller-sized NPs which lead to cell lysis. The release of H_2O_2 from the surface of chitosan NPs also reported as the possible mechanism for bactericidal activity. The generation of H_2O_2 is highly dependent on the surface area of chitosan and the generated H_2O_2 penetrates the cell membrane and causes damage to kill the bacteria. The presence of alkaloids, terpenoids, flavonoids, tannins, carbohydrates, sterols, saponins, proteins, and amino acids in *Plectranthus vettiveroides* root extract showed potential bio reducing activity and also bactericidal activity against the tested bacteria which could be useful for biomedical application

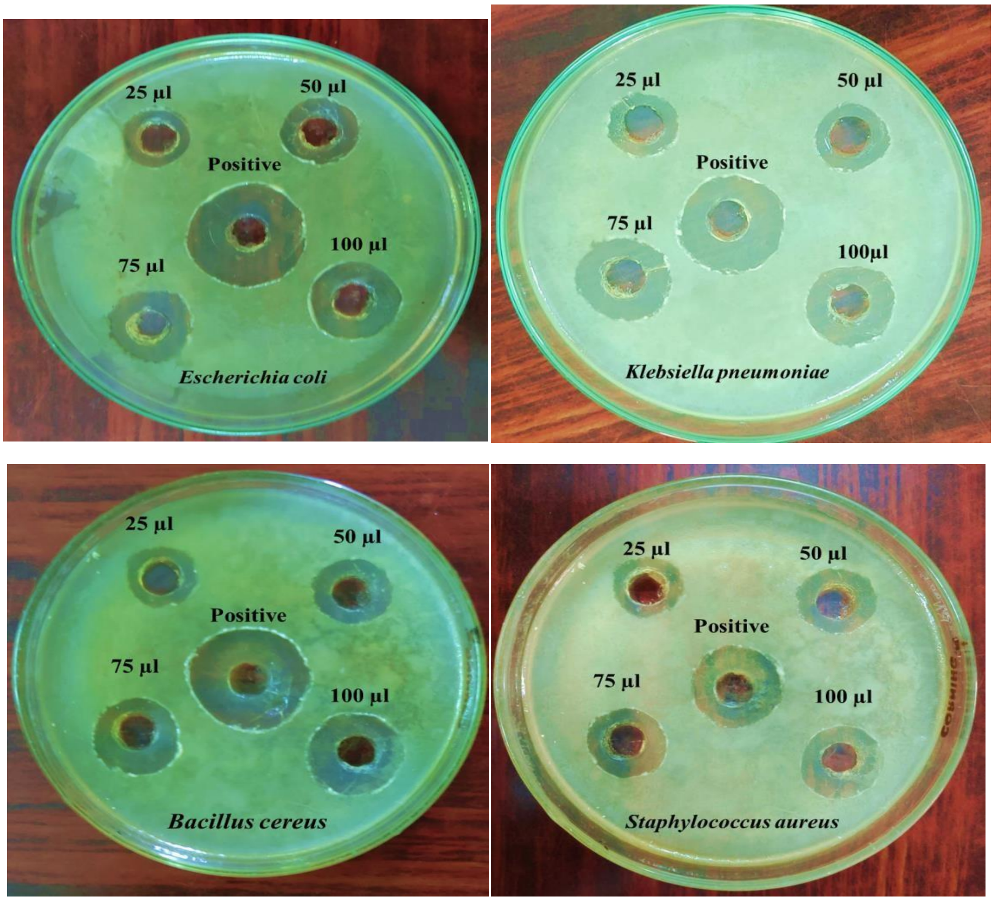

Fig no:7 Antibacterial activity CNPs synthesized using *Plectranthus vettiveroides* root extract.

Table.1. Zone of inhibition of CNPs synthesized using *Plectranthus vettiveroides* root extract.

Bacterial pathogens	Zone of inhibition mm				
	25 µl	50 µl	75 µl	100 µl	Positive
Klebsiella pneumoniae	9	13	18	22	28
Escherichia coli	8	12	16	21	27
Bacillus cereus	8	10	14	19	30
Staphylococcus aureus	7	11	13	17	25

4. CONCLUSION

Chitosan nanoparticles were successfully synthesized via a biogenic approach using the ethanolic root extract of *Plectranthus vettiveroides*. UV–Visible spectroscopy confirmed nanoparticle formation with a characteristic absorption peak at 234 nm, while SEM analysis revealed predominantly spherical morphology. The biosynthesized nanoparticles exhibited significant antibacterial activity against *Klebsiella pneumoniae*, *Escherichia coli*, *Bacillus cereus*, and *Staphylococcus aureus*, with the strongest effect observed against *Klebsiella pneumoniae*. These results demonstrate that *Plectranthus vettiveroides*-mediated chitosan nanoparticles are a promising, eco-friendly option for developing effective antimicrobial agents with potential applications in pharmaceutical and biomedical fields.

5. REFERENCE

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