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RESEARCH PAPER

Pulsed Laser Ablation Synthesis of Chitosan-ZnO-TiO_2 Nanocomposites for Bacterial Inhibition

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ABSTRACT

This study investigated the antimicrobial properties of chitosan-based nanocomposites against E. coli and Porphyromonas bacteria. The nanocomposites, including CS, CS-ZnO, CS-TiO_2, and CS-ZnO-TiO_2, were synthesized using pulsed laser ablation in liquid (PLAL). Characterization techniques confirmed the formation of nanoparticles with varying sizes and distributions. Antimicrobial assays demonstrated that the CS-ZnO-TiO_2 nanocomposite exhibited superior inhibitory activity against both bacterial strains compared to the individual components or their binary combinations. The inhibition percentage increased with the concentration of the nanomaterials, highlighting the dose-dependent antibacterial effect. Statistical analysis confirmed significant differences in the antibacterial activity of the different nanomaterials. These findings suggest that the CS-ZnO-TiO_2 nanocomposite has potential as a broad-spectrum antimicrobial agent, warranting further investigation into its mechanism of action and optimization for various applications.

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INTRODUCTION

Chitosan, a well-known material with antibacterial, biodegradable and biocompatible properties, can be used to produce thin films. Nonetheless, nanomaterial infusion has been investigated by scientists so as to enhance its antibiotic efficacy against various bacterial strains in order to meet particular needs [1, 2, 3]. An example of this is the incorporation of zinc oxide (ZnO) and titanium oxide (TiO2) nanostructures having distinct characteristics which are usable in diverse industries [4, 5, 6]. In fact, zinc oxide nanoparticles show a direct energy gap width of 3.36 eV and possess such attributes as strong ultraviolet radiation absorption; photocatalytic activity; stability; antibacterial potency; non-toxicity on one hand while titanium oxide nanoparticles, which can be under 100 nanometers in size generally, enhance the mechanical properties of materials like corrosion/oxidation resistance and exhibit remarkable electrical/magnetic traits making them appropriate for industrial as well as technical applications.

Various techniques may be employed such as using green compounds to decorate chitosan matrices with titanium thus conferring antimicrobial properties for synthesizing these nanomaterials. Moreover, creation of zinc oxide and titanium dioxide nanoparticles within chitosan solutions can be done using laser ablation with strict control over particle size reduction and stability enhancement. Pulsed laser ablation in liquid technology (PLAL) is a potent method that has some advantages over alternative methods,

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where nanoparticle size and structure depend on the pulse duration, wavelength and energy [7, 8]. The diverse applications are driving the rapid development of nanoparticles production especially nanocomposites for better performance. In this study we propose a new synthesis of nanocomposite by combining chitosan with zinc oxide and titanium oxide through laser ablation to reduce processing time. This approach may be used to combine the synergistic properties of these composites leading to multifunctional materials having wide applicability [9, 10].

This study aims to address these limitations by employing a green and efficient synthesis approach, pulsed laser ablation in liquid (PLAL), to create chitosan-ZnO-TiO_2 nanocomposites. By combining the antimicrobial properties of chitosan with the photocatalytic and antibacterial characteristics of ZnO and TiO_2, we hypothesize that the resulting nanocomposite will exhibit enhanced antimicrobial activity against a broad spectrum of bacteria.

MATERIALS AND METHODS

Materials

Chitosan powder (purchased from Life Sciences, GP5053). Zinc and titanium plates (purity 99.8%) were purchased from the commercial market as the target.

Preparation of the Nanocomposite

Dissolve 0.5 g of chitosan powder in a solution containing 670 ml of water and 70 ml of acetic acid.

For the synthesis of zinc oxide (ZnO) nanoparticles, employ pulsed laser ablation in liquids. Submerge a zinc plate in the prepared chitosan solution. Use an Nd: YAG laser operating at a 1064 nm wavelength, with a pulse energy of 500 mJ, duration of exposure of one minute per pulse, and a pulse frequency of 6 Hz, delivering 1500 pulses onto the plate. After removing the zinc plate from the chitosan-ZnO (CS-ZnO) solution, submerge the titanium plate in the CS-ZnO solution to obtain TiO_2 nanoparticles using the same ablation parameters. The composite CS-ZnO-TiO_2 is successfully synthesized. The laser beam should be directed perpendicular to both the zinc and titanium plates, facilitated by a convex lens featuring a focal length of 10 cm.

Distinctly, CS-ZnO and CS-TiO_2 nanocomposites should be individually prepared within the same chitosan solution, employing the previously specified laser parameters for each sample (CS-ZnO, CS-TiO_2, and CS-ZnO-TiO_2).

The compound should be placed in plastic tubes and sent for examination using transmission electron microscopy (TEM). These samples were placed within quartz cells for UV-visible spectroscopy testing.

Antimicrobial Section

To examine the effectiveness of the nanocomposite, two types of clinical isolates (*E. coli*) (gram-negative) and Porphyromonas (gram-positive) were used. The bacterial isolates were transferred to the appropriate culture medium for bacterial growth and placed in an incubator for 24 hours at 37 C^0. The activity of the nanocomposite was examined by making four small holes into which nanomaterials were poured in succession. The first hole was for the solvent, which is chitosan (CS), the second hole was for (CS –TiO_2), the third was for (CS-ZnO), and the fourth was for the nanocomposite (CS-ZnO-TiO_2). Different concentrations of the aforementioned materials were added, and then the dishes were placed in the incubator for 24 hours [11, 12].

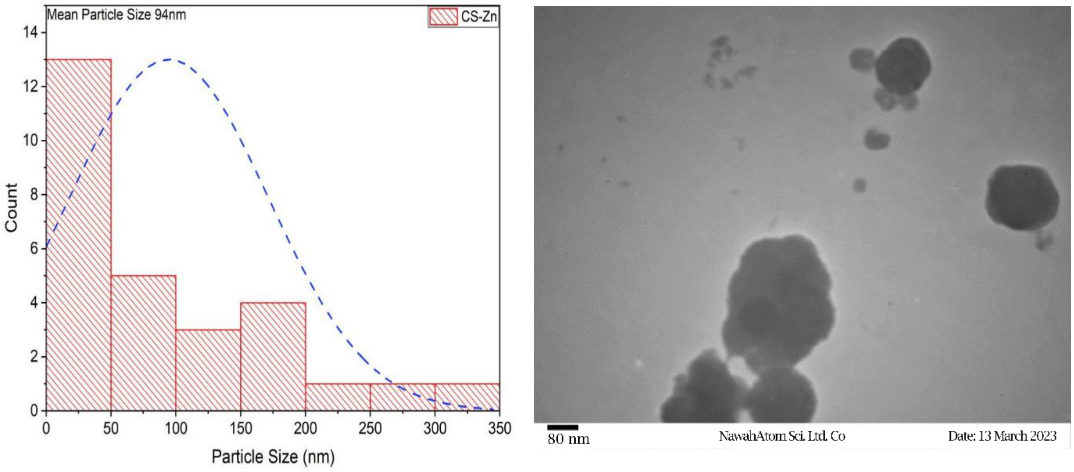
RESULTS AND DISCUSSION

The TEM images in Fig. 1 revealed that all the examined samples consisted of spherical nanoparticles, with the average size of the zinc particles being 94 nm, the medium size of the titanium being 15 nm, and the average size of the compound being 12 nm. The images also indicated that the zinc and titanium were linked with the polymer lattice. The synthesis of titanium and zinc with chitosan to produce spherical nanoparticles has been an active area of research and development, with potential benefits including a large surface area, improved mechanical and microscopic properties, and enhanced electrochemical properties, making them suitable for medical uses

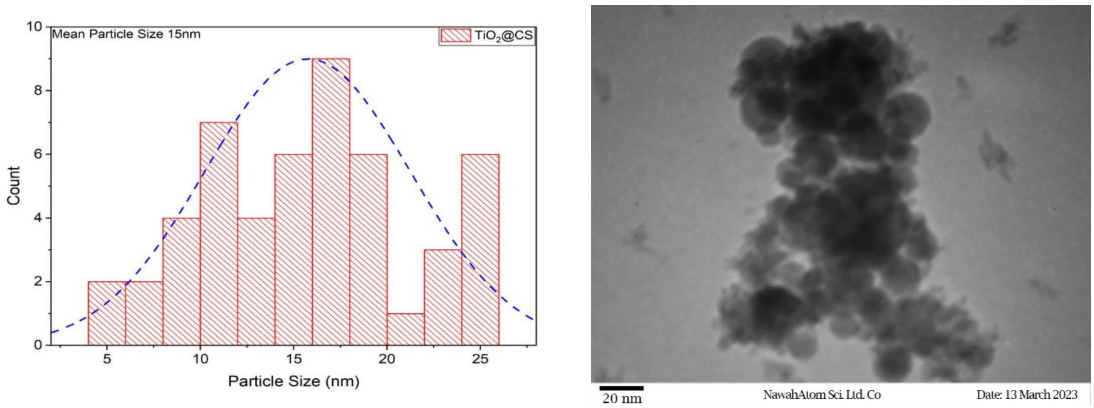
In general, the smaller the size of nanoparticles, the greater their potential benefits in multiple fields, such as technology, medical sciences, and materials. However, precautions must be taken when handling nanoparticles due to their small size impact on safety and impact on the environment [13].

The UV-visible spectroscopy is a very useful and reliable technique in the preliminary characterization of synthesized nanoparticles and is also used to monitor the synthesis and stability of nanoparticles.

Fig. 2 shows that the peaks (230-240) nm represent zinc and titanium, respectively. This indicates that the zinc and titanium nanoparticles



A.The TEM images for (CS-ZnO)



B. The TEM images for (CS-TiO_2)

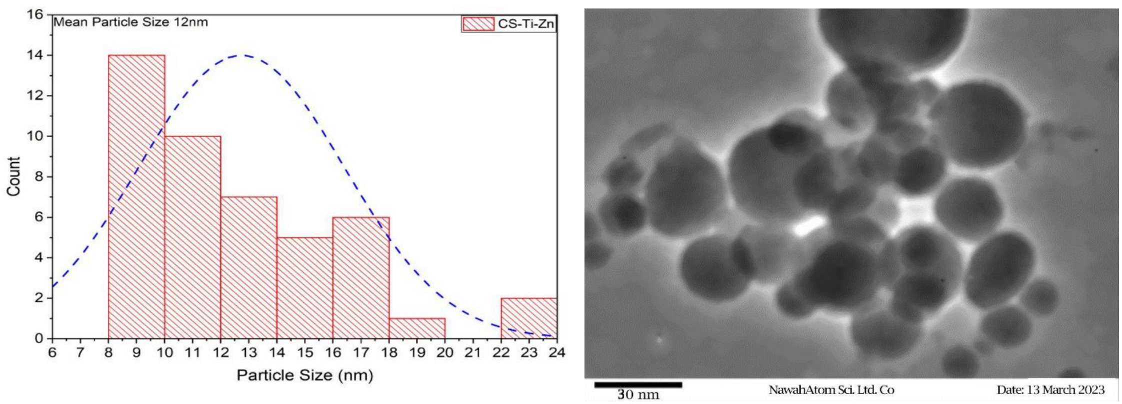


Fig.1. A, B, C the TEM examination for (CS-ZnO) (CS –TiO_2) (CS-ZnO-TiO_2).

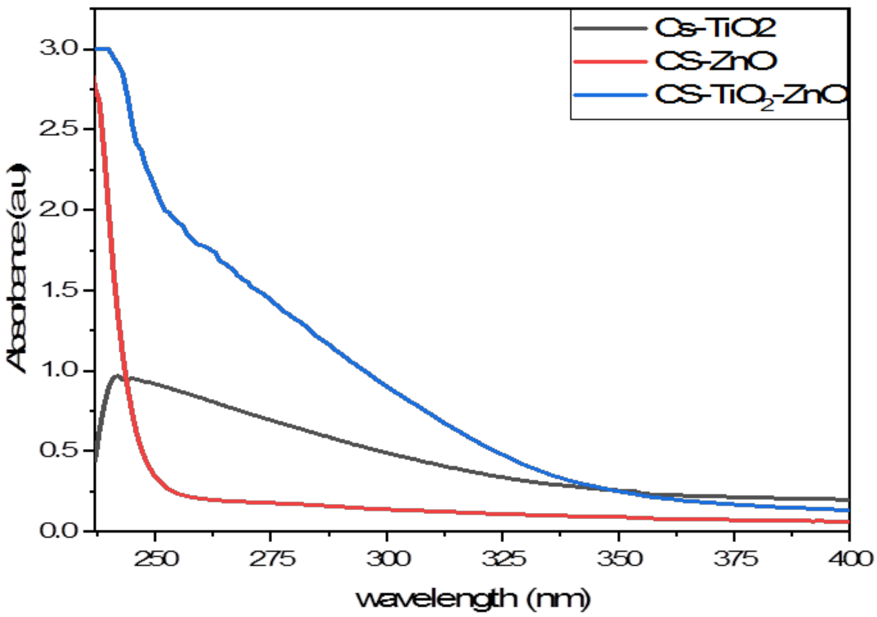


Fig. 2. The absorbance for (CS-ZnO), (CS-TiO_2), (CS-ZnO-TiO_2).

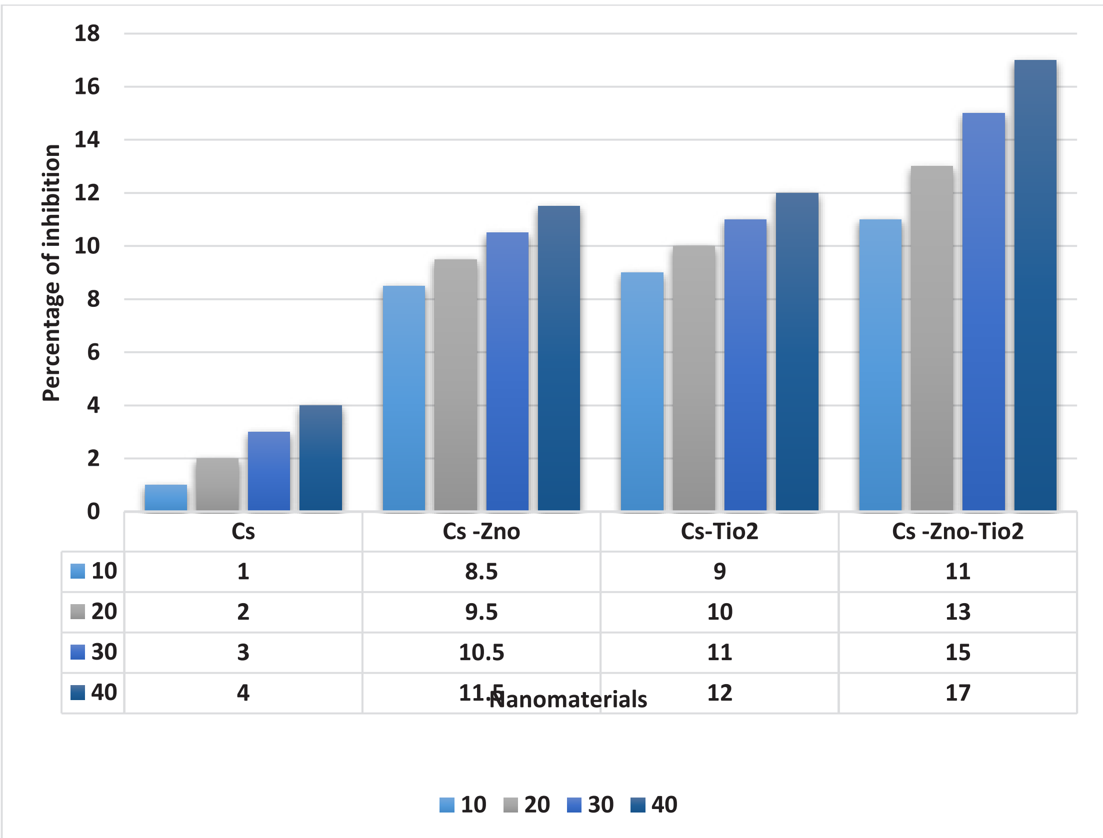


Fig. 3. the Nanomaterials at different amounts (10, 20, 30, 40) µl which inhibited E. coli bacteria to certain percentages.

have been linked to the polymer chain, and this is consistent with the research.

Antibacterial activity assay of the nanocomposite

Figs. 3-7 illustrate the antibacterial effects

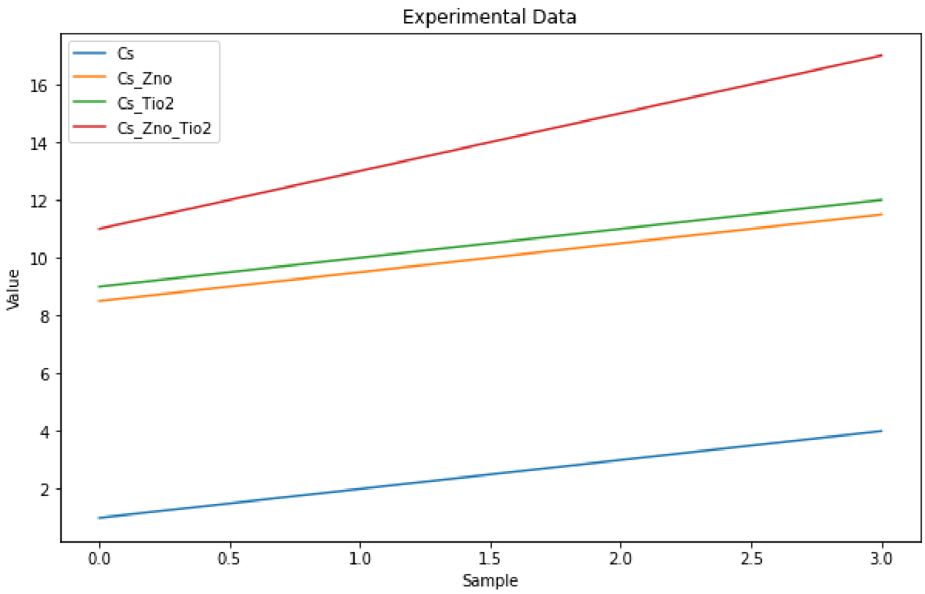


Fig. 4. Statistics analysis for the four Nanomaterials (CS, CS-ZnO, CS-TiO_2 and CS-ZnO-TiO_2) inhibited E. coli bacteria.

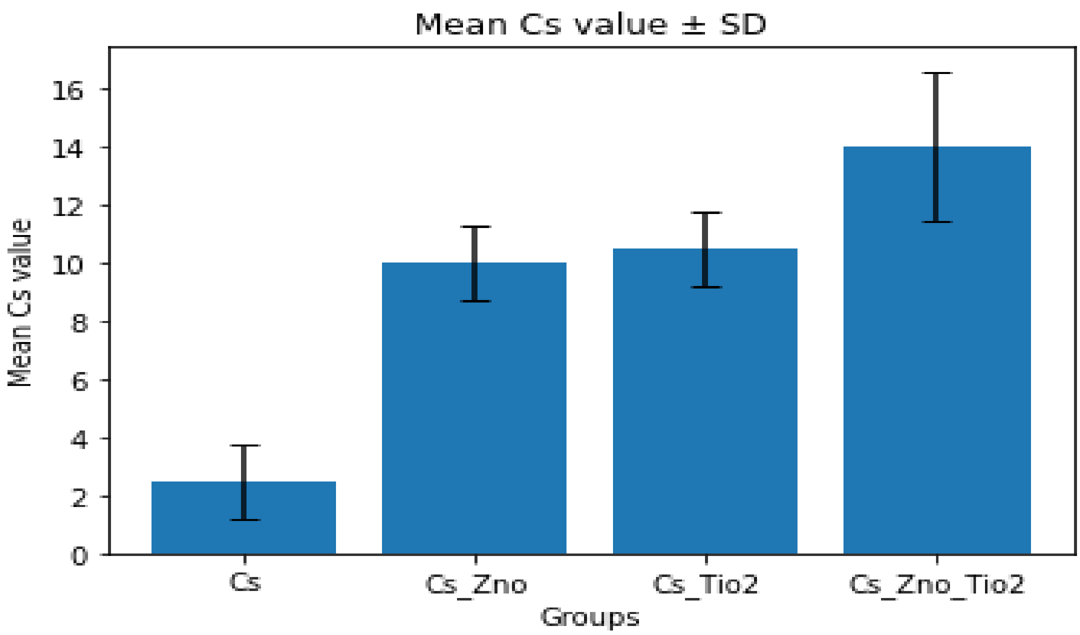


Fig. 5. t-Test analysis presented as the mean ± SD for the four Nanomaterials (CS, CS-ZnO, CS-TiO_2 and CS-ZnO-TiO_2) inhibited E. coli bacteria.

of the synthesized nanomaterials (CS, CS-ZnO, CS-TiO_2, and CS-ZnO-TiO_2) against E. coli and Porphyromonas, respectively. The results demonstrate a concentration-dependent antibacterial activity, with the CS-ZnO-TiO2 nanocomposite exhibiting the most potent inhibition for both bacterial strains [13, 14]. This composite effectively inhibited bacterial growth

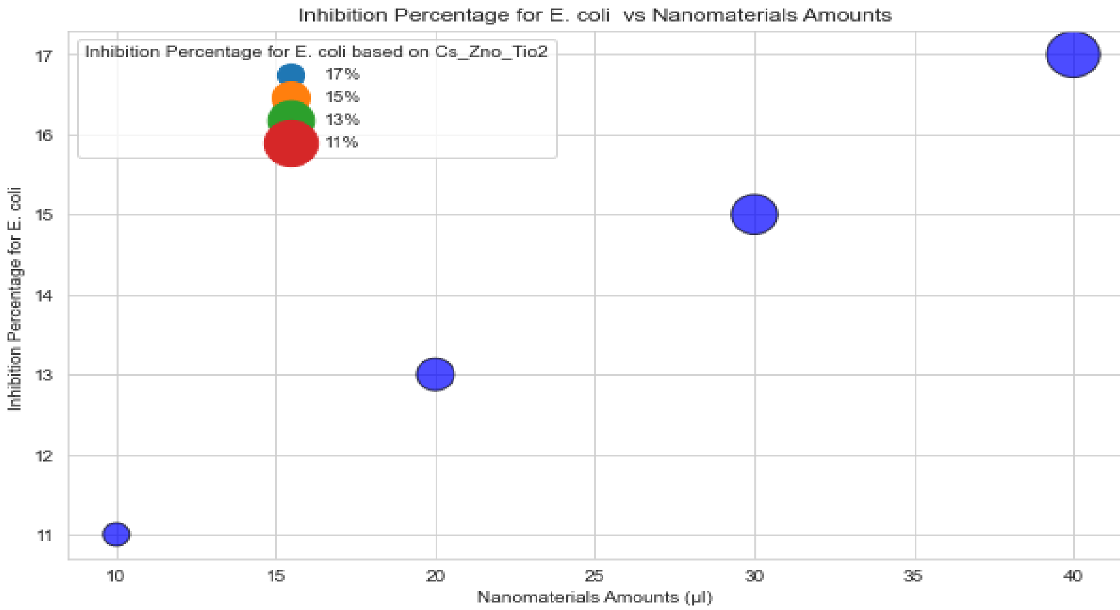


Fig.6. Inhibition Percentage of E. coli bacteria based on CS-ZnO-TiO_2 as a Nanomaterial.

Table 1. The Basic Statistics of the Nanomaterials at different amounts (10, 20, 30, 40) µl which inhibited E. coli bacteria to certain percentages.

Nanomaterial	Count	Mean	Std	Min	25%	50%	75%	Max
CS	4	2.5	1.29	1	1.75	2.5	3.25	4
CS-ZnO	4	10	1.29	8.5	9.25	10	10.75	11.5
CS-TiO_2	4	10.5	1.29	9	9.75	10.5	11.25	12
CS-ZnO-TiO_2	4	14	2.58	11	12.5	14	15.5	17

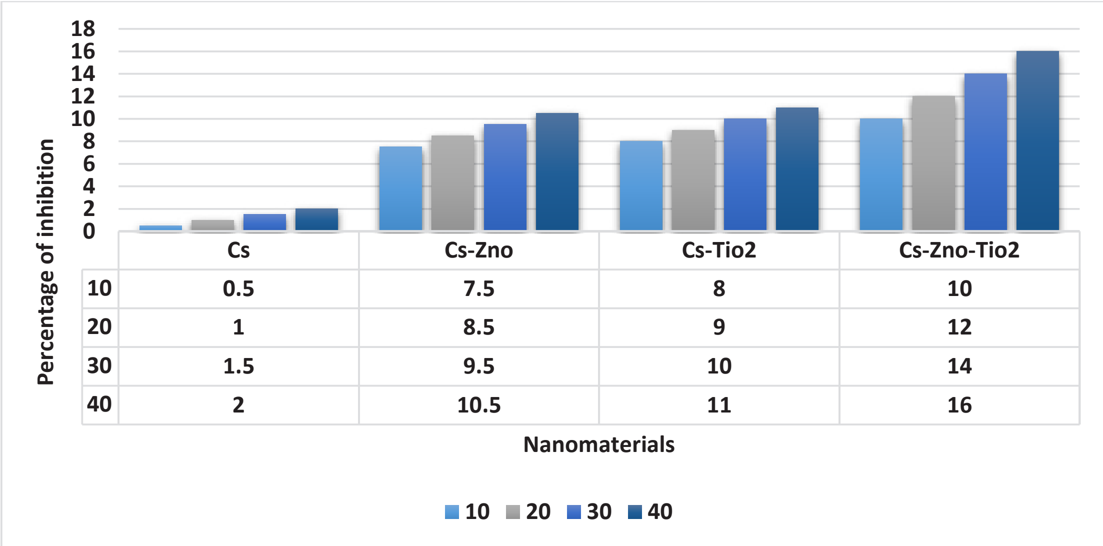
Table 2. The Basic Statistics of the Nanomaterials at different amounts (10, 20, 30, 40) µl which inhibited Porphyromonas to certain percentages.

	CS	CS-ZnO	CS_TiO_2	CS_ZnO_TiO_2
count	4	4	4	4
mean	1.25	9	9.5	13
std	0.645497	1.290994	1.290994	2.581989
min	0.5	7.5	8	10
25%	0.875	8.25	8.75	11.5
50%	1.25	9	9.5	13
75%	1.625	9.75	10.25	14.5
max	2	10.5	11	16

at concentrations as low as 40 µL, suggesting its potential as a broad-spectrum antimicrobial agent. The observed superior performance of the nanocomposite compared to its individual components highlights the synergistic effects of combining chitosan, zinc oxide, and titanium oxide. These findings underscore the potential application of this nanocomposite in addressing various infections caused by both Gram-negative (E. coli) and Gram-positive (Porphyromonas) bacteria [15, 16].

Statistical Analysis of Antimicrobial Activity

To rigorously evaluate the antimicrobial efficacy of the synthesized nanomaterials (CS, CS-ZnO, CS-TiO_2, and CS-ZnO-TiO_2), statistical analysis was



	Cs	Cs-Zno	Cs-Tio2	Cs-Zno-Tio2
10	0.5	7.5	8	10
20	1	8.5	9	12
30	1.5	9.5	10	14
40	2	10.5	11	16

Fig. 7. The Nanomaterials at different amounts (10, 20, 30, 40) µl which inhibited Porphyromonas bacteria to certain percentages.

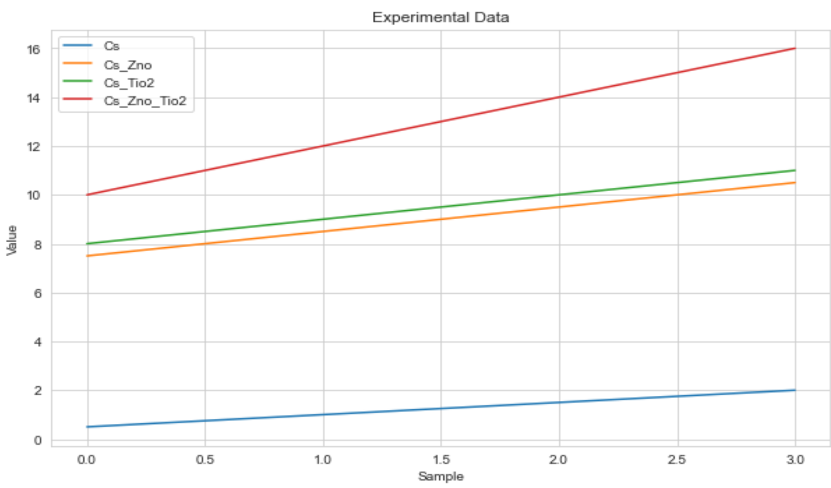


Fig. 8. Statistics analysis for the four Nanomaterials (CS, CS-ZnO- CS-TiO_2 and CS-ZnO-TiO_2) inhibited Porphyromonas.

performed as in Figs. 4, 5, 6, 9 and 10 and Tables 1 and 2. A test revealed significant differences in the mean inhibition percentages among the four nanomaterials ($p < 0.05$). The Cs-ZnO-TiO_2 nanocomposite demonstrated significantly higher inhibition rates compared to the other groups ($p < 0.05$), confirming its superior antibacterial properties.

Correlation analysis between nanomaterial concentration and inhibition percentage indicated a strong positive correlation ($r > 0.9$) for all nanomaterials, suggesting a dose-dependent antibacterial effect. However, the slope of the regression line for CS-ZnO-TiO_2 was significantly steeper than for the other nanomaterials, further emphasizing its potency.

The statistical analysis confirms the visual observations from the figures, demonstrating that the CS-ZnO-TiO_2 nanocomposite exhibits superior antibacterial activity against both E. coli and Porphyromonas compared to the individual components or their binary combinations. The

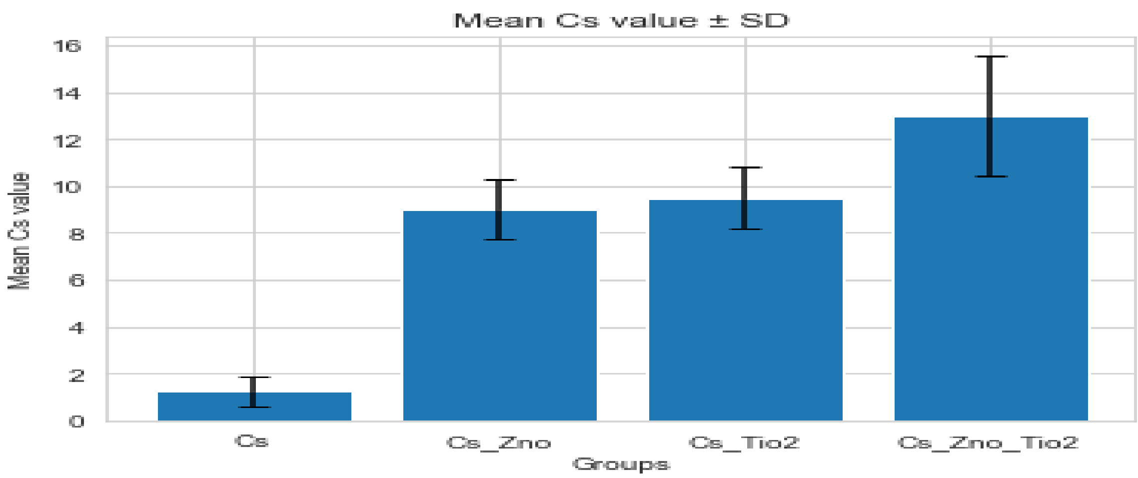


Fig. 9. t-Test analysis presented as the mean ± SD for the four Nanomaterials (CS, Cs-ZnO, CS-TiO_2 and CSs-ZnO-TiO_2) inhibited Porphyromonas.

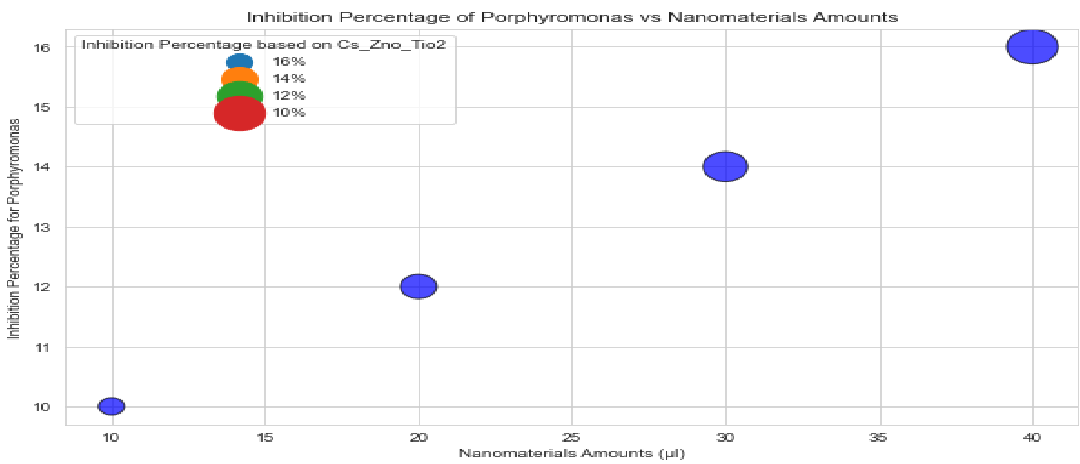


Fig. 10. Inhibition Percentage of Porphyromonas based on CS-ZnO-TiO_2 as a Nanomaterial.

dose-dependent response suggests that increasing the concentration of the nanocomposite can further enhance its inhibitory effect.

These findings highlight the potential of the CS-ZnO-TiO_2 nanocomposite as a promising antimicrobial agent.

CONCLUSION

This study successfully synthesized chitosan-based nanocomposites using the PLAL technique and evaluated their antimicrobial properties against E. coli and Porphyromonas bacteria. The results demonstrated that the CS-ZnO-TiO_2 nanocomposite exhibited the most potent antibacterial activity compared to the individual components or their binary combinations. The observed dose-dependent inhibition and the superior performance of the nanocomposite highlight its potential as a promising antimicrobial agent. Further research should focus on elucidating the underlying mechanisms of antibacterial action, optimizing the synthesis process for enhanced efficacy, and evaluating the safety and biocompatibility of the nanocomposite for potential biomedical applications.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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