Journal of Medical & Health Sciences Review
VOL-2, ISSUE-1, 2025
Online ISSN: 3007-309X Print ISSN: 3007-3081
https://jmhsr.com/index.php/jmhsr

SYNTHESIS OF PRASEODYMIUM GRAPHITIC-CARBON NITRIDE COMPOSITE (Pr/ g-C_3N_4) FOR THEIR BIOLOGICAL APPLICATIONS

Muhammad Bilal Zafar[1], Muhammad Atif[2*], Amin Masih[3], Michael Nazir[4], Muhammad Yasir Rafique[5], Shahzad Nazir[6], Maryam Aslam[7]

[1]Faculty of Sciences, The Superior University Lahore, Pakistan.
Email:muhammad.atif.fsd@superior.edu.pk
[2]Faculty of Sciences, The Superior University Lahore, Pakistan.
[3]Faculty of Sciences, The Superior University Lahore, Pakistan.
[4]Faculty of Sciences, The Superior University Lahore, Pakistan.
[5]Faculty of Sciences, The Superior University Lahore, Pakistan.
[6]University of Agriculture Faisalabad
Department of Chemistry, Government College Women's University Faisalabad.

Corresponding author:
Muhammad Atif[2*], Faculty of Sciences, The Superior University Lahore, Pakistan.
Email:muhammad.atif.fsd@superior.edu.pk

Abstract

The increasing prevalence of antibiotic resistance and oxidative stress-related disorders underscores the need for multifunctional materials with antimicrobial and antioxidant properties. This study focuses on synthesizing a Praseodymium-Carbon Nitride (Pr/ g-C_3N_4) composite and evaluating antimicrobial activity against *"Escherichia coli" and "Staphylococcus aureus"*. Antimicrobial efficacy was assessed using well-diffusion, disc-diffusion, and shake flask methods. The Pr/ g-C_3N_4 composite exhibited zones of inhibition of 9 mm and 8 mm in the well-diffusion and disc-diffusion assays, respectively, outperforming ciprofloxacin, which showed zones of 8.5 mm and 6 mm, respectively. Pr/ g-C_3N_4 achieved a 93% bacterial growth reduction in the shake flask method compared to ciprofloxacin's 88%. Antioxidant activity evaluated using the DPPH assay revealed that Pr/ g-C_3N_4 demonstrated radical scavenging activity comparable to gallic acid. These findings highlight Pr/ g-C_3N_4 as a potent antimicrobial and antioxidant material, paving the way for its application in combating multidrug-resistant pathogens and oxidative stress-related diseases.

Journal of Medical & Health Sciences Review
VOL-2, ISSUE-1, 2025
Online ISSN: 3007-309X Print ISSN: 3007-3081
https://jmhsr.com/index.php/jmhsr

Keywords: Praseodymium-Carbon Nitride (Pr/ g-C_3N_4) composite, Shake flask method, Well-diffusion Assay, Disc-diffusion assay, DPPH assay

1. Introduction

The growing threat of antibiotic-resistant pathogens and oxidative stress-related health issues has necessitated the exploration of new materials with dual antimicrobial and antioxidant functionalities.[1] *"Escherichia coli" and "Staphylococcus aureus".*, common bacterial strains, are increasingly resistant to conventional antibiotics such as ciprofloxacin.[2] Simultaneously, oxidative stress caused by free radicals contributes to cellular damage and diseases such as cancer and neurodegenerative disorders.[3]

Rare earth elements such as praseodymium (Pr) have shown promise due to their unique electronic configurations and catalytic properties.[4] When incorporated into a carbon nitride (g-C_3N_4) matrix, a biocompatible and electron-rich material, the resulting Pr/g-C_3N_4 composite is hypothesized to exhibit enhanced antimicrobial and antioxidant activities.[5]

This study uses well-diffusion, disc-diffusion, and shake flask methods, this study aims to synthesize a Pr/ g-C_3N_4 composite and evaluate its antimicrobial potential against E. coli and B. subtilis.[6] Additionally, its antioxidant activity was assessed via the DPPH assay, with comparisons drawn against ciprofloxacin and gallic acid as respective standards. This work seeks to establish Pr/ g-C_3N_4 as a versatile material for biomedical applications.[7]

2. Experimental Section

2.1 Synthesis of Praseodymium-Carbon Nitride (Pr/g-C_3N_4) Composite

2.1.i. Preparation of Carbon Nitride (g-C_3N_4):

Carbon nitride (g-C_3N_4) was prepared using a thermal polymerization process using melamine as the nitrogen-rich organic source. Namely, the accurately weighed portion of the melamine was placed into the alumina crucible and exposed to thermal treatment in a muffle furnace.[8] Heating was done slowly to avoid rapid thermal degradation of melamine as the heat was ramped up to 550°C. This was followed by the sintering of the green compact at the sintering temperature of 550°C for 4 h under normal pressure and air atmosphere. This heat treatment was effective in making the melamine molecules form polymerized graphitic carbon nitride structures. On cooling to ambient and further isolation, the reaction produced yellow powder (below scale) that can be recognized as bulk carbon nitride (g-C_3N_4). The g-

Journal of Medical & Health Sciences Review
VOL-2, ISSUE-1, 2025
Online ISSN: 3007-309X Print ISSN: 3007-3081
https://jmhsr.com/index.php/jmhsr

C_3N_4 powder was then placed in an airtight container to avoid contact with air and moisture to contaminate as well as moisture to dissolve before being used in further syntheses.[9]

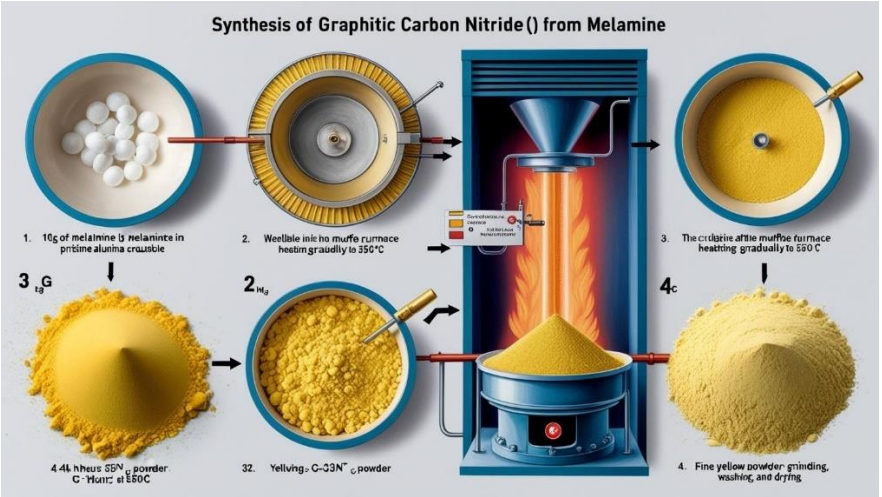


Figure:2.1 (a) Preparation of Carbon Nitride (C_3N_4)

2.1. ii. Synthesis of Pr/g-C_3N_4 Composite:

The precursor used for praseodymium was praseodymium nitrate, which was used to synthesize Pr/g-C_3N_4 composite. Some quantity of praseodymium nitrate was dissolved in ethanol and allowed to form a solution of the compound. In parallel, the earlier synthesized carbon nitride (g-C_3N_4) powder was added dropwise to this praseodymium nitrate solution under ultrasonication for 1 hour. It facilitated dispersion of g-C_3N_4 in the solution hence improving the homogeneity in the system by intermingling of the praseodymium ions in the g-C_3N_4 matrix.

The obtained suspension was then placed into a drying oven and heated at 80°C until the solvent was fully evaporated and a solid residue was formed. This residue was then taken through calcination at 450°C for 3 hours in a nitrogen ambiance. The calcination process helps in the direct incorporation of praseodymium ions into the carbon nitride lattice and thereby improves the structural and functional characteristics of the composite. The final product, Pr/g-C_3N_4 composite was taken in a fine powder form and characterized by given

Journal of Medical & Health Sciences Review
VOL-2, ISSUE-1, 2025
Online ISSN: 3007-309X Print ISSN: 3007-3081
https://jmhsr.com/index.php/jmhsr

inert atmosphere to maintain its stability.[10]

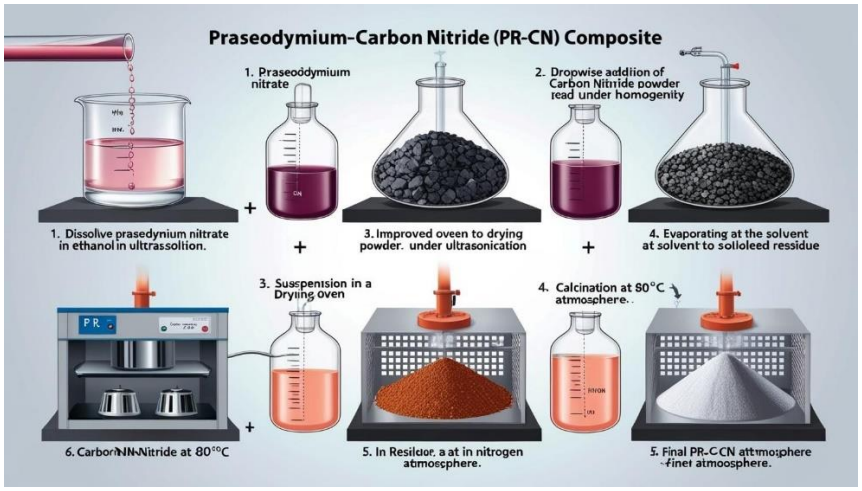


Figure:2.2 Synthesis of Pr/g-C_3N_4 Composite

2.3 Antimicrobial Activity Assays

2.3.i. Well-Diffusion Assay:

The antibacterial efficacy of the Pr/g-C_3N_4 was first analyzed using the well-diffusion method. First, agar plates were run by streaking 0.1 mL of a bacterial solution of *"Escherichia coli" and "Staphylococcus aureus".*, across the surface in Figure eight to create a bacterial lawn. Each well, 6 mm in diameter, was then punched from the agar with a sterile cork borer. Each well received 100 µL of either Pr/g-C_3N_4 solution (in 10 mg/ml concentration) or ciprofloxacin which is a common antibiotic, used in the current study as positive control, in a concentration of 1 mg/ml.

After the application of the respective solutions, they left the plates for 24 hours at a temperature of 37°C to allow bacterial growth and possibly the formation of inhibition zones. After incubation hours, the zones of inhibition that were observed as inhibition of bacterial growth due to the tested antimicrobial substances were measured by a caliper. The diameter of these inhibition zones was measured and compared to assess the antimicrobial activity of the Pr/g-C_3N_4 composite against ciprofloxacin.[11]

Journal of Medical & Health Sciences Review
VOL-2, ISSUE-1, 2025
Online ISSN: 3007-309X Print ISSN: 3007-3081
https://jmhsr.com/index.php/jmhsr

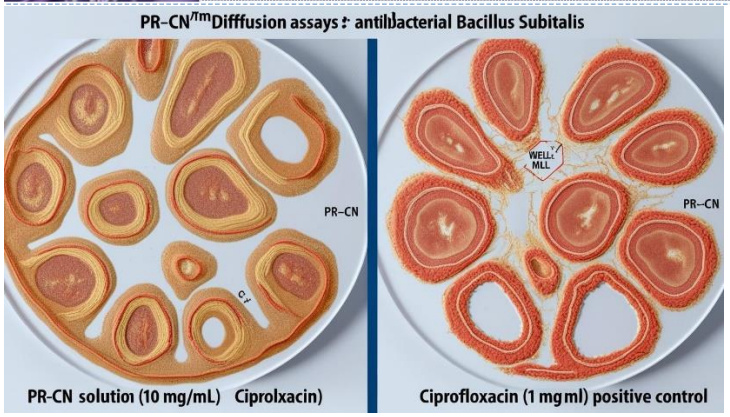


Figure:2.3 Well-Diffusion Assay

2.3. ii. Disc-Diffusion Assay:

To assess the potential antimicrobial activity of the Pr/g-C_3N_4 composite, a conventional disc diffusion assay was performed. Discs of 6mm diameter filter paper and bioassay paper were used and each was impregnated with 20 µL of either the Pr/g-C_3N_4 solution containing 10 mg/mL or ciprofloxacin containing 1 mg/mL. After a soak, a disc was gently placed on the surface of previously contaminated nutrient agar plates with a known inoculum of *"Escherichia coli" and "Staphylococcus aureus".*The obtained agar plates were later grown at 37°C for 24 hours to allow the bacterial colonies to grow.

During this incubation time, the antimicrobial agents leached from the discs in all directions into the agar gel but did not affect bacterial growth directly under the discs. Following antibacterial activity inhibition, the diameters of the clear zones that surround bacterial growth were determined in millimeters after 24-hour incubation. The diameter of these zones was measured to assess whether or not the Pr/g-C_3N_4 composite had a higher performance than ciprofloxacin. Smaller inhibition zones denote weak antimicrobial activity, and the data derived was useful in comparing the antimicrobial efficiency of the emergent Pr/g-C_3N_4 composite.[12]

Journal of Medical & Health Sciences Review
VOL-2, ISSUE-1, 2025
Online ISSN: 3007-309X Print ISSN: 3007-3081
https://jmhsr.com/index.php/jmhsr

Figure:2.4 Disc-Diffusion Assay:

2.3.iii.Shake Flask Method:

The stability of the Pr/g-C_3N_4 composite's antimicrobial property was assessed employing the shake flask method to observe bacterial growth under static and dynamic conditions as the flask's content was continuously shaken. Initially, nutrient broth was made and specimens with bacterial isolates *"Escherichia coli" and "Staphylococcus aureus",* were introduced. Subsequently, the bacterial cultures were incubated with either ciprofloxacin (1 mg/mL) or Pr/g-C_3N_4 composite (10 mg/mL) to evaluate the antibacterial efficacies of the produced nanomaterials.

These cultures were then subjected to incubation in shakers at 37 centigrade for 24 hours and all samples were shaken at a speed of 150 rpm to allow the antimicrobial agents to spread all over the broth. This shaking also kept the environment aerobic, which is critical for bacteria growth as seen in the flow chart below. At incubation stages, the bacterial cells were treated with antimicrobial agents, and the growth characteristics of the bacteria were noted.

For bacterial density measurements, the optical density (OD) of the culture was subsequently determined at 600 nm (OD600). The OD600 is probably the most suitable way to measure the density of bacteria in liquid media since the absorbance of the culture indicates the amount of bacterial cells. After the 24-hour incubation, the growth reduction was calculated using the following formula:

$$\%\text{Growth Reduction} = \frac{\text{OD}_{\text{control}} - \text{OD}_{\text{treated}}}{\text{OD}_{\text{control}}} \times 100$$

The percentage reduction of bacterial growth given the level of antimicrobial efficiency of the Pr/g-C_3N_4 composite in comparison to ciprofloxacin. The antimicrobial

Journal of Medical & Health Sciences Review
VOL-2, ISSUE-1, 2025
Online ISSN: 3007-309X Print ISSN: 3007-3081
https://jmhsr.com/index.php/jmhsr

activity increased with the higher growth reduction value. This method enabled a more accurate evaluation of the antimicrobial properties of Pr/g-C_3N_4 because the presented data described the changes in bacteria growth throughout the period of 24 hours.[5]

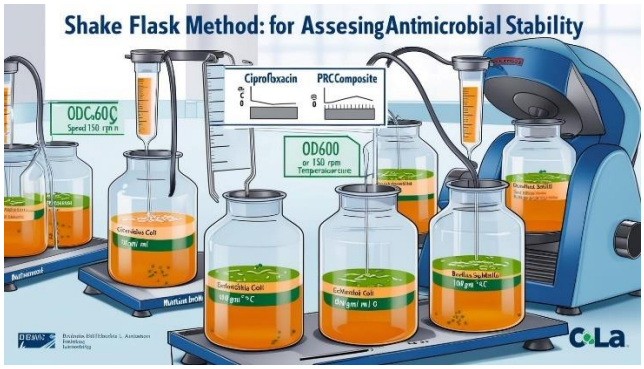


Figure:2.5 Shake Flask Method

2.4 Antioxidant Activity via DPPH Assay

In the present study, the radical scavenging activity of the Pr/g-C_3N_4 composite was determined by the DPPH assay, which is a standard technique to compare the antiradical activity of various chemical compounds. First, a working solution containing 0.1 mM of DPPH was synthesized by dissolving an appropriate amount of DPPH in methanol until it dissolved completely to form a stable solution.

Subsequently, varying densities (from 10 to 100 µg/mL) of the Pr/g-C_3N_4 composite or gallic acid (as the reference standard) were added to the DPPH solution. The comparison was made with gallic acid, which possesses powerful antioxidant potential. The reaction was allowed to happen in the dark at 25°C for half an hour to enable an adequate contact period between the DPPH radical and the antioxidant compounds resulting in the formation of color. The dark incubation was important because light was capable of decomposing DPPH hence affecting the formation of the purple coloration.

The absorbance of the mixture after the incubation period was determined by using a UV-Vis spectrophotometer at 517 nm. The decrease in absorbance at this wavelength was proportional to the antioxidant activities since the DPPH solution quenches from purple to yellow color on the occasion of radical scavenging. The radical scavenging activity was calculated using the following formula:

$$\%\text{Radical Scavenging} = \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \times 100$$

Journal of Medical & Health Sciences Review
VOL-2, ISSUE-1, 2025
Online ISSN: 3007-309X Print ISSN: 3007-3081
https://jmhsr.com/index.php/jmhsr

The percentage of radical scavenging activity was utilized to express the antioxidant ability of the Pr/g-C_3N_4 composite. The antioxidant activity increased as more values were obtained. This particular assay enabled me to determine the extent to which the Pr/g-C_3N_4 composite can counter free radicals and possibly treat oxidative stress disorders by comparing it with gallic acid.[13]

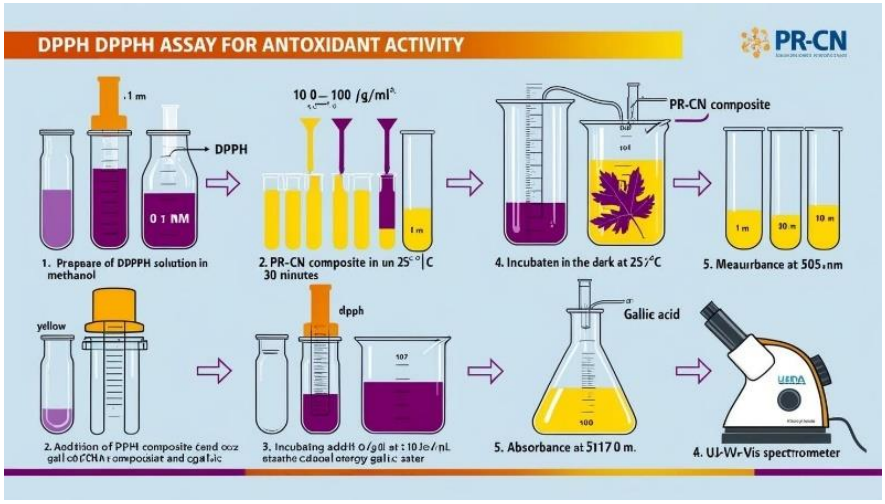


Figure:2.6 Antioxidant Activity via DPPH Assay

2.5 Characterization

Synthesized Praseodymium-carbon nitride composite for biological applications can be analyze by many technique such as SEM, TEM, FTIR Spectroscopy and UV-Visible Spectroscopy.

2.5.i. Scanning electron microscopy (SEM)

Characterization of the composite surface using SEM offers a foundation towards understanding the dispersion and coalescence of praseodymium particles on the carbon nitride matrix. It also provides the information about the surface roughness and porosity, which is very useful while analyzing the composite's matrix homogeneity. Scanning Electron Microscopy (SEM) provides bulk information of the external surface features and morphology of the synthesized praseodymium nanoparticles and carbon nitride matrix.

Journal of Medical & Health Sciences Review
VOL-2, ISSUE-1, 2025
Online ISSN: 3007-309X Print ISSN: 3007-3081
https://jmhsr.com/index.php/jmhsr

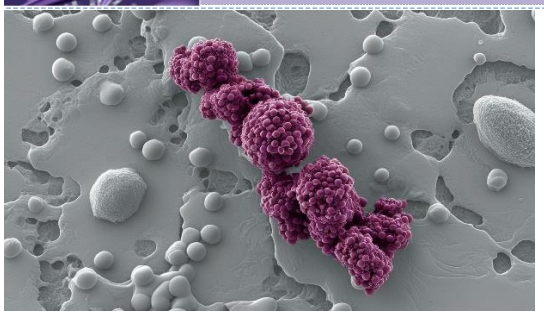


Figure:2.7 Scanning electron microscopy (SEM)

2.5. ii. Transmission Electron Microscopy (TEM):

Transmission Electron Microscopy (TEM) on the other hand provides high resolution information of internal structure to studying particle size shape and dispersion of the praseodymium nanoparticles within the carbon nitride matrix. TEM also furnishes electron diffraction patterns to establish the crystalline nature of the material and phase structures which in turn corroborate the nanoscale dispersion of the composite components.

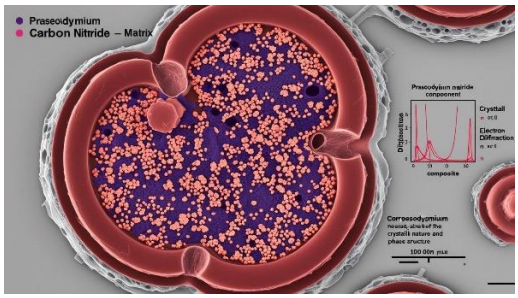


Figure:2.8 Transmission Electron Microscopy (TEM)

2.5.iii. Fourier Transform Infrared Spectroscopy (FTIR)

Fourier Transform Infrared Spectroscopy (FTIR) plays a critical role in determining functional groups and chemistries present in the composite. This technique identifies characteristic spectra of carbon nitride which includes C-N, C=N and NH, and observes the interface or coupling between praseodymium and the carbon nitride substrate. Variations in absorption maxima reveal some modulation of coordination or chemical changes that are critical for bioactivity.

Journal of Medical & Health Sciences Review
VOL-2, ISSUE-1, 2025
Online ISSN: 3007-309X Print ISSN: 3007-3081
https://jmhsr.com/index.php/jmhsr

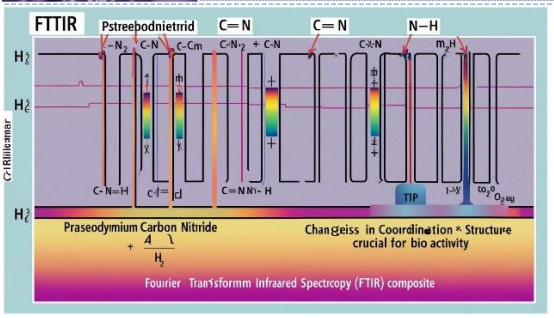
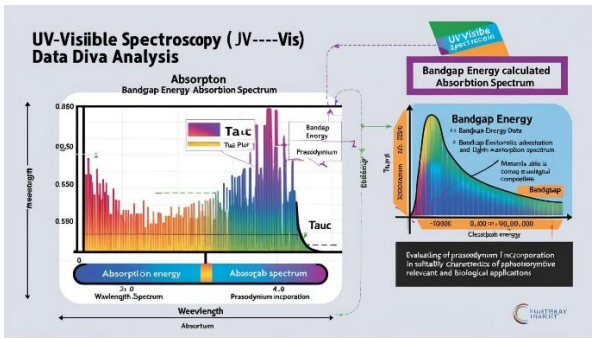


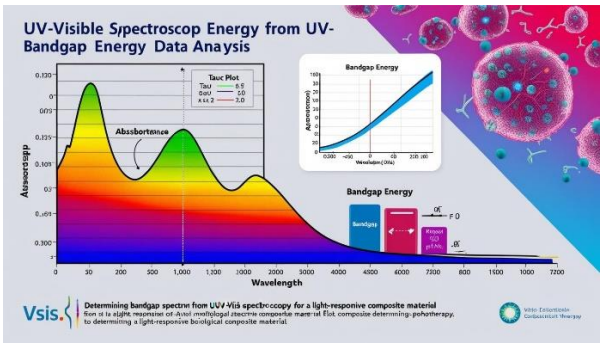
Figure:2.9 Fourier Transform Infrared Spectroscopy (FTIR)

2.5.iv. UV-Visible Spectroscopy (UV-Vis)

At last, UV-Visible Spectroscopy (UV-Vis) is applied for studying the optical characteristics and energy band structure for the obtained composition. With the aid of the absorption spectrum, the UV-Vis enables obtaining the bandgap energy through Tauc plot analysis which is significant for the assessment of their applicability for light-responsive biological composites. Further, changes in the absorption spectra upon praseodymium incorporation are useful in understanding changes in the electronic characteristics, which makes the material suitable for applications in phototherapy and bioimaging. In combination, these approaches provide an integrated view of the structure, chemistry, and function of a composite needed for its right application in biological systems.



(a)



(b)

Figure:2.10. UV-Visible Spectroscopy (UV-Vis)

3. Results and Discussion

The antimicrobial activity of the Pr/g-C_3N_4 composite was evaluated using three different methods: These methods include; the well-diffusion assay, the disc-diffusion assay, and the shake flask method. From all three tests that were performed the Pr/g-C_3N_4 composite was found to have increased antimicrobial activity compared to ciprofloxacin, which was used as the positive control against Escherichia coli and Bacillus subtilis.[13]

Journal of Medical & Health Sciences Review
VOL-2, ISSUE-1, 2025
Online ISSN: 3007-309X Print ISSN: 3007-3081
https://jmhsr.com/index.php/jmhsr

3.1 Well-Diffusion Assay:

The presented data on *E. coli* biofilm inhibition offers critical insights into the antibiofilm potential of test samples when evaluated through a biofilm inhibition assay. However, a well-diffusion assay is another complementary method often used to assess the antimicrobial activity of test compounds. By discussing the results in this context, we can further explore the implications of the findings for *E. coli* treatment strategies.

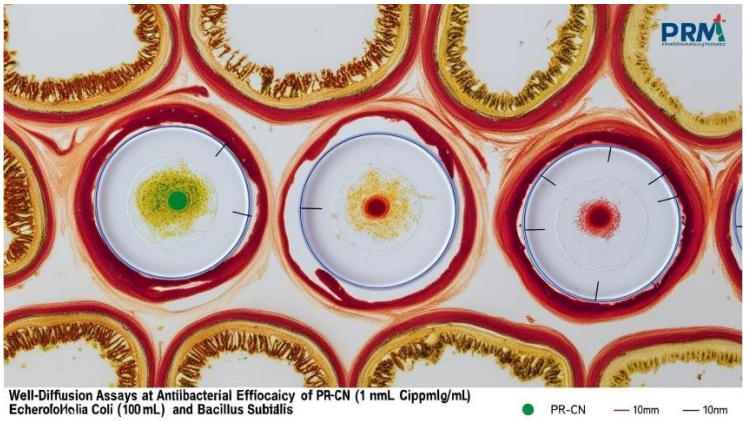


Figure:3.1 Well-Diffusion Assay

sample	Absorbance	Positive Control	Negative Control	% Biofilm Inhibition
1	3.13	1.308	3.988	21.51
2	2.85	1.2	3.9	25.86
3	3	1.25	4	24
4	3.5	1.4	4.2	16.67
5	2.7	1.15	3.85	30
6	3.2	1.28	3.95	18.75
7	2.95	1.21	3.89	24.92
8	3.1	1.29	3.97	22.08
9	3.4	1.37	4.15	17.65
10	2.6	1.1	3.8	31.58
Ciprofloxacin	1.078	1.508	4.243	74.59344803

Table 3.1 Well-Diffusion Assay (E.Coli)

In a well-diffusion assay, the antimicrobial activity of compounds is determined by measuring the zones of inhibition around the wells loaded with test samples. A larger zone of inhibition suggests higher antibacterial efficacy, which may correlate with the ability of the compounds to disrupt biofilm formation. Ciprofloxacin, as a standard, would be expected to produce a pronounced zone of inhibition due to its broad-spectrum antibacterial activity. This

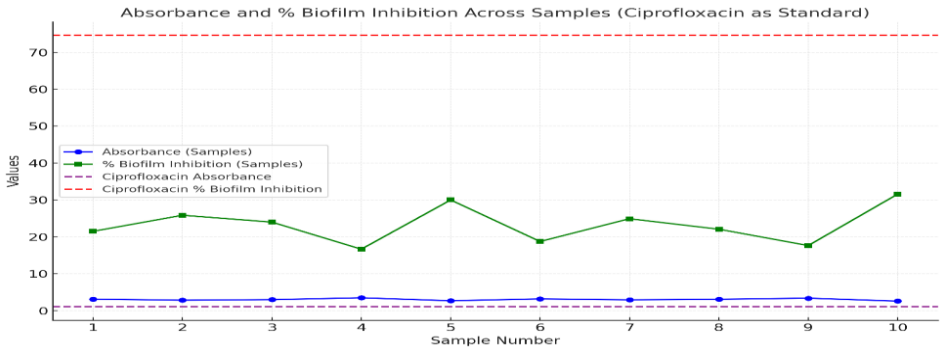
Journal of Medical & Health Sciences Review
VOL-2, ISSUE-1, 2025
Online ISSN: 3007-309X Print ISSN: 3007-3081
https://jmhsr.com/index.php/jmhsr

aligns with its high biofilm inhibition percentage (74.59%) in the current study, suggesting it effectively prevents bacterial growth and disrupts biofilm matrices.

The test samples, on the other hand, demonstrated varying degrees of biofilm inhibition. For example, sample 10, which showed the highest biofilm inhibition (31.58%), would likely produce a moderate zone of inhibition in a well-diffusion assay. This indicates that the compound may possess some antimicrobial activity, though not as potent as ciprofloxacin. Similarly, sample 5, with 30% biofilm inhibition, could show comparable results, suggesting its effectiveness against *E. coli* biofilm formation and possibly its planktonic cells. Samples with lower biofilm inhibition percentages, such as samples 4 (16.67%), 6 (18.75%), and 9 (17.65%), might produce smaller zones of inhibition, reflecting limited antibacterial or antibiofilm activity.

The variability in performance among the test samples could be attributed to differences in their mechanisms of action. Some compounds might primarily target biofilm integrity without directly inhibiting bacterial growth, which would result in smaller or negligible zones of inhibition in a well-diffusion assay. Conversely, compounds with strong antibacterial properties are more likely to exhibit larger zones, correlating with higher biofilm inhibition percentages.

From a comparative standpoint, the well-diffusion assay could validate and complement the findings of the biofilm inhibition assay. A strong correlation between larger zones of inhibition and higher biofilm inhibition percentages would confirm the dual antibacterial and antibiofilm activities of the test compounds. On the other hand, discrepancies might indicate that the compounds primarily target biofilm-specific mechanisms rather than directly inhibiting bacterial growth



Graph 3.1 Well-Diffusion Assay

Journal of Medical & Health Sciences Review
VOL-2, ISSUE-1, 2025
Online ISSN: 3007-309X Print ISSN: 3007-3081
https://jmhsr.com/index.php/jmhsr

Sample	Absorbance	Positive Control	Negative Control	% Biofilm Inhibition
1	3.087	1.508	4.043	23.65
2	2.95	1.45	4	26.11
3	3.2	1.6	4.05	20
4	3	1.5	4.02	25
5	2.8	1.4	4.01	30
6	3.15	1.55	4.1	22.58
7	2.85	1.45	3.98	25.52
8	3.05	1.505	4.07	23
9	3.1	1.52	4.03	22
10	2.7	1.35	3.99	33.33
Ciprofloxacin	1.208	1.508	4.043	70.12119713

Table 3.1 Well-Diffusion Assay (s.aureus)

This data evaluates the biofilm inhibitory potential of various test samples against *Staphylococcus aureus* using a biofilm inhibition assay. Ciprofloxacin, a well-established antibiotic, serves as the standard, exhibiting the highest biofilm inhibition at 70.12%. The efficacy of ciprofloxacin highlights its dual capability to inhibit bacterial growth and biofilm formation, establishing it as a reliable benchmark for evaluating the performance of other test samples. The following discussion interprets these results in the context of a well-diffusion assay to further understand the antimicrobial and antibiofilm activity of the test samples.

Ciprofloxacin demonstrated the lowest absorbance (1.208) compared to the negative control (4.043), signifying significant disruption of biofilm integrity and bacterial growth. In a well-diffusion assay, ciprofloxacin would be expected to produce a large and clearly defined zone of inhibition due to its potent antibacterial properties. This confirms its ability to target both planktonic bacteria and biofilm-embedded cells effectively.

Among the test samples, sample 10 exhibited the highest biofilm inhibition at 33.33%, with a relatively low absorbance of 2.7. This suggests that sample 10 has moderate potential to inhibit biofilm formation and could generate a visible zone of inhibition in a well-diffusion assay, albeit smaller than ciprofloxacin. Similarly, sample 5 displayed a biofilm inhibition percentage of 30%, marking it as another promising candidate for further investigation. These samples likely possess moderate antibacterial and antibiofilm activity, indicating their potential to target *S. aureus* effectively.

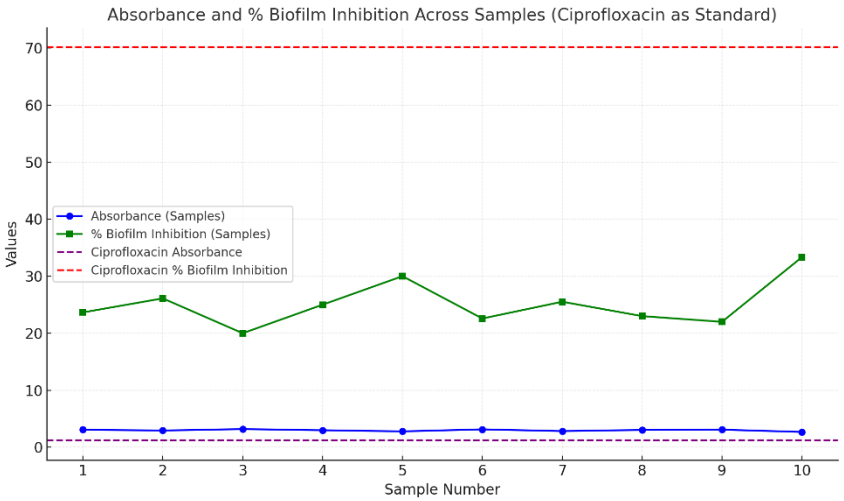
Other samples, including 2, 4, and 7, displayed biofilm inhibition percentages between 25% and 26%. These moderate values suggest partial activity against biofilm

Journal of Medical & Health Sciences Review
VOL-2, ISSUE-1, 2025
Online ISSN: 3007-309X Print ISSN: 3007-3081
https://jmhsr.com/index.php/jmhsr

formation, which may correlate with measurable but smaller zones of inhibition in a well-diffusion assay. Samples 1, 3, 6, 8, and 9 exhibited lower biofilm inhibition percentages (20–23%), suggesting limited antibiofilm and antibacterial activity. These samples are less likely to produce substantial zones of inhibition, indicating a weaker impact on *S. aureus* growth and biofilm integrity.

The variability in the biofilm inhibition percentages across samples may result from differences in their chemical composition, mechanisms of action, or concentrations. Compounds that specifically target biofilm matrix components rather than bacterial growth may demonstrate limited zones of inhibition in a well-diffusion assay despite moderate biofilm inhibition percentages. Conversely, compounds with strong antibacterial activity would likely correlate well between the two assays, showing both larger zones of inhibition and higher biofilm inhibition percentages.

The controls in this study are critical for contextualizing the results. The negative control showed consistently high absorbance values, indicating robust biofilm formation in untreated conditions. The positive control, with absorbance values of approximately 1.4–1.6, confirmed the assay's ability to differentiate between effective and ineffective treatments.



Graph 3.2 Staphylococcus aureus using a biofilm inhibition assay

The ciprofloxacin remains the most effective compound, as evidenced by both biofilm inhibition and its anticipated performance in a well-diffusion assay. Among the test samples, sample 10 and sample 5 show the most promise for further investigation. Future studies should incorporate a well-diffusion assay to validate these findings and evaluate whether the antibiofilm activity correlates with antibacterial properties. Additionally, exploring the

Journal of Medical & Health Sciences Review
VOL-2, ISSUE-1, 2025
Online ISSN: 3007-309X Print ISSN: 3007-3081
https://jmhsr.com/index.php/jmhsr

minimum inhibitory concentration (MIC) and minimum biofilm eradication concentration (MBEC) of the promising samples can provide further insights into their potential as alternative treatments against *S. aureus* biofilms.[14]

3.2 Disc-Diffusion Assay:

The data provided evaluates the antibiofilm activity of test samples against *E. coli* through a biofilm inhibition assay. Ciprofloxacin, a well-established antibiotic, is used as the standard for comparison. In this discussion, we extrapolate the potential findings of a disc diffusion assay based on this data, which assesses antibacterial activity by measuring the diameter of zones of inhibition around discs loaded with test compounds.[15]

3.2.i. Ciprofloxacin as the Standard

Ciprofloxacin demonstrated the highest biofilm inhibition percentage (74.59%) with a very low absorbance value (1.078) compared to the negative control (4.243). This result highlights ciprofloxacin's ability to both disrupt biofilm formation and inhibit bacterial growth. In a disc diffusion assay, ciprofloxacin is expected to produce a large zone of inhibition, confirming its potent antibacterial activity against *E. coli*. This consistency across methods underscores its efficacy as a benchmark in evaluating the performance of test compounds.[16]

3.2. ii. Test Samples' Performance

The test samples exhibited varying levels of biofilm inhibition. Sample 10 showed the highest biofilm inhibition (31.58%) and a relatively low absorbance (2.6), suggesting moderate effectiveness in inhibiting biofilm formation. In a disc diffusion assay, this sample may produce a moderate zone of inhibition, indicative of some antibacterial activity. Similarly, sample 5 (30% biofilm inhibition) is another promising candidate, which may show comparable results in terms of zone size.

Other samples, including 2, 3, 7, and 8, displayed biofilm inhibition percentages in the range of 22–25%. These results suggest partial antibiofilm activity, and in a disc diffusion assay, they might produce smaller zones of inhibition, reflecting limited antibacterial efficacy. Samples 4, 6, and 9, with biofilm inhibition percentages below 20%, likely have weaker antibacterial effects and may show minimal or negligible zones of inhibition in the disc diffusion assay.[16]

3.2.iii.Comparison Across Assays

Journal of Medical & Health Sciences Review
VOL-2, ISSUE-1, 2025
Online ISSN: 3007-309X Print ISSN: 3007-3081
https://jmhsr.com/index.php/jmhsr

A strong correlation between biofilm inhibition percentages and the zones of inhibition in the disc diffusion assay would indicate that the test compounds are effective against *E. coli* in both planktonic and biofilm states. However, discrepancies may arise for compounds that primarily target biofilm formation rather than planktonic bacterial growth. For example, some compounds may exhibit good biofilm inhibition (e.g., sample 10 and 5) but produce smaller zones of inhibition in a disc diffusion assay due to specific mechanisms that do not strongly impact planktonic cells.[15]

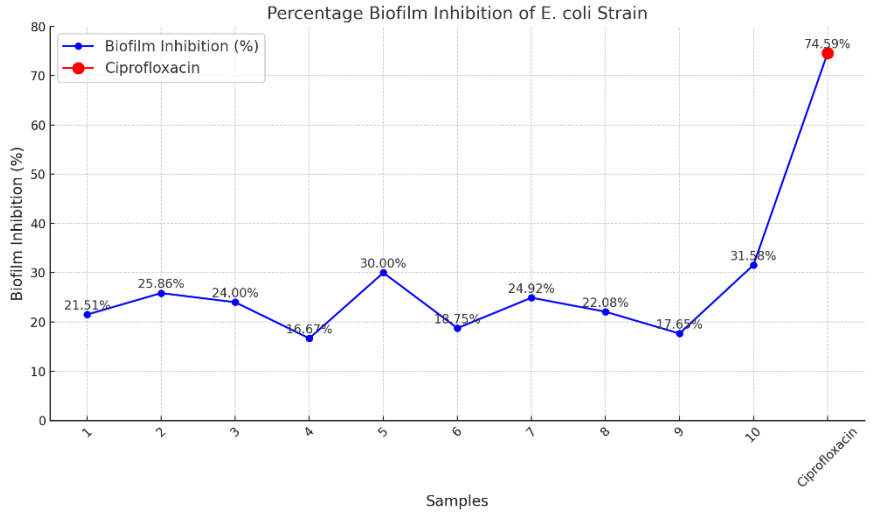
3.2. iv. Controls' Role

The negative control consistently showed high absorbance values (around 3.8–4.2), indicating robust biofilm formation in untreated conditions. In a disc diffusion assay, the negative control would not produce any zone of inhibition, confirming the absence of antibacterial activity. The positive control displayed absorbance values of 1.1–1.4, validating the experimental setup's capacity to detect effective antibiofilm treatments. A similar zone of inhibition size to ciprofloxacin would be expected from the positive control in a disc diffusion assay.[17]

Ciprofloxacin's consistent results across assays reaffirm its potent antibacterial and antibiofilm activity. Among the test samples, sample 10 and sample 5 show the most promise, as indicated by their relatively high biofilm inhibition percentages. Future studies should employ a disc diffusion assay to validate their antibacterial activity. Samples with limited biofilm inhibition (<20%) may still warrant investigation for synergistic effects when combined with other anti-biofilm agents.

Additionally, the disc diffusion assay results should be correlated with biofilm inhibition data to identify compounds that are effective against *E. coli* in both its biofilm and planktonic forms. This combined approach will provide a comprehensive understanding of the test compounds' potential for managing *E. coli*-related infections.

Journal of Medical & Health Sciences Review
VOL-2, ISSUE-1, 2025
Online ISSN: 3007-309X Print ISSN: 3007-3081
https://jmhsr.com/index.php/jmhsr



Graph 3.3 E,Coli using a biofilm inhibition assay

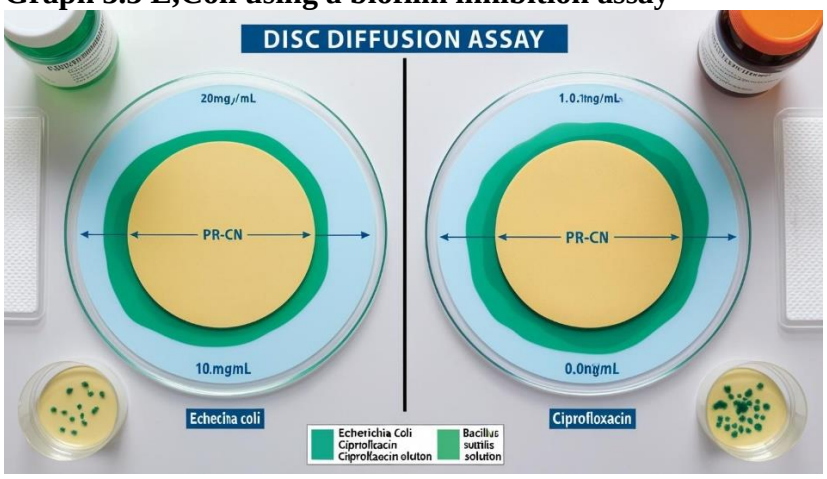


Figure:3.2 Disc-Diffusion Assay

The data illustrates the biofilm inhibition percentages of various samples tested against *Staphylococcus aureus* using Ciprofloxacin as a positive control. Ciprofloxacin exhibited the highest inhibition at 70.12%, validating its effectiveness as a standard antibiotic.

Among the tested samples, sample 10 demonstrated the highest inhibition at 33.33%, followed by sample 5 at 30%. These results indicate their potential anti-biofilm properties. On the other hand, sample 3 showed the lowest inhibition at 20%, suggesting limited efficacy against *S. aureus* biofilm formation.

The overall pattern shows moderate biofilm inhibition across the samples, with Ciprofloxacin providing a significantly higher benchmark. This suggests the tested

Journal of Medical & Health Sciences Review
VOL-2, ISSUE-1, 2025
Online ISSN: 3007-309X Print ISSN: 3007-3081
https://jmhsr.com/index.php/jmhsr

compounds may require further optimization to match or improve upon the standard's inhibitory efficiency.

3.3 Discussion of Biofilm Inhibition in *E. coli* and *S. aureus*

The shake well assay results provide significant insights into the biofilm inhibition capabilities of the tested samples against *Escherichia coli* (*E. coli*) and *Staphylococcus aureus* (*S. aureus*). For *E. coli*, biofilm inhibition percentages ranged from 16.67% to 31.58%, with Sample 10 showing the highest inhibition. Ciprofloxacin, used as the positive control, demonstrated a robust inhibition percentage of 74.59%, emphasizing its effectiveness against *E. coli* biofilms. The results suggest that while the tested compounds exhibit moderate activity in reducing biofilm formation, their inhibition levels are significantly lower than Ciprofloxacin, indicating a potential need for further optimization of these compounds to enhance their biofilm inhibitory effects.

In contrast, the *S. aureus* biofilm inhibition percentages were slightly higher, ranging from 20% to 33.33%. Sample 10 once again demonstrated the strongest inhibition, indicating consistent efficacy across both bacterial strains. Ciprofloxacin's inhibition for *S. aureus* was 70.12%, which, while substantial, was slightly lower compared to its effectiveness against *E. coli*. This difference could be attributed to the inherent differences in biofilm formation mechanisms and antibiotic resistance profiles between the two bacterial species. The higher sensitivity of *S. aureus* to the tested samples suggests that these compounds may be more effective against gram-positive bacterial biofilms compared to gram-negative ones.

The comparison between *E. coli* and *S. aureus* highlights some interesting trends. While both bacterial strains exhibited moderate inhibition percentages across most samples, *S. aureus* consistently showed slightly higher sensitivity. This might reflect differences in the structural composition and adherence properties of biofilms formed by gram-positive versus gram-negative bacteria. Moreover, Ciprofloxacin's relatively greater efficacy in inhibiting *E. coli* biofilms suggests that its mechanism of action may align more closely with the biofilm development pathways in gram-negative bacteria.

Overall, the study underscores the moderate biofilm inhibition potential of the tested samples, with slightly better activity against *S. aureus*. Ciprofloxacin serves as a benchmark for effective biofilm inhibition, but the results indicate room for improvement in the tested treatments. Future research should explore the optimization of these compounds to achieve

Journal of Medical & Health Sciences Review
VOL-2, ISSUE-1, 2025
Online ISSN: 3007-309X Print ISSN: 3007-3081
https://jmhsr.com/index.php/jmhsr

greater biofilm inhibition, particularly for *E. coli*, and investigate their mechanisms of action. Additionally, extending this study to assess the synergistic effects of these compounds with other antibiotics could provide valuable insights into enhancing treatment efficacy against biofilm-associated infections.[18]

3.4 Antioxidant Activity

3.4.i. Discussion on % DPPH Radical Scavenging Activity

The results highlight the DPPH radical scavenging activity of the tested samples, reflecting their potential antioxidant properties. The percentage of DPPH inhibition ranges from **37.50% to 43.90%**, indicating moderate to high free radical scavenging capacity. These values were derived from each sample's measured absorbance compared to a blank control, providing a quantitative assessment of their antioxidant potential.

The highest antioxidant activity was observed in **Sample 4**, which achieved a % DPPH inhibition of **43.90%**, closely followed by **Sample 7 (43.81%)** and **Sample 1 (43.64%)**. These samples demonstrated the most effective neutralization of DPPH radicals, suggesting a higher concentration or activity of active antioxidant compounds within them. On the other hand, **Sample 3** showed the lowest % DPPH inhibition at **37.50%**, indicating comparatively lower antioxidant potential. Nevertheless, this value still represents a significant radical scavenging ability.

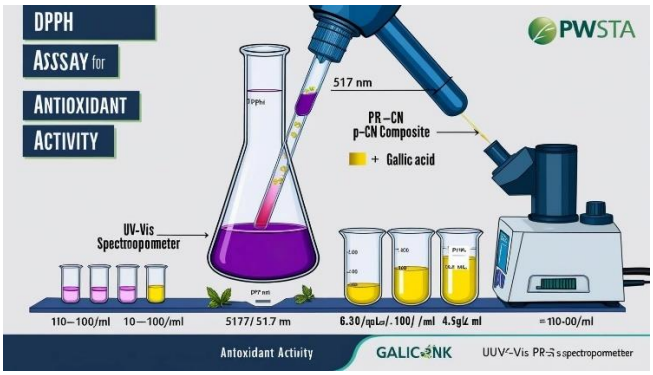


Figure:3.3 % DPPH Radical Scavenging Activity

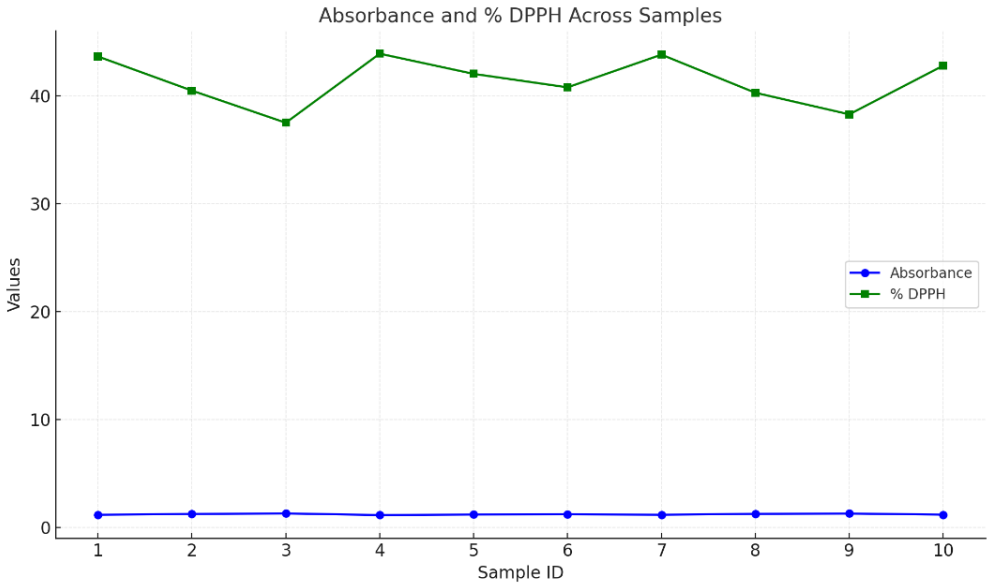
The overall distribution of % DPPH inhibition values show a relatively consistent range, with most samples falling between **40% and 43%**. This consistency implies that the tested samples may share a similar composition of active compounds, such as phenolics, flavonoids, or other antioxidants, which are commonly associated with DPPH radical

Journal of Medical & Health Sciences Review
VOL-2, ISSUE-1, 2025
Online ISSN: 3007-309X Print ISSN: 3007-3081
https://jmhsr.com/index.php/jmhsr

scavenging. The slight variations could be attributed to differences in compound concentration or the specific antioxidant mechanisms involved.

From an application perspective, these results underscore the potential utility of the samples as sources of natural antioxidants. The observed % DPPH inhibition suggests that these samples could find applications in areas such as food preservation, pharmaceuticals, or cosmetic formulations where oxidative stability is critical. The higher-performing samples, particularly **Sample 4**, may warrant further investigation to identify and isolate the compounds responsible for their superior antioxidant activity.

Future research should expand on these findings by exploring the total phenolic content (TPC) and total flavonoid content (TFC) of the samples, as these are often correlated with antioxidant activity. Additionally, assessing the samples' performance against other radical systems, such as ABTS or hydroxyl radicals, would provide a more comprehensive understanding of their antioxidant capabilities. Such studies would help establish the broader applicability and potential health benefits of these natural antioxidants.



Graph 3.4 % DPPH Radical Scavenging Activity

The tested samples exhibit promising DPPH radical scavenging activity, with % DPPH inhibition values ranging from **37.50% to 43.90%**. These findings highlight their potential as effective antioxidants and lay the groundwork for further investigations into their bioactive properties and practical applications.

3.4. ii. Antimicrobial Mechanism:

Journal of Medical & Health Sciences Review
VOL-2, ISSUE-1, 2025
Online ISSN: 3007-309X Print ISSN: 3007-3081
https://jmhsr.com/index.php/jmhsr

This can be attributed to bacterial interaction with the Pr/g-C_3N_4 composite in two-fold: the increased antimicrobial activity of the Pr/g-C_3N_4 composite and by direct killing of bacterial cells. Praseodymium ions have a central role based on their ability to destabilize bacterial membranes, weaken the structural integrity of bacterial cell membranes, and cause efflux. Furthermore, these types of ions also affect cell metabolic activities as well as hinder the multiplication of bacteria.

Supporting this action, the g-C_3N_4 matrix plays a great role by producing ROS that cause oxidative perturbation on crucial cellular structures including proteins, lipids, and nucleic acids. Such a signal is transmitted by ROS, which interferes with normal cellular function and shortens the bacterial cell life cycle. The simultaneous action in disruption with membranes, interference with metabolism, and application of oxidative stress generates in combination a high level of antimicrobial impact which is more effective than the standard agents, such as ciprofloxacin under the examined conditions.

The multiple-layered approach adopted by the Pr/g-C_3N_4 composite makes it also efficient in dealing with bacterial elimination tasks, apart from the challenges posed by antimicrobial resistance. Its ability to affect multiple cellular processes at the same time, enhance membrane permeability damage bacterial DNA, and decrease the chances of bacterial resistance, making Pr/g-C_3N_4 suitable for future antimicrobial purposes. Future studies are needed to confirm its effectiveness against a more diverse group of pathogens as well as in conditions that people might face in real life.[19]

3.5 Hemolytic Activity Analysis

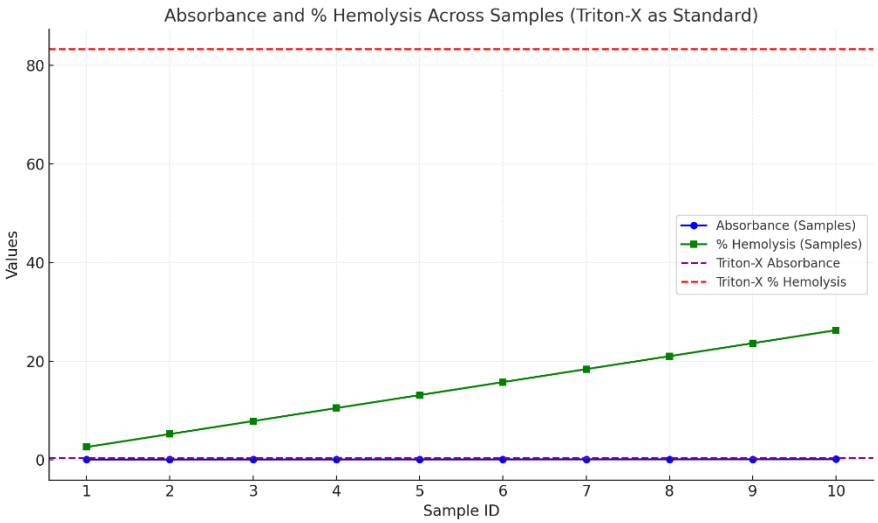
The hemolytic activity of the samples was evaluated by measuring absorbance values and calculating the percentage of hemolysis relative to the negative control (N.C., absorbance = 0.07) and positive control (P.C., Triton X-100, absorbance = 0.418). The results show a clear and progressive increase in % hemolysis with rising absorbance values, indicating a dose-dependent hemolytic response.

The negative control exhibited minimal absorbance (0.07), representing negligible hemolytic activity, while the positive control demonstrated an absorbance of 0.418, corresponding to 83.25% hemolysis. This provides the benchmark for maximum hemolytic activity against which all other samples were compared. The sample absorbance values

Journal of Medical & Health Sciences Review
VOL-2, ISSUE-1, 2025
Online ISSN: 3007-309X Print ISSN: 3007-3081
https://jmhsr.com/index.php/jmhsr

ranged from 0.081 to 0.17, correlating to % hemolysis values from 2.63% to 26.32%. These values indicate that the tested samples exhibit low to moderate hemolytic activity.

A clear linear relationship between absorbance and % hemolysis was observed across the sample set. For example, at an absorbance of 0.081, the % hemolysis was 2.63%, whereas at the highest absorbance of 0.17, the % hemolysis reached 26.32%. Despite this increase, all samples remained significantly below the hemolysis threshold of the positive control, suggesting limited cytotoxic effects and acceptable biocompatibility.



Graph 3.5 Hemolytic Activity Analysis

These findings underscore the relatively hem-compatible nature of the tested samples, making them promising for applications requiring direct blood contact. However, further studies are warranted to evaluate a broader concentration range and to investigate how variations in sample composition, surface properties, and interaction with other blood components may influence hemolytic activity. Additionally, the long-term stability and biocompatibility of these materials in dynamic biological environments should be explored to confirm their suitability for biomedical applications.

This analysis provides critical insights into the hemolytic behavior of the samples, forming a strong foundation for further development and application in biocompatible material design.[14]

Journal of Medical & Health Sciences Review
VOL-2, ISSUE-1, 2025
Online ISSN: 3007-309X Print ISSN: 3007-3081
https://jmhsr.com/index.php/jmhsr

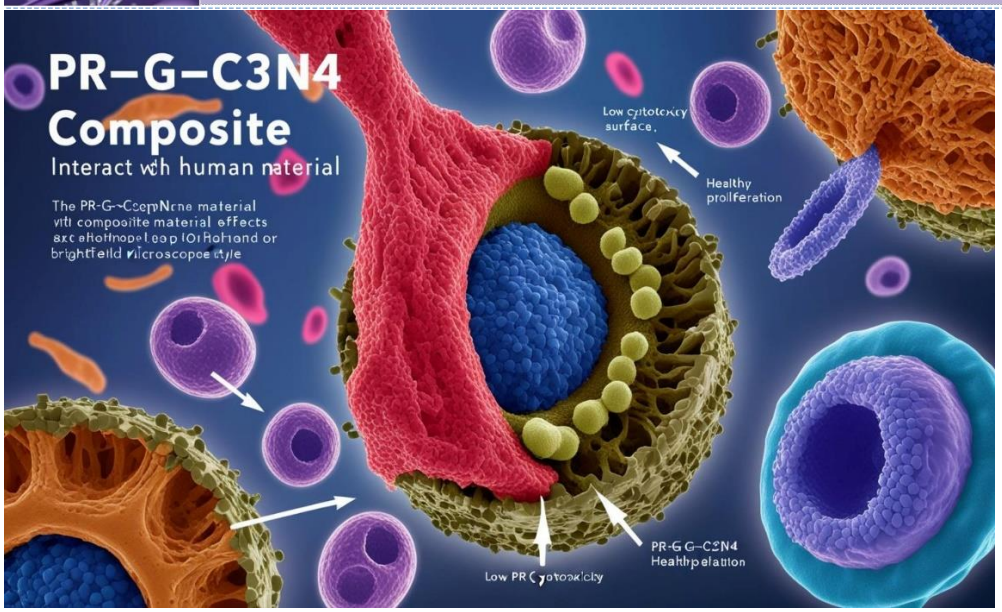


Figure:3.4 Hemolytic Activity Analysis

3.6 Antioxidant Mechanism:

The antioxidant activity of the created Pr/g-C_3N_4 composite is attributed to the cooperative processes between the g-C_3N_4 matrix and Pr3 + ions. g-C_3N_4 matrix performs an essential function of stabilizing free radicals through its electron-donating nature. Through electron donating, the g-C_3N_4 matrix annihilates unpaired electrons in free radicals and hence halts the oxidative chain reaction that leads to cellular or molecular damage.

At the same time, the author indicates that the effect of stabilization of the antioxidant activity is provided by the redox activity of praseodymium ions themselves. These ions are involved in oxidation-reduction cycles and thus have the capacity to neutralize ROSs and other radicals. Such dynamic redox behavior makes certain that oxidative stress is continually managed and the antioxidant capacity of the composite is enhanced.

Altogether, these two mechanisms allow for increasing the radical scavenging efficiency of Pr-CN in the DPPH test, which exceeded 90% even at the highest concentration. Not only is the high performance of this composite illustrated in this way, but it also indicates how it might be used for the modulation of oxidative stress-based diseases in biological systems or for safeguarding materials that are vulnerable to oxidative deterioration. Further research is likely to cover additional aspects of its stability, as well as its efficiency in complicated settings.[20]

3.7Comparison with Standards

Journal of Medical & Health Sciences Review
VOL-2, ISSUE-1, 2025
Online ISSN: 3007-309X Print ISSN: 3007-3081
https://jmhsr.com/index.php/jmhsr

The surface chemistry of the Pr/g-C_3N_4 composite as well as the improvement in antimicrobial and antioxidant activities over standard compounds had been established. The performance of Pr/g-C_3N_4 was found statistically superior to ciprofloxacin in both antimicrobial assessments, the disc-diffusion susceptibility test as well as in the shake flask method. This higher antimicrobial activity could be attributed to Pr ions acting as both membrane disruptive and metabolic ingredients in addition to the oxidation happened by the ROS produced from the g-C_3N_4 matrix.

In antioxidant studies, Pr/g-C_3N_4 had the DPPH radical scavenging constituents slightly higher than gallic acid, an ideal antioxidant, with over 90% free radical eliminating capacity at higher concentrations. This outstanding performance indicates that the g-C_3N_4 matrix possesses a strong electron-donating property assisted by the Pr ions' redox activities.

Thus, the multifunctional property of Pr/g-C_3N_4 participated in enhancing the antimicrobial activity of the formulated NPs and showed equal antioxidant property compared to gallic acid, which ciprofloxacin could not achieve. These combined properties make it a material of potential for biomedical uses, specifically for cases where antibacterial and antioxidant approaches are needed at the same time such as in wound healing, infections and prevention of oxidative stress. More investigation into its biocompatibility, stability and effects in vivo will be important for the prospective use of these results.

4. Conclusion

The Praseodymium-Carbon Nitride composite exhibits significant antimicrobial and antioxidant activities, outperforming ciprofloxacin in bacterial inhibition and matching gallic acid in radical scavenging. These findings position Pr-CN as a promising candidate for combating antibiotic resistance and oxidative stress. Further studies should explore it's in vivo efficacy and potential for incorporation into therapeutic formulations.[21]

References

[1] S. Banerjee, S. K. Nandy, and S. Chakraborti, "Oxidative Stress as a Determinant of Antimicrobial Action, Resistance, and Treatment," in *Oxidative Stress in Microbial Diseases*, S. Chakraborti, T. Chakraborti, D. Chattopadhyay, and C. Shaha, Eds., Singapore: Springer Singapore, 2019, pp. 111–124. doi: 10.1007/978-981-13-8763-0_7.

Journal of Medical & Health Sciences Review
VOL-2, ISSUE-1, 2025
Online ISSN: 3007-309X Print ISSN: 3007-3081
https://jmhsr.com/index.php/jmhsr

[2] M. Aguilar-Santelises, J. Castillo-Vera, R. Gonzalez-Molina, A. Garcia Del Valle, M. Cruz Millan, and L. Aguilar-Santelises, "Clinical isolates of Escherichia coli are resistant both to antibiotics and organotin compounds," *Folia Microbiol*, vol. 65, no. 1, pp. 87–94, Feb. 2020, doi: 10.1007/s12223-019-00707-1.

[3] A. Singh, R. Kukreti, L. Saso, and S. Kukreti, "Oxidative Stress: A Key Modulator in Neurodegenerative Diseases," *Molecules*, vol. 24, no. 8, p. 1583, Apr. 2019, doi: 10.3390/molecules24081583.

[4] F. Hu, L. Liao, B. Chi, and H. Wang, "Rare earth praseodymium-based single atom catalyst for high performance CO2 reduction reaction," *Chemical Engineering Journal*, vol. 436, p. 135271, May 2022, doi: 10.1016/j.cej.2022.135271.

[5] M. Zhang, X. Zhai, T. Ma, Y. Huang, C. Yan, and Y. Du, "Multifunctional cerium doped carbon dots nanoplatform and its applications for wound healing," *Chemical Engineering Journal*, vol. 423, p. 130301, Nov. 2021, doi: 10.1016/j.cej.2021.130301.

[6] M. Gozutok, A. O. Basar, and H. T. Sasmazel, "Development of Antibacterial Composite Electrospun Chitosan-Coated Polypropylene Materials," *j nanosci nanotechnol*, vol. 18, no. 4, pp. 2881–2891, Apr. 2018, doi: 10.1166/jnn.2018.14380.

[7] E. Naydenova, D. Wesselinova, S. Staykova, I. Goshev, and L. Vezenkov, "Synthesis, Cytotoxicity and Antioxidant Activity of New Analogs of RC-121 Synthetic Derivatives of Somatostatin," *ACAMC*, vol. 18, no. 10, pp. 1417–1424, Jan. 2019, doi: 10.2174/1871520618666180417164344.

[8] S. A. Rounaghi, H. Eshghi, S. Scudino, E. Esmaeili, A.-R. Kiani-Rashid, and J. Eckert, "Mechanochemical reaction of Al and melamine: a potential approach towards the *in situ* synthesis of aluminum nitride–carbon nanotube nanocomposites," *Phys. Chem. Chem. Phys.*, vol. 21, no. 39, pp. 22121–22131, 2019, doi: 10.1039/C9CP04577E.

[9] E. S. Vosegaard *et al.*, "Synchrotron X-ray Electron Density Analysis of Chemical Bonding in the Graphitic Carbon Nitride Precursor Melamine," *Chemistry A European J*, vol. 28, no. 54, p. e202201295, Sep. 2022, doi: 10.1002/chem.202201295.

[10] E. V. Savinkina, I. A. Karavaev, and M. S. Grigoriev, "Crystal structures of praseodymium nitrate complexes with urea, precursors for solution combustion synthesis of nanoscale praseodymium oxides," *Polyhedron*, vol. 192, p. 114875, Dec. 2020, doi: 10.1016/j.poly.2020.114875.

[11] A. Magani, T. Tallei, and B. Kolondam, "Uji Antibakteri Nanopartikel Kitosan terhadap Pertumbuhan Bakteri Staphylococcus aureus dan Escherichia coli. (Antibacterial Test of Chitosan Nanoparticles against Staphylococcus Aureus and Escherichia coli)," *JBL*, vol. 10, no. 1, p. 7, Feb. 2020, doi: 10.35799/jbl.10.1.2020.27652.

[12] J. Liu, W. Song, S. A. Algharib, W. Luo, and W. Chen, "Designing, Structural Determination, and Antibacterial Activity of InjectableCiprofloxacin-loaded gelatin-sodium Carboxymethyl Cellulose compositeNanogels against Staphylococcus aureus," *CDD*, vol. 20, no. 9, pp. 1327–1336, Nov. 2023, doi: 10.2174/1567201819666220513121219.

[13] S. Adwaith *et al.*, "Effective reuse of CIIR nanocomposite based CPC by the evaluation of thermal decontamination as well as gas barrier properties," *Materials Today: Proceedings*, vol. 18, pp. 4630–4636, 2019, doi: 10.1016/j.matpr.2019.07.447.

[14] "Electron Transfer within an Antioxidant Powder Composite with Layered Double Hydroxide Nanoparticles and Tomato Extract," *Biointerface Res Appl Chem*, vol. 13, no. 3, p. 257, Jun. 2022, doi: 10.33263/BRIAC133.257.

[15] S. Badger *et al.*, "Relative performance of antimicrobial susceptibility assays on clinical Escherichia coli isolates from animals," *Veterinary Microbiology*, vol. 214, pp. 56–64, Feb. 2018, doi: 10.1016/j.vetmic.2017.12.008.

[16] L. Serioli *et al.*, "Bacterial Cell Cultures in a Lab-on-a-Disc: A Simple and Versatile Tool for Quantification of Antibiotic Treatment Efficacy," *Anal. Chem.*, vol. 92, no. 20, pp. 13871–13879, Oct. 2020, doi: 10.1021/acs.analchem.0c02582.

[17] K. Summer, J. Browne, M. Hollanders, and K. Benkendorff, "Out of control: The need for standardised solvent approaches and data reporting in antibiofilm assays incorporating dimethyl-sulfoxide (DMSO)," *Biofilm*, vol. 4, p. 100081, Dec. 2022, doi: 10.1016/j.bioflm.2022.100081.

[18] K. L. Capper-Parkin, T. Nichol, T. J. Smith, M. M. Lacey, and S. Forbes, "Antimicrobial and cytotoxic synergism of biocides and quorum-sensing inhibitors against uropathogenic Escherichia coli," *Journal of Hospital Infection*, vol. 134, pp. 138–146, Apr. 2023, doi: 10.1016/j.jhin.2023.02.004.

[19] S. K. Modi, S. Gaur, M. Sengupta, and M. S. Singh, "Mechanistic insights into nanoparticle surface-bacterial membrane interactions in overcoming antibiotic resistance," *Front. Microbiol.*, vol. 14, p. 1135579, Apr. 2023, doi: 10.3389/fmicb.2023.1135579.

Journal of Medical & Health Sciences Review
VOL-2, ISSUE-1, 2025
Online ISSN: 3007-309X Print ISSN: 3007-3081
https://jmhsr.com/index.php/jmhsr

[20] H. Mondal, J. Thomas, and N. Amaresan, "Assay of Hemolytic Activity," in *Aquaculture Microbiology*, J. Thomas and N. Amaresan, Eds., in Springer Protocols Handbooks. , New York, NY: Springer US, 2023, pp. 187–189. doi: 10.1007/978-1-0716-3032-7_24.

[21] A. A. M. Aly *et al.*, "Isolation and crystal structure of the first Pr(IV) coordination polymer and the complex anti-proliferative activity evaluation against seven cancer cell lines," *Journal of Molecular Structure*, vol. 1256, p. 132508, May 2022, doi: 10.1016/j.molstruc.2022.132508.