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Antibacterial Activity of Cu Decorated TiO_2 Nanorods

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ABSTRACT

Global public health is seriously threatened by the spread of infectious illnesses in general, particularly by the appearance of bacterial strains that are resistant to antibiotics. New antibacterial drugs are likely a result of recent developments in the field of nanobiotechnologies, particularly the ability to make metal oxide nanomaterials with specific morphologies. Using antibiotics for a long time period will show antibiotic resistance in host cells, which means the drug does not kill the pathogen anymore. As a solution to this problem, nanoparticles can be used. Researchers may find nanoparticles with high antibacterial activity which can kill the pathogen. This research shows the antibacterial activity of Cu decorated TiO_2 nanoparticles against *Klebsiella pneumoniae.* In here the nanoparticles were synthesized in three weight ratios with TiO_2 and CuO using hydrothermal method. Pure CuO and TiO_2 were synthesized as controls. Then antibacterial activity was checked by the well diffusion method. After incubation the inhibition zones were measured, and the results were recorded. The antibacterial effect can be determined with the size of the inhibition zone. The synthesized nanoparticles were characterized using XRD to analyze physical properties such as phase composition, crystal structure. The value for inhibition zone of the best performing sample

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which the sample concentration is 40mg/ml is 13.17±1.53 mm which contains TiO_2 : CuO (1:2) weight ratio. Therefore it can be determined that the best performing sample which has the highest antibacterial activity against *Klebsiella pneumoniae* is G3 which contains TiO_2 : CuO (1:2) weight ratio.

1. INTRODUCTION

The testing of antibacterial activity of Cu decorated TiO_2 nanorods is a novel method that used nanotechnology to test the antibacterial activity. *Klebsiella pneumoniae* was used to test antibacterial activity. A gram -negative, encapsulated, non-motile bacteria known as *Klebsiella pneumoniae* is present in the environment and has been linked to pneumonia in patients with diabetes mellitus or alcohol use disorder. The bacterium usually colonizes the oropharynx and gastrointestinal (GI) tract mucosa of humans. The bacterium can exhibit high levels of virulence and antibiotic resistance once it has entered the bloodstream. (Ashurst JV, 2023).

Due to their high activity, antimicrobial nanoparticles, particularly metal oxide nanoparticles, have been investigated. In this regard, titanium dioxide nanoparticles are among the antibacterial nanoparticles (NPs) whose research has attracted attention in recent years. TiO_2 is a chemical substance with high photocatalytic activity that is thermally stable, biocompatible, and has demonstrated promising outcomes against bacterial contamination. (López de Dicastillo et al) TiO_2 nanotubes demonstrated interesting antimicrobial decrease due to the increase in specific surface area. This fact can be explained by the properties of titanium dioxide, one of which is the production of reactive oxygen species (ROS) on its surface during the photocatalytic reaction when subjected to light of the proper wavelength. It is essential to note that some studies have shown that TiO_2 nanoparticles' antimicrobial activity increased when they were exposed to UV-A light because of the oxide's photocatalytic properties. Reactive oxygen species (ROS) with high oxidative potentials generated under band-gap irradiation photo-induces charge in the presence of O_2 are frequently linked to the mechanism of TiO_2's antimicrobial action. ROS have various effects on bacterial organisms that cause their demise. To combat the MDR (multidrug resistance) caused by conventional antibiotic site-specific, antimicrobial agents with wide spectrum action against microorganisms (Gram-negative and Gram-positive bacteria) are particularly crucial. Researchers proved that the Cu NPs show high antibacterial properties since nanoparticles may be smaller in size than bacterial pores, they will have a unique ability to penetrate the cell membrane. Therefore, Cu decorated TiO_2 NPs show huge antibacterial properties which can be a solution in antibacterial resistance. Antibacterial resistance happens when bacteria develop the ability to defeat the drugs designed to kill them. That means the germs are not killed and continue to grow.

Resistant infections can be difficult, and sometimes impossible, to treat. Therefore, novel methods with nanoparticles will help in this matter.

2. MATERIALS AND METHODS

2.1. PREPARATION OF NANOPARTICLES

2.1.1. SYNTHESIS OF PURE CUO NANOPARTICLES

A mass of 6.24 g of $CuSO_4.5H_2O$ was measured. It was dissolved in minimum amount of DI water. A 10 M NaOH solution was added to the $CuSO_4.5H_2O$ solution dropwise while continuous stirring until a blue color precipitate was formed. Then the solution was heated to 180 °C for 24 hours using hydrothermal method. After the hydrothermal treatment, the solution was filtered and washed with deionized water until negative to . After the washing procedure, the precipitate was kept in dry oven at 180 °C until it dried.

2.1.2. SYNTHESIS OF PURE TIO_2 NANOPARTICLES

A mass of 2 g of P_{25} was measured. It was dispersed in 10 M NaOH solution, and the solution was sonicated for 1 hour. After the solution was heated to 180 °C for 24 hours using hydrothermal method. After the hydrothermal treatment, the solution was filtered and washed with deionized water until the pH to neutral. After the washing procedure, the precipitate was kept in dry oven at 180 °C until it dried.

2.1.3. SYNTHESIS OF NANOCOMPOSITES

A mass of 2 g of P_{25} was measured. It was dispersed in minimum amount of 10 M NaOH solution. A mass of 6.24 g of $CuSO_4.5H_2O$ was measured to synthesize TiO_2 : CuO (1:1) ratio. It was dissolved in minimum amount of DI water. A 10 M NaOH solution was added to the above solution dropwise. The solution was stirred well overnight until it formed a black color precipitate. After that $CuSO_4.5H_2O$ solution and P_{25} solution were mixed, and the solution was sonicated for 30 mins. Then the solution was stirred for 2-3 hours. The solution was heated to 180 °C for 24 hours using hydrothermal method. After the hydrothermal treatment, the solution was filtered and washed with deionized water until negative to . The resultant solution was checked for the pH to neutral. After the washing procedure, the precipitate was kept in dry oven at 180 °C until it dried. The nanocomposites of TiO_2 : CuO (1:2) and TiO_2 : CuO (2:1) were synthesized using the above procedure with accurate weights. For TiO_2 : CuO (1:2) the measured $CuSO_4.5H_2O$ weight was 12.48 g. The $CuSO_4.5H_2O$ weight for TiO_2 : CuO (2:1) was 3.12 g and the P_{25} weight was 2 g for all nanocomposites.

2.2. ANTIBACTERIAL ACTIVITY

Well diffusion method was performed for *Klebsiella pneumoniae* (gram negative) bacterial strain. First, freshly prepared culture of the bacteria was obtained. The bacteria were inoculated from pure culture to a separate flask which contained freshly prepared Luria Bertani broth. Then the broth culture was kept in incubator for 24 hours in 37 °C temperature to pathogen to grow well. Then the freshly prepared broth culture with the bacterial strain was adjusted to 1×10^8 CFU/ml concentration. After that the bacterial strain was streaked separately on Mueller Hinton agar plates using cotton swabs. Then the wells were cut using pipette tips. After that the prepared samples with the nanoparticles (40 mg/ml) were inserted into the wells accordingly. Then the petri plates were incubated for 24 hours in 37 °C temperature. The process was triplicated. Finally, the inhibition zones were measured in each well in petri plates. In addition to that the control experiments were also performed well method using Amoxicillin as the positive control and Distilled water as the negative control for the bacteria. Zone of inhibitions was compared, and the best sample was selected.

3. RESULTS AND DISCUSSION

3.1. ANTIBACTERIAL ACTIVITY

3.1.1. WELL DIFFUSION METHOD

Microorganism	Zone of Inhibition (mm)				
	G1	G2	G3	G4	G5
Klebsiella Pneumoniae	10.83±1.15	12.5±0.5	13.17±1.53	12±0.87	10±0.87

Table 1. Inhibition zones against *Klebsiella pneumoniae* with 40 mg/ml concentration of G1: Pure CuO, G2: TiO_2:CuO (1:1), G3: TiO_2:CuO (1:2), G4: TiO_2:CuO (2:1), G5: Pure TiO_2 using well diffusion method

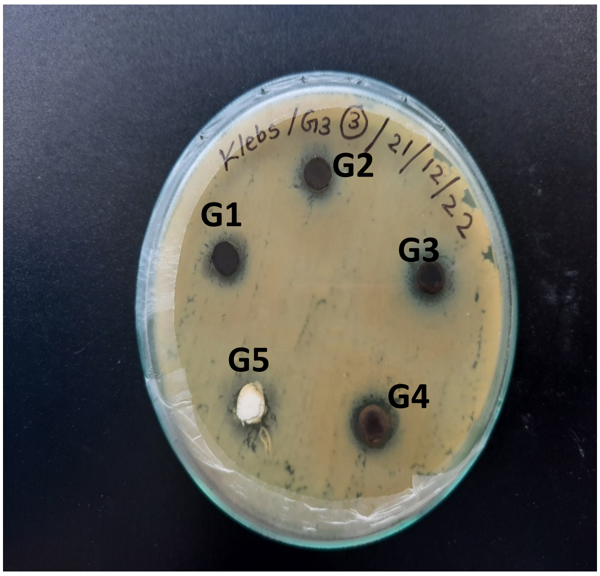

Figure 1. Inhibition zones of *Klebsiella pneumoniae* with 40 mg/ml concentration of G1: Pure CuO, G2: TiO_2:CuO (1:1), G3: TiO_2:CuO (1:2), G4: TiO_2:CuO (2:1), G5: Pure TiO_2 using well diffusion method

According to the diameters of inhibition zones in Table 1 the diameters vary according to the nanomaterial. The zone of inhibitions for G1, G2, G3, G4 and G5 are 10.83±1.15 mm, 12.5±0.5 mm, 13.17±1.53 mm, 12±0.87 mm and 10±0.87 mm respectively. Among them, the highest zone of inhibition value was exhibited by G3 which contains TiO_2 : CuO (1:2) weight ratio. Therefore, it can be determined that the best performing sample for *Klebsiella pneumoniae* is G3.

3.2. XRD ANALYSIS

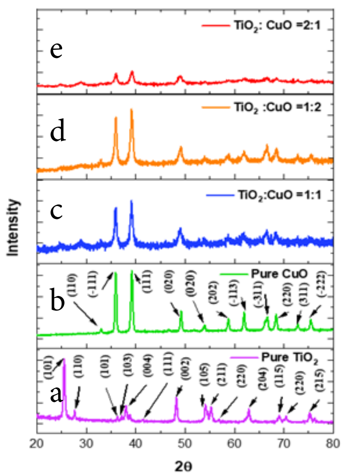

Figure 2. XRD analysis of synthesized nano compounds

X-ray diffraction patterns were gathered to understand the crystal nature of the synthesized composites. In XRD it is used to determine the type of material. The substance may be regarded as crystalline for sharp peaks. If there is a larger peak, the material may be polycrystalline, but if there is a noisy pattern without a peak, the material is said to be amorphous. In order to comprehend the crystal nature of the produced composites, X-ray diffraction patterns were obtained. The XRD patterns of the synthesized compounds are depicted in Figure 2. XRD pattern of P25 TiO_2 show peaks at 7.57°, 36.18°, 41.37°and 56.73° which are attributed to (110), (101), (111) and (220) planes of the Rutile phase and the peaks at 25.46°, 37.95°, 38.72°, 39.31°,48.17°, 54.07°, 55.18°, 62.83°, 68.94°, 70.34° and 75.21°assigned to (101), (103), (004), (112), (002), (105), (211),(204), (115), (220) and (215) the Anatase phase (Figure 2a). XRD pattern of pure CuO consists of peaks at 32.45°, 35.57° , 38.69° , 48.77° , 53.57° , 58.75° , 61.72° , 66.04° , 68.24° , 72.4° , 75.16° assigned to (110), (-111), (111), (020), (020), (202), (-113), (-311), (220), (311), (-222) shows in Figure 2b.

The composites showed crystalline phase peaks of both TiO_2 and CuO which confirmed the presence of both phases in the composite. According to the Figure 2c, 2d and 2e XRD graphs, it does not show a clear peak of TiO_2. The higher effect of CuO suppresses the effect of TiO_2. Therefore, it does not show a clear peak of TiO_2. But it shows all the crystalline phase peaks for CuO.

4. CONCLUSION

Antibacterial activity of Cu decorated TiO_2 nanorods exhibited a positive result against *Klebsiella Pneumoniae.* Comparing the zone of inhibitions with the synthesized nanomaterials, the highest antibacterial activity was showed by G3 which contains TiO_2 : CuO (1:2) weight ratio. The nanocomposite which has high Cu amount showed the highest antibacterial activity against *Klebsiella Pneumoniae.* It can also conclude that nanocomposites show higher antibacterial activity than pure nanoparticles. Analyzing the XRD patterns it can determine that the crystal structure of the synthesized nanocomposites is in good condition. This best performing nanocomposite (G3) can be used to cure diseases which are caused by *Klebsiella Pneumoniae* by conducting further studies and experiments.

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