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Biotemplated Synthesis of N doped TiO_2 Nanoparticles and their Antimicrobial Activity

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Abstract

Pure and nitrogen doped titanium dioxide nanoparticles were synthesized using a bio-template assisted method. The resulting nanoparticles were subjected to annealing at a temperature of 500ºC for duration of 2 hours. Comprehensive investigations of the nanoparticles such as structural, morphological and optical properties were conducted using a variety of scientific techniques including Powder X-ray diffraction, scanning electron microscopy, UV-diffuse reflectance spectroscopy and Fourier transform Infrared spectroscopy. The findings indicate that N doped TiO_2 (N@TiO_2) demonstrates superior antibacterial properties compared to bare TiO_2, exhibiting a larger inhibition zone for Gram positive microorganisms. Importantly, the controlled synthesis method and the utilization of natural biotemplates offer a sustainable and environmentally friendly strategy for the development of antimicrobial nanomaterials, thereby opening up new avenues for research and innovation within the realm of nanotechnology. The recently formulated N@TiO_2 compound has demonstrated impressive antibacterial abilities, particularly targeting *S. aureus*. In the upcoming days, N doped TiO_2 has the potential to be utilized as a substitute therapy or in conjunction with other substances to enhance the effectiveness of treating gram positive bacterial infections.

Keywords: *Bio-template synthesis, Titanium dioxide nanoparticles, Crystal structure, Morphology, Antimicrobial activity*

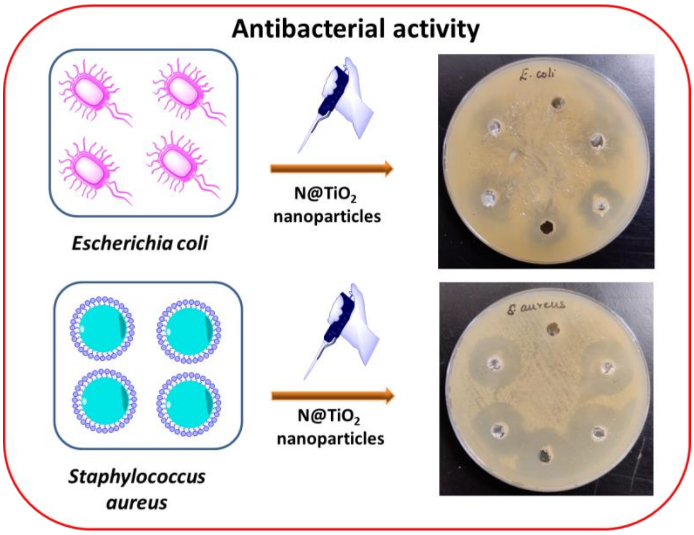

Figure 1: Graphical Abstract

1. Introduction

In recent years, the emergence of antibiotic-resistant bacteria has become a global health concern (Razavi et al., 2023; Seo and Park 2020). Traditional antibacterial agents are losing their effectiveness against these resistant strains, leading researchers to explore novel approaches to combat microbial infections (Dey et al., 2023). Nanotechnology has emerged as a promising field for developing new antimicrobial agents due to its unique properties and potential applications (Rout et al., 2024). Among various nanomaterials, titanium dioxide (TiO_2) nanoparticles have gained significant attention due to their excellent antimicrobial properties and potential for various biomedical applications (Dessai et al., 2022; Mani et al., 2021). TiO_2 is a widely studied material in the field of nanotechnology (Nilavukkarasi et al., 2022) because of it's unique physiochemical properties, including high stability, low toxicity and excellent photo catalytic activity (Cao et al., 2021). These properties make it an ideal candidate for various applications, such as photocatalysis (Motamedi et al., 2022; Alghamdi et al., 2022), dye sensitized solar cells (Ahmadi et al., 2024) and antimicrobial coatings (Mirzapoor et al., 2023). However, the pure form of TiO_2 has limited antimicrobial activity under visible light due to its large band gap (Razavi et al., 2023). To enhance its antimicrobial properties, researchers have focused on TiO_2 nanoparticles doping with heteroatom like S, N etc,. Several studies have demonstrated the successful synthesis of nanoparticles using biotemplates green tea extract (Kumar et al., 2024) chitosan (Packirisamy et al., 2019), egg shell membrane (Yang et al., 2002; Zhang et al., 2024), skeletal plates (Guo et al., 2023), doped with Ag, Fe, Co, Cu [Nguyen et al., 2022; Rathi and Jeice 2024; Rao et al., 2019; Nagaraj et al., 2023; Moradi et al., 2019; Anu et al., 2023; Mishra et al., 2023; Yilmaz et al., 2021; Prajapat et al., 2024) and their enhanced antimicrobial activity. In recent years, researchers have explored novel and sustainable approaches for the synthesis of TiO_2 nanoparticles (Baamer et al., 2024) to overcome the limitations associated with conventional methods. One such approach is the utilization of biotemplates, which are natural materials that can serve as a template for the synthesis of nanoparticles. These biotemplates offer several advantages, including cost-effectiveness, environmentally friendly synthesis process and the ability to produce nanoparticles with unique morphologies and properties (Prajapat et al., 2024). Tomato peel, a waste product generated from the food processing industry, has gained attention as a potential biotemplate for the synthesis of TiO_2 and N@TiO_2 nanoparticles and it contains various bioactive compounds, including polyphenols, flavonoids and carotenoids, which can serve as reducing and stabilizing agents during nanoparticle synthesis. Furthermore, tomato peel is abundantly available; making it an attractive and sustainable option for nanoparticle production (Wu et al., 2023). The global demand for tomatoes has been steadily increasing over the years, leading to excess production in many regions (Shao et al., 2023). While this abundance may seem like a blessing for farmers, it also presents challenges in terms of storage and preventing spoilage. However, recent research has revealed an innovative solution that not only addresses this issue but also benefits the farmer community by repurposing tomato peels as a biotemplate for preparing nanoparticles.

The objective of this study is to synthesize TiO_2 and N doped TiO_2 nanoparticles using tomato peel as a biotemplate and investigate their antimicrobial activity against pathogenic bacteria. The utilization of tomato peel as a biotemplate for nanoparticle synthesis offers several advantages, including its cost-effectiveness, environmentally friendly nature, and potential for bacterial inhibition due to the presence of bioactive compounds (Shakya et al., 2019). Finally, we will discuss the implications of this study and the potential applications of tomato peel assisted synthesis of TiO_2 and N doped TiO_2 nanoparticles for antimicrobial activity.

2. Experimental section

Synthesis of TiO_2 and N@TiO_2 nanomaterials

All chemicals utilized in this experiment were of analytical grade. In a round bottom flask, 1.5 mL of H_2O and 1.5 mL of NH_4OH were combined with 150 mL of acetonitrile and 250 mL of ethanol. The mixture was thoroughly stirred and then 5 mL of titanium isopropoxide was gradually added drop by drop to the aforementioned solution, resulting in the formation of a milky suspension. The reaction mixture was left to stir for approximately 2 hours. Following this, tomato peels were immersed in the suspension for a period of 24 hours. Subsequently, the peels were removed from the solution and air-dried at ambient temperature. The dried peels were subjected to calcination at a temperature of 500˚C, yielding titanium dioxide nanopowders. Similarly, for the production of Ndoped TiO_2, urea was introduced into the precursor solution. The peels were then soaked, dried and calcined at 500˚C to obtain the same material.

Characterization of doped and N doped TiO_2

The crystallite size and phase structure of the prepared samples (TiO_2 and N@TiO_2) were verified using an X-ray diffractometer (Model JDX-8030, JEOL). In order to determine the composition, the infrared spectra of the samples were obtained as potassium bromide (KBR) discs using the Nicolet Avatar model FT-IR spectrophotometer, covering a range of 400-4000 cm^{-1}. To assess the absorption ranges of the prepared samples, a UV-DRS spectrophotometer was employed. Scanning electron microscopy (SEM) images were captured using the JEOL-JSM-6390 instrument, equipped with energy dispersive X-ray analysis (EDAX) capability.

Antibacterial activity

The effectiveness of synthesized titanium dioxide (TiO_2) and N-doped TiO_2 nanoparticles in fighting against *Staphylococcus aureus* (gram-positive bacteria) and *E. coli* (gram-negative bacteria) was examined. The Kirby-Bauer disc diffusion method was used to assess their antimicrobial properties. In a sterile environment, the test organisms were cultured on nutrient agar plates using a cotton swab. After allowing the plates to sit for 10 minutes to let the cultures absorb, 8 mm wells were made in nutrient agar plates to evaluate the antimicrobial effects of the TiO_2 and N-doped nanoparticles. To prevent any leakage of the nanomaterials, the wells were sealed with molten agar (0.8% agar). 30 µl of each sample with varying concentrations (50µg/ml and 100µg/ml) of the nanoparticle suspension was added to the wells on petri plates using a micropipette. The plates were then placed in an incubator at 37 °C for 24 hours to allow the bacteria to grow. After the incubation period, the size of the inhibition zone was measured.

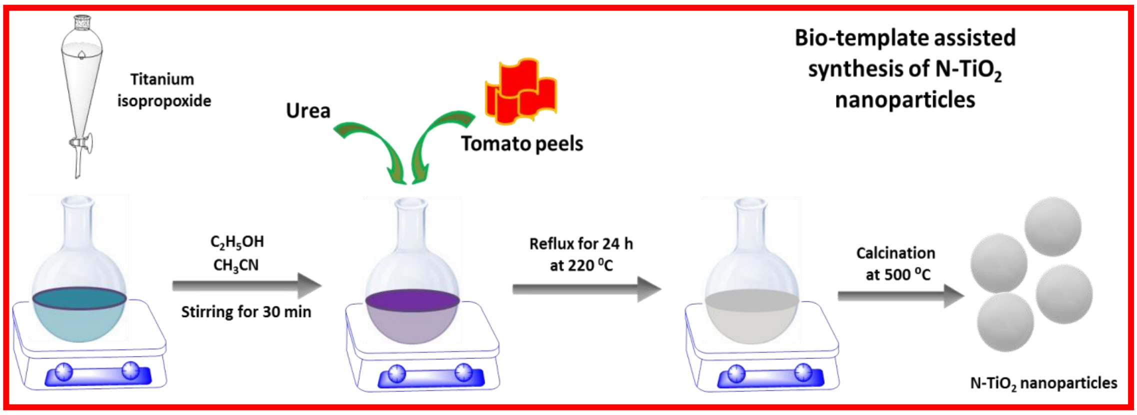

Figure 2: Experimental work

3. Results and discussion

3.1 X-ray diffraction studies

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In this study, X-ray diffraction (XRD) was used to investigate the chemical composition and structural properties of titanium dioxide (TiO_2) and nitrogen-doped titanium dioxide (N doped TiO_2) nanoparticles. The purpose of the study was also to evaluate the crystallinity and size of the nanocrystals. Figure 1 shows the powder X-ray diffraction pattern of pure TiO_2 and N-doped TiO_2 nanoparticles. The observed diffractions were attributed to the pure anatase phase, with peaks occurring at 25.41, 37.84, 48.13, 53.90, 55.13, 62.66, 68.76, 70.31, and 75.06. These peaks correspond to the tetragonal phase of TiO_2 nanoparticles as defined in JCPDS File No. 21-1272. In contrast, no changes in peak positions were observed in the N-doped TiO_2 samples, but a slight decrease in intensity was observed. In addition, the synthesized nanospheres had excellent crystallinity and were mainly composed of the anatase phase of TiO_2 (Ashraful Alam et al., 2024; Thakuret al., 2024;Mathew et al., 2020).

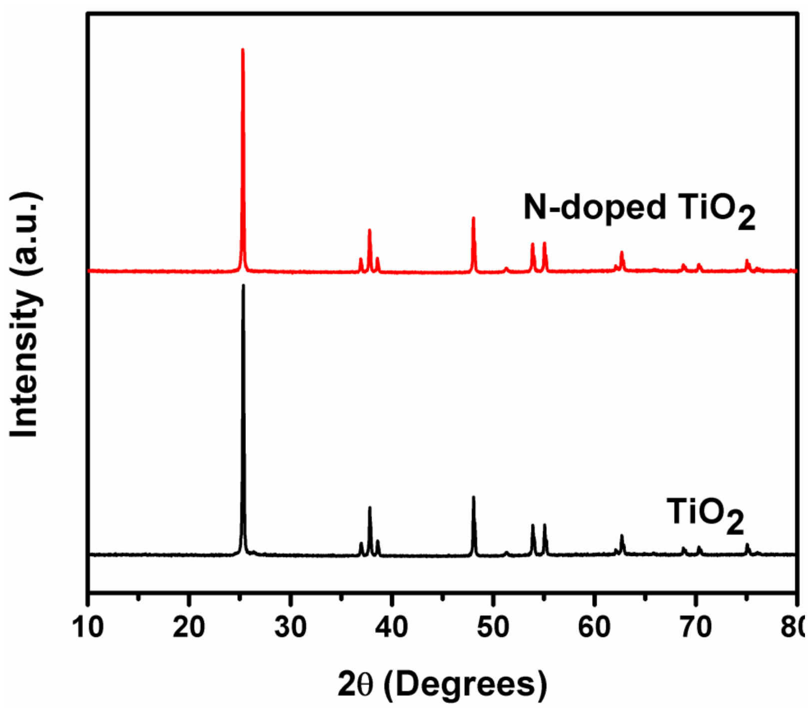

Figure 3: Powder X-ray diffraction pattern for TiO_2 and N-doped TiO_2

3.2 UV-Diffuse Reflectance spectroscopy

Using UV diffuse reflectance spectroscopy, the photoabsorbance characteristics of TiO_2 and N doped TiO_2 nanoparticles were examined in the wavelength range of 200–800 nm. The absorption band of TiO_2 within the 300-400 nm wavelength range arises from metal-ligand charge transfer between the conduction and valence band. Nevertheless, TiO_2 shows noteworthy light reflection in the visible range of 400–800 nm. As demonstrated in earlier research (Lal et al., 2022; Pragathiswaran et al., 2021). Figure 2 illustrates that the introduction of nitrogen doping in TiO_2 nanoparticles leads to the red shift of the absorption edge towards longer wavelengths. This phenomenon can be attributed to either reduced band gaps resulting from nitrogen-induced defects in the TiO_2 lattice structure or an increase in the crystal structure.

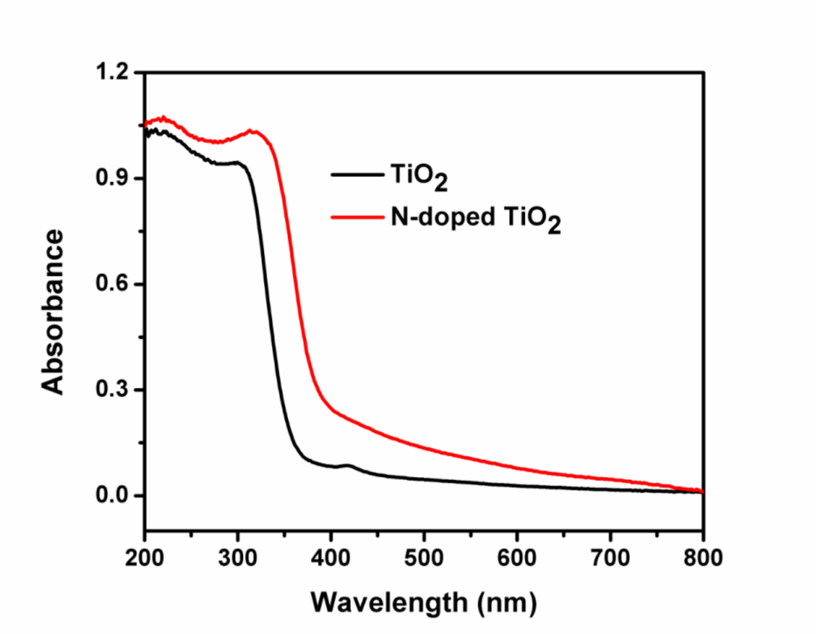

Figure 4: UV-DRS for TiO_2 and N-doped TiO_2

3.3 Fourier Transform Infra red spectroscopy

The identification of the different functional groups and composition of TiO_2 and N doped TiO_2 was achieved using FT-IR spectra. The observed peaks around 3536 cm^{-1} and 1634 cm^{-1} are due to the presence of surface hydroxyl groups and the bending mode of water molecules. The wide band pure around 400–700 $cm^{-1}$is due to is ascribed to stretching vibration of Ti-O and Ti-O-Ti modes (Balakumar et al., 2022; Tharuman et al., 2023). TiO_2 exhibited a strong wideband at. The peak near 2842 cm^{-1} is related to the stretching-vibration mode of CH bonds, while the peak at 2450 cm^{-1} is due to the presence of N atoms incorporated on the TiO_2 lattice or on its surface (Divyasri et al., 2021; Chang et al., 2023). In addition, the signals observed at 1560 cm^{-1} correspond to the nitrite peak, which indicates the successful nitrogen doping in the TiO_2 framework, implying both the achievement of nitrogen doping and the existence of a stable structure.

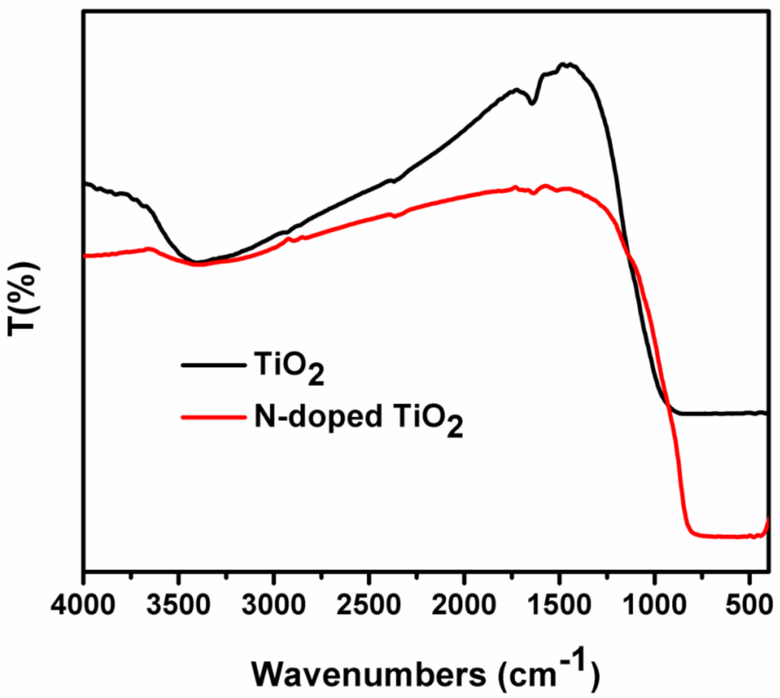

Figure 5: FT-IR forTiO_2 and N doped TiO_2

3.4 Scanning electron microscopy

SEM images of pure TiO_2 nanoparticles showed a spherical morphology with an average particle size of 50 nm. The surface of the nanoparticles appeared smooth and evenly distributed. For N-doped TiO_2 nanoparticles, SEM images showed a similar spherical morphology but a slightly larger average particle size of 70 nm (Divyasri et al., 2021). The N doped TiO_2 nanoparticles also had a smooth surface and relatively uniform distribution. These results indicate that the doping process did not significantly affect the morphology or distribution of the nanoparticles. It is worth noting that the observed irregularities did not compromise the overall integrity of the nanoparticles. This is useful in antimicrobial applications because it allows effective diffusion and coating of the surface to be protected. The slightly larger particle size of the N doped TiO_2 nanoparticles may be due to the doping process that introduced nitrogen atoms into the TiO_2 network. The presence of nitrogen can lead to small changes in the crystal structure that affect particle size. However, these changes are within an acceptable range and do not significantly change the overall morphology or distribution.

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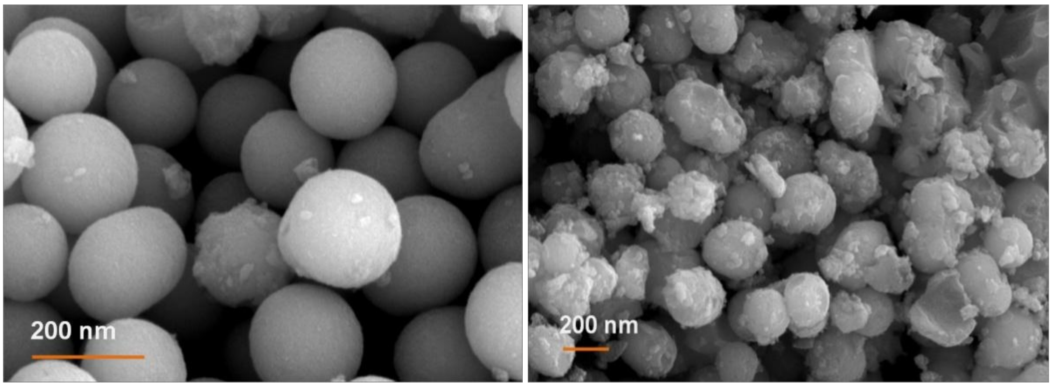

Figure 6: SEM image of TiO_2 and N doped TiO_2

3.5 Antimicrobial activity for N doped TiO_2

Titanium dioxide nanoparticles (TiO_2 NPs) and nitrogen doped titanium dioxide nanoparticles (N doped TiO_2) were synthesized and their antibiotic sensitivity was tested at concentrations of 50 and 100 µg/ml using neomycin sulphate as a control. Addition of urea to TiO2 has been found to improve its antibacterial properties against both Gram-positive and Gram-negative bacterial strains, particularly *S. aureus* and *E. coli*. The ability of these nanoparticles to inhibit the growth of *S. aureus* and *E. coli* were investigated and are shown in Figures A and B. This illustrates that the inhibition zone of N doped TiO_2 was significantly higher compared to the control and the same was done for TiO_2 NPs. The zone of inhibition increases to 10 mm for *S. aureus* and 7 mm for *E. coli*. The addition of urea to TiO_2 gave promising results, possibly due to N-type doping, which changed the energy band structure. *E. coli* infection usually lasts a short time and clears quickly from the body, while *S. aureus* infection tends to persist and can become chronic. As the prevalence of multidrug resistance in bacterial strains has increased, the synthesized N doped TiO_2 nanoparticles show promise as alternative antimicrobial agents, especially against *S. aureus*, in the near future. Thus, N doped TiO_2 may be an alternative therapy or used in combination with other compounds for a more effective treatment of grampositive bacterial infections. As shown by previous reports, the antimicrobial activity of Ag-TiO_2 was found to be effective against both Gram-positive and Gram-negative bacteria. Compared with the previous studies mentioned above, our result showed that N-doped TiO_2 is effective for *S. aureus* and *E. coli* bacteria (Liza et al., 2024; Jiang et al., 2023; Mohammadi et al., 2023).

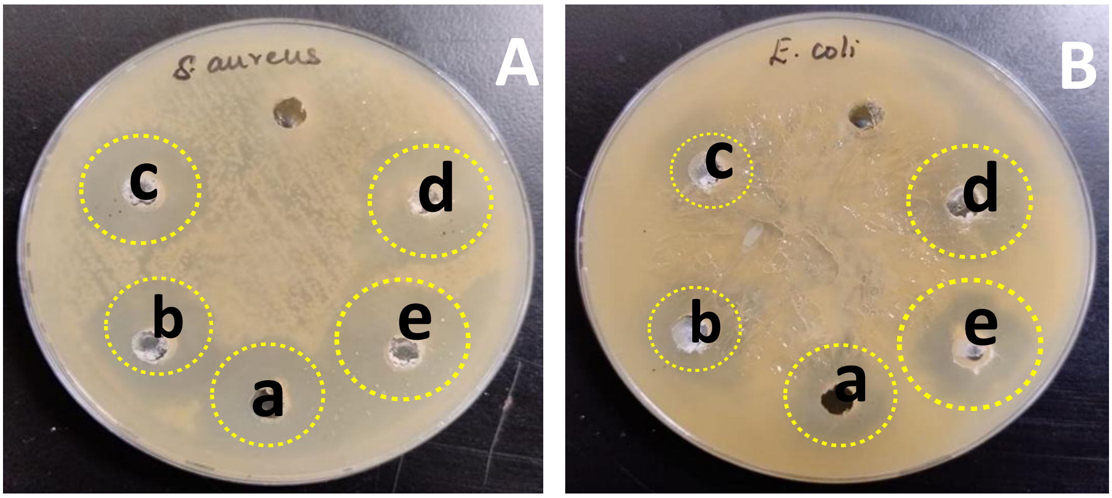

Figure: 7 Antimicrobial activity of TiO_2 and N doped TiO_2 against *S. aureus*and *E. Coli*(Fig A&B)

4. Conclusion

In conclusion, the utilization of biotemplates, such as tomato peel, for the synthesis of nanoparticles offers a sustainable and eco-friendly approach for developing antimicrobial agents. The unique properties of TiO_2 and N doped TiO_2 nanoparticles make them ideal candidates for various biomedical applications, including bacterial inhibition. The synthesis of these nanoparticles using tomato peel as a biotemplate not only provides an alternative to conventional methods but also presents an opportunity to utilize waste materials for nanoparticles production. In this article, we have discussed the biotemplate-assisted synthesis of TiO_2 and N-doped TiO_2 nanoparticles and their potential applications in antimicrobial activity. With further research and development, biotemplate TiO_2 and N-doped TiO_2 nanoparticles may emerge as efficient and sustainable antimicrobial agents that can address the growing threat of antibiotic resistance.

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