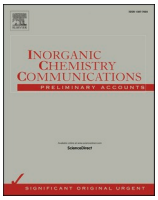
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Short communication



Biosynthesis of Ag and TiO_2 nanoparticles and the evaluation of their antibacterial activities

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ABSTRACT

In this study, *Pseudomonas aeruginosa* was used for the synthesis of silver and titanium oxide nanoparticles (Ag and TiO_2 NPs). The nanoparticles were prepared by mixing the supernatant solution of the cultured organism and the solution of silver nitrate and titanium tetrabutoxide. UV–Vis spectroscopic analysis gave absorption peak at wavelength of 414 nm and 390 nm for Ag and TiO_2 NPs respectively. The FTIR spectra gave absorption bands attributed to N—H stretch of the peptide linkages, O—H stretch of carboxylic acid, amide bend, and —C═O stretch which may have emanated from the micro-organism. The powdered X-ray diffraction (PXRD) studies revealed the crystalline nature of AgNPs and TiO_2 NPs while microscopic studies showed spherical and dispersed morphologies with average particle size of 7.27 nm and 6.83 nm for Ag and TiO_2 NPs respectively. Antibacterial analysis of the synthesized nanoparticles showed broad spectrum inhibition against disease causing microorganisms with TiO_2NPs exhibiting excellent results more than AgNPs and the control drugs.

1. Introduction

Nanotechnology has gained a new trend of research with various applications in biomedical, medicine, engineering, corrosion, and science in general [6,9,18]. Among several properties associated with nanomaterials, their large surface area and stability are key factors responsible for their vast activities and applications. These unique properties explain higher reactivity and stability for longer period than bulk materials. Iravani *et al.* [11] and Wang et al., [31] have reported that various methods can be employed for the synthesis of NPs and that these methods are broadly divided into two main classes which are the bottom-up and top-down approaches.These two classes can be by chemical, physical, and biological methods. The physical methods include laser ablation, mechanical milling, sputtering, ultrasound etc while the chemical methods include chemical reduction, solvothermal, sol–gel synthesis, chemical vapour deposition, microwave irradiation etc. The time frame for the synthesis of nanoparticles using the chemical means usually requires a short period for a large quantity of the nanoparticles. However the chemical method requires stabilization and capping agent, and the reagents used for such purpose are usually toxic and not eco-friendly. To avoid this challenge, there is need to adopt non-poisionous, ecofriendly synthetic protocols for nanosynthesis. This has led to the development of biological approaches that are void of the use of harmful chemicals, thus, increasing the demand for "green nanotechnology" [26].

The use of plant materials is one of the most studied biological method since the plant extracts contain metabolites (eg starch, proteins, amino acids, alkaloids, terpenoids and other polyphenolic compounds) which initiate reduction process and can also act as stabilizing and capping agents. There are reports on the use of plant materials such as *Tinospora cordifolia* [21], *Ocimum Sanctum* [13],umbrella plant [7] for synthesis of silver nanoparticles whereas *Withania somnifera* [2], *Acorus calamus* [3], jasmine flower [4], *Azadirachta indica* [28] have been used for the synthesisis of TiO_2 nanoparticles.

The microorganisms assisted method which is a bottom-up approach and also another green technique have also gained success in nano synthesis and recently found as possible eco-friendly nano factories,

These green protocols are currently replacing the established physical and chemical meethods because they do not emit toxic chemicals and do not involve extreme reaction conditions leading to easy scale up

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[17]. Formation of metal nanoparticles in microorganisms including bacteria, yeasts, fungi have been reported to be viable [19]. However, on few classes of microbes as possible "nano-factories" have been done so far. This microbial utilization in the biosynthesis of metallic nanoparticles of silver and titanium oxide have opened up new ways for researchers to delve into. Several researchers have shown that bacteria, like *Pseudomonas deceptionensis* [14], *Weissella oryzae* [25], *Bacillus methylotrophicus* [30], *Brevibacterium frigoritolerans* and *Bhargavaea indica*, have been explored for silver and gold nanoparticle synthesis [24].

Kumar et al. [16], have earlier reported the synthesis of silver nanoparticles using *Pseudomomas aeruginosa* but no report on the TiO_2NPs using the same organism. This research work therefore reaffirms the properties of the AgNPs and compares with the TiONPs while verifying their different antibacterial properties on gram-positive and gram-negative bacteria. It is envisaged that the study will benefit the science community by providing evidence-based conclusions on the ability of *Pseudomona aeruginosa* to facilitate the synthesis, development, and activity of nanoparticles.

2. Materials and method

2.1. Materials

The chemical and reagents used are NaOH, HCl, $AgNO_3$ (BDH chemicals, England), TiO_2 (Anatase), Titanium tetrabutoxide [$Ti(OBu)_4$], ethanol, chloromethane, nutrient Agar (Sigma-Aldrich, USA), distilled deionized water, Nutrient broth (Sigma-Aldrich, USA), and were of Analytical grade. Bacteria strains of *Pseudomonas aeruginosa* was collected from Microbiology laboratory, Kwara State University, Malete, Kwara state, Nigeria.

2.2. Synthesis of nanoparticles using P.aeruginosa

The method of synthesis employed was followed using the procedure explained by [12] but with slight modifications. Nutrient agar was prepared, autoclaved, and cooled to about 45 °C. To the prepared nutrient agar, a loopful of 24 h test culture of *P. aeruginosa* was inoculated and incubated at about 37 °C for 24 h. After this, the culture was centrifuged and the supernatant was obtained for the synthesis of AgNPs and TiO_2 NPs. The collected supernatant of *P. aeruginosa* culture was dispensed in a different conical flask, after which aqueous $AgNO_3$ and titanium tetrabutoxide [$Ti(OBu)_4$] solution with concentration 0.001 M and 0.02 M were added respectively, in the ratio of 1:3. This mixture was transferred with stirring in a shaker at 100 rpm at 37 °C in bright conditions. The reduction of silver and titanium ions in the solution by *P. aeruginosa* was monitored at regular intervals by sampling 1 mL of the reaction mixture and measuring the absorbance using a UV–Vis spectrophotometer at the wavelength range of 300–600 nm [16]. Nanoparticles were formed at this wavelength range and physical observations of color were seen from green to dark brown for AgNPs. The harvested nanoparticles were purified by methanol and chloromethane in 1:1 proportion and gently washed with deionized water. The nanoparticles were centrifuged and oven-dried at an optimum temperature of 60 °C. The diagrammatic representation of the experimental set up for the bacterial-aided synthesis of Ag and TiO_2 nanoparticles is shown in Figs. 1 and 2..

2.3. Antimicrobial test analysis

The clinical test organisms of *Bacillus subtilis, Bacillus anthrasis, Escherichia coli* and *Staphylococcus aureus* used to study the biological activity of the synthesized Ag and TiO_2 nanoparticles were procured from the Microbiology laboratory, Kwara State University, Malete, Kwara state, Nigeria. The antibacterial activity of the Ag and TiO_2 nanoparticles was studied against gram positive and gram negative bacteria using a well diffusion method at varying concentration of both nanoparticles in relation to the ciproflaxin antibiotic, which serves as the control experiment. The cultures were grown on nutrient agar slants at 37 °C for 24 h and maintained at 4 °C. The Fig. 3below show the agar plate used for the antimicrobial analysis:

3. Results and discussion

3.1. UV–Visible spectra of Ag and TiO_2 nanoparticles

The UV–Vis spectral analysis gives important information on the formation of nanoparticles. It is usually the first instrumental technique to determine the progress of nano-synthesis. Different absorption wavelengths are observed for different nanoparticles. For a particular metal or semiconductor nanoparticles, wavelength of absorption is observed in a particular range but there could be little red or blue shift depending on the substrate used, concentration or other reaction conditions. During reaction times, a change in colour to brown was recorded for the AgNPs whereas milky-white coloured sample was obtained for TiONPs. These colour variations and intensities are reflection of the absorption, the number and size distribution of the nanoparticles [33]. Absorption wavelengths at 414 nm and 390 nm were obtained for the synthesized Ag and TiO_2 nanoparticles respectively as shown in Figs. 4 and 5. This is in accordance with the reports of [8]; Malar, 2019 who got peak absorptions at wavelength 420 nm for AgNPs and 400 nm for TiO_2NPs. The outcome explains that there is a reduction of silver from Ag^+ to Ag^0 (dark brown) after contact with the reducing agent produced

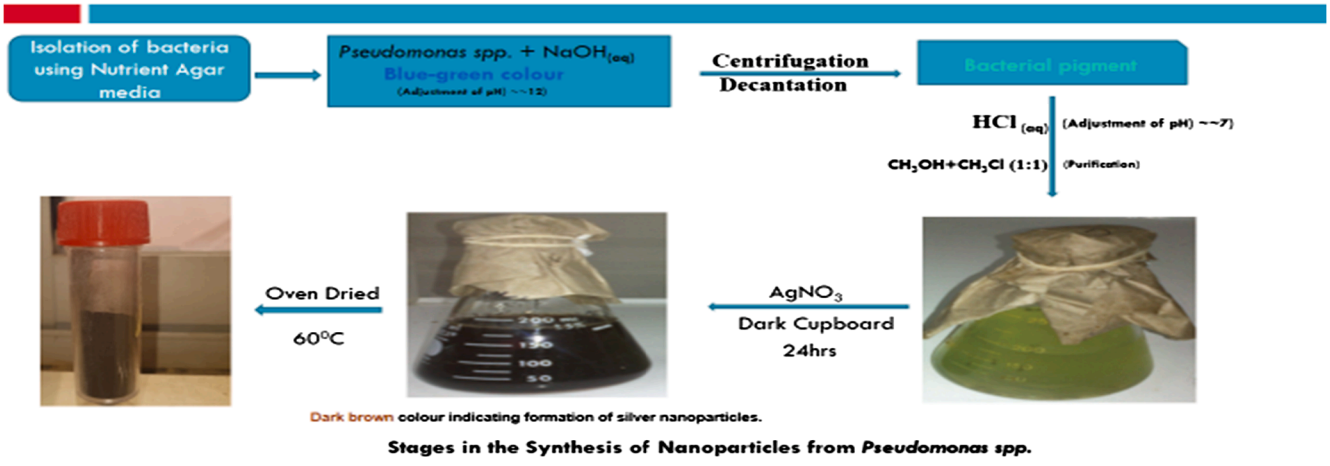


Fig. 1. Stages in the extraction and synthesis of AgNPs.

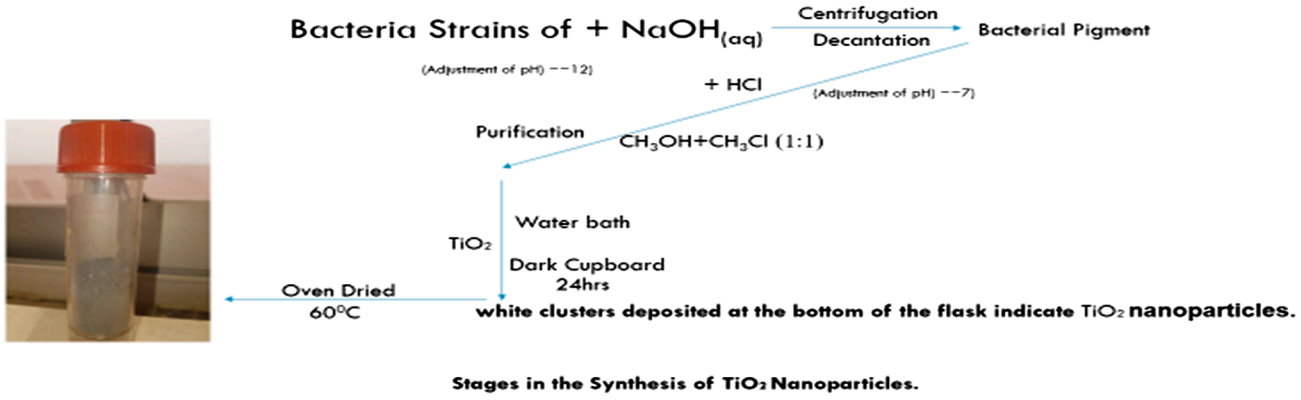


Fig. 2. Stages in the extraction and synthesis of TiO_2 NPs.

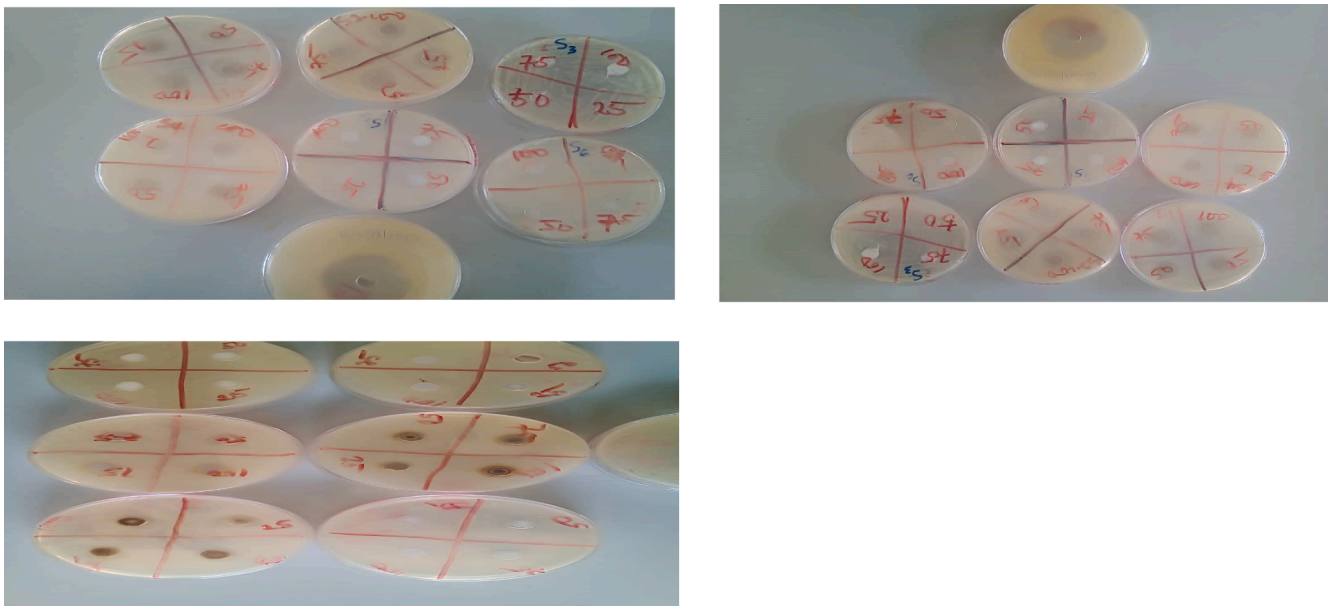


Fig. 3. Antimicrobial analysis.

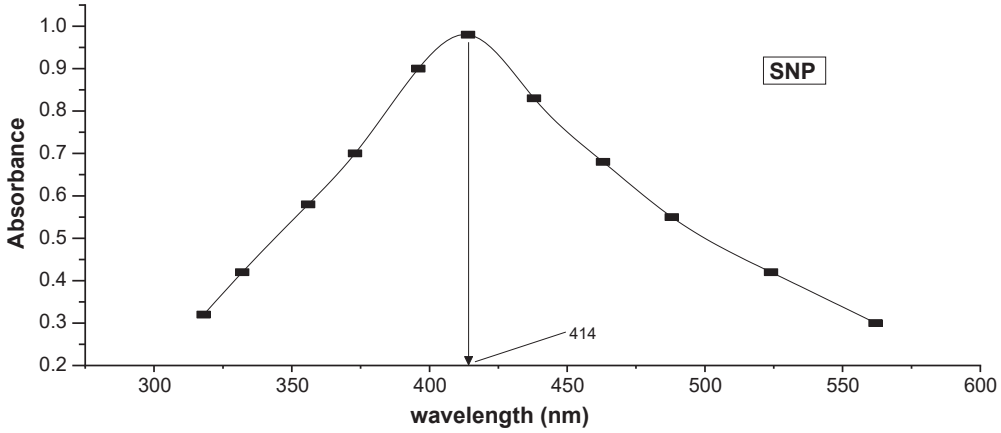


Fig. 4. UV–VIS Spectra for synthesized AgNPs as (SNP).

extracellularly by the bacteria [23].

$Ag^{+} + e^{-}$ (bacterial enzymes) → Ag^{0} (dark brown) (1)

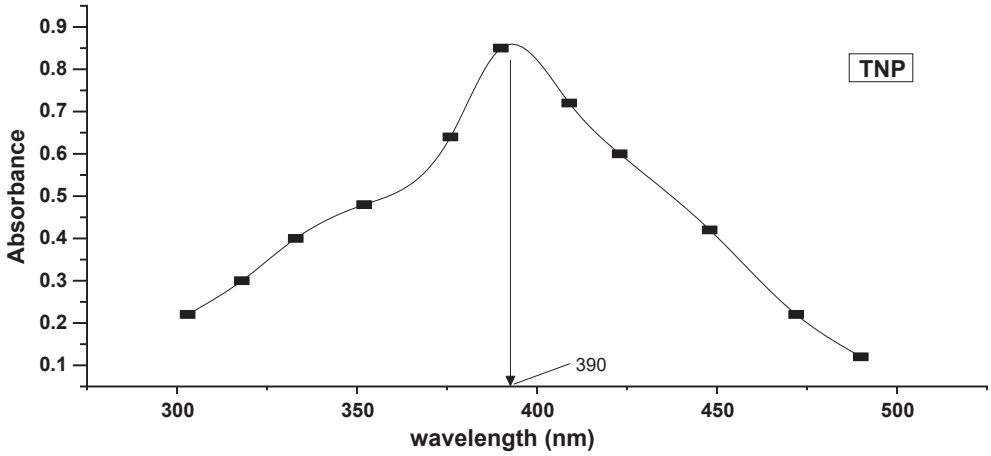


Fig. 5. UV–Vis Spectra for synthesized TiO_2NPs as (TNP). **(Where SNP** = Silver nanoparticles **TNP** = Titanium oxide nanoparticles).

3.2. FTIR analysis

The functional groups from the *P. aeruginosa* responsible for the reduction reaction and capping effect were analysed using the FTIR method. Figs. 6 and 7 showed the spectra results for the synthesized nanoparticles of silver and titanium oxide. From the spectra results, AgNPs showed absorption bands at 3422.72, 2900.11, 1654.10, 1384.31 cm^{-1} and TiO_2 NPs at 3448.58, 1636.94, 1400 cm^{-1}. The broad absorbance bands observed at 3422.72 and 3448.58 cm^{-1} represent the N—H stretch of the peptide linkages and O—H stretch vibration of the carboxylic acid group [29]. The band observed at 1654.10 and 1636 cm^{-1} represents amide bend, indicating the presence of protein moiety in the sample. The analytical data could be seen to suggest the presence of capping protein around the nanoparticles. The protein molecules thus is responsible for the reduction and capping of the nanoparticles, preventing aggregation and providing stability to the nanoparticles [22].

3.3. X-ray diffraction studies (XRD)

The crystallinity of nanoparticles were determined by the powder X-ray diffraction studies (PXRD) and diffraction peaks obtained for Ag and TiO_2 nanoparticles as shown in Figs. 8 and 9. For AgNPs, the diffractions are seen at 38.56°, 45.50, 65.70° and 77.80°, which correspond to the 2θ index of (111), (200), (220), and (311) diffraction planes of the silver respectively. The clarity of the peaks reveals the formation, purity and crystallinity of silver nanoparticles showing face-centered cubic (fcc) structure in tandem with the reports of [1,5,23].

Also, the peaks for TiO_2 were observed at 25.3°, 37.8°, 48.0°, 53.9°, 55.1°, 62.7°, 68.8°, 70.3° and 75.1° corresponding to miller indices (101), (004), (200), (105), (211), (204), (116), (220) and (215) respectively and the peak values corroborates with the results of [10].

However, the presence of the other peaks in the plane could be attributed to the presence of capping protein around the nanoparticles [23].

3.4. Scanning electron microscopy (SEM) and energy dispersive X-ray (EDX) analysis

The surface morphologies of the Ag and TiO_2 NPs look similar and are shown in Figs. 10 and 11. The images give interesting interpretation of the surface chemistry as they can be seen to have porous surfaces which aids their adsorption properties and determines vast applications. However, there is difficulties in recognizing the images and their shapes possibly due to their small particulate nature.

The EDX analysis of the Ag and TiO_2 NPs revealed the elemental constituents of the synthesized nanoparticles and peak signals showed the presence of elemental silver and titanium as shown in Fig. 10. The Ag and TiO_2 NPs displayed band peaks at 3 keV and 4.5kev respectively, which is typical for the absorption of metallic silver and Titanium nanocrystals as also observed by [5,15]. However, other elemental signals such as C, Cl, Si, Na, Sn, and O were also recorded at a normal mode which could be attributed to the emission from the capping protein.

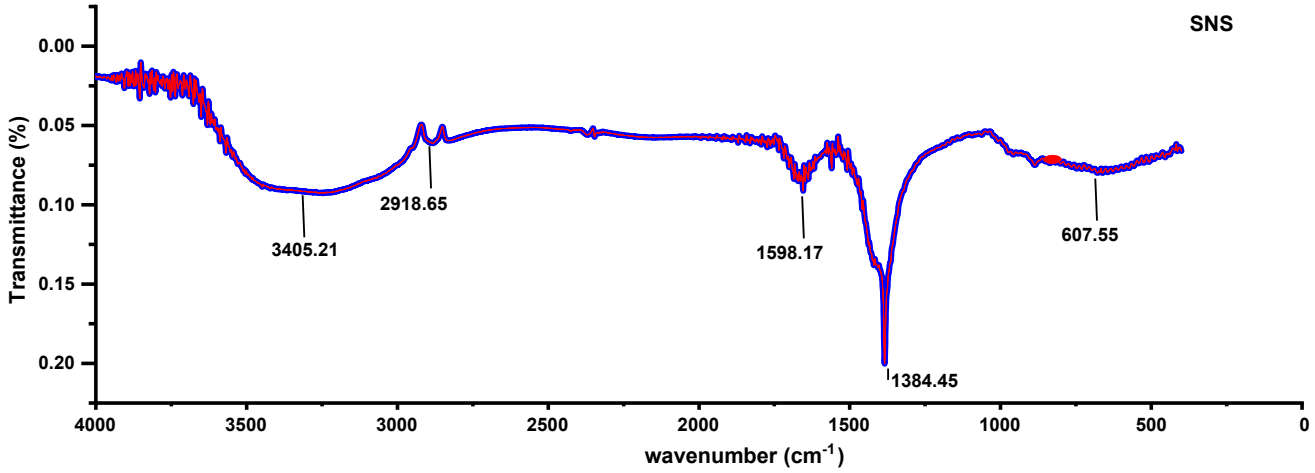


Fig. 6. FTIR spectra of AgNPs.

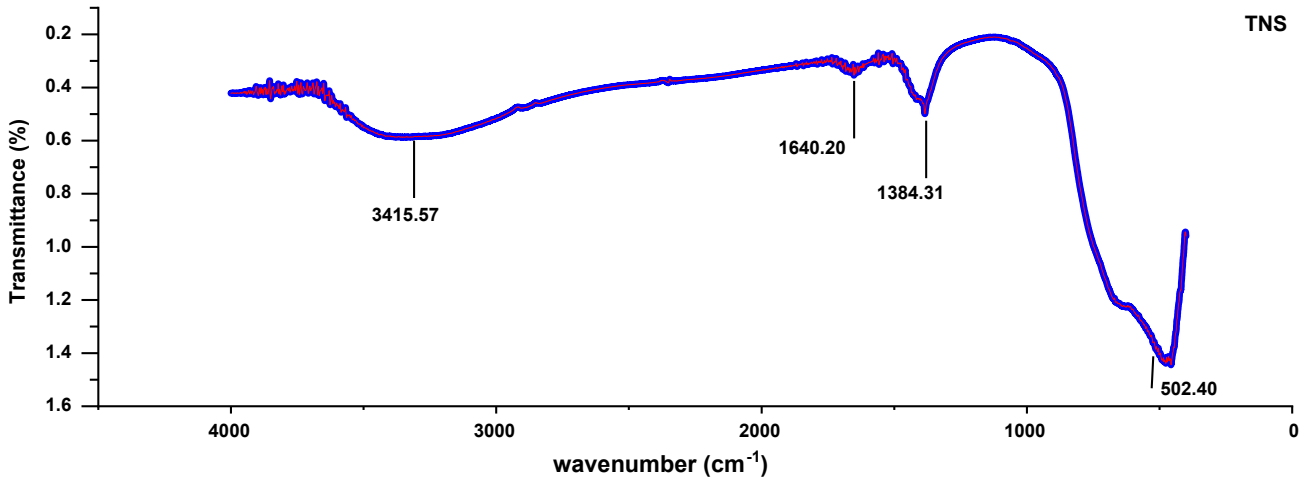


Fig. 7. FTIR Spectra of TiO_2 NPs.

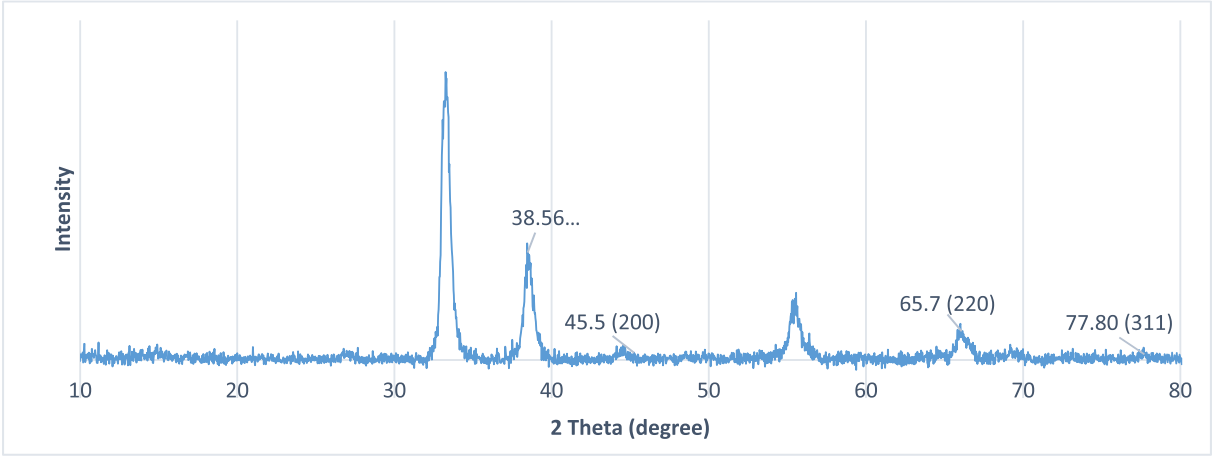


Fig. 8. XRD for AgNPs.

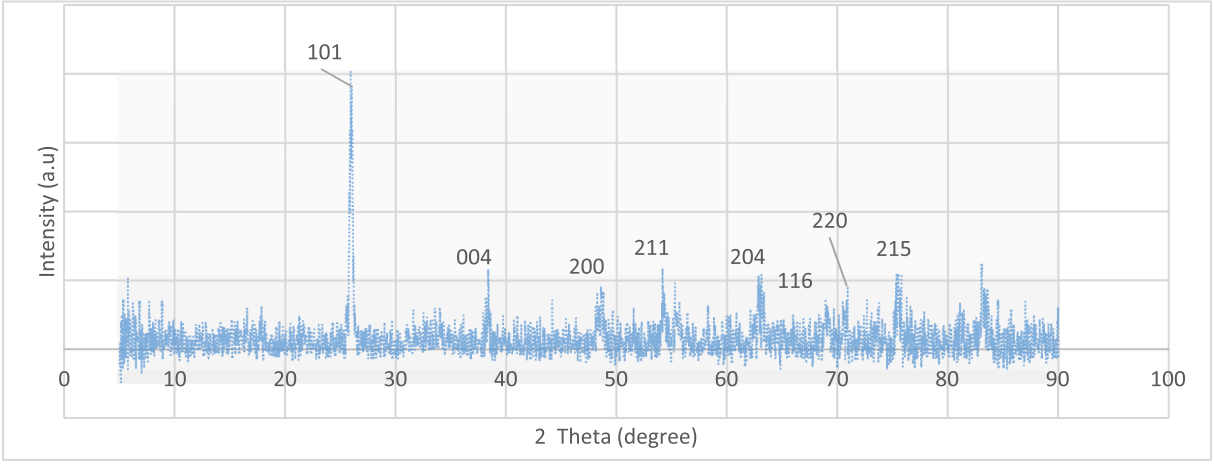


Fig. 9. XRD for TiO_2 NPs.

3.5. TEM analysis

The Ag and TiO_2 NPs produced were spherical and well dispersed without agglomerations as shown in Fig. 12. The size distribution was also shown by the histogram accompanying the images. The crystalline sizes obtained using Image J and Origin software are 7.27 and 6.83 nm for Ag and TiO_2NPs respectively. The size of nanoparticles synthesized in this work is very small and the TiO_2 NPs could be reffered to as quantum dots. In a similar synthesis by [32] using marine *Streptomyces*-MS 26, the synthesized AgNPs were spherical and polydispersed with

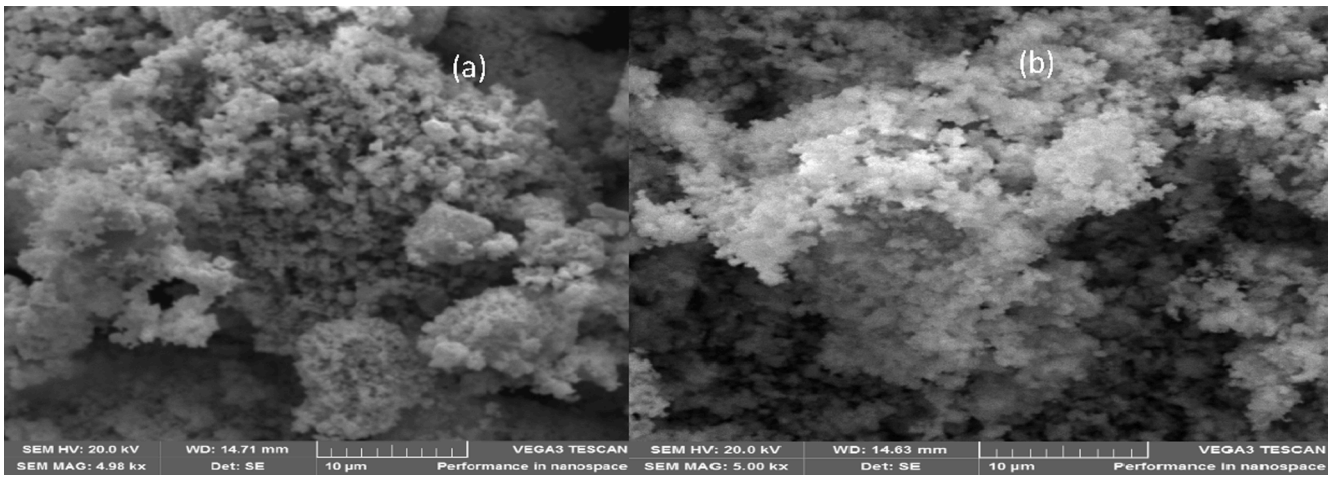


Fig. 10. SEM micrograph of (a)AgNPs and (b)TiO_2 NPs.

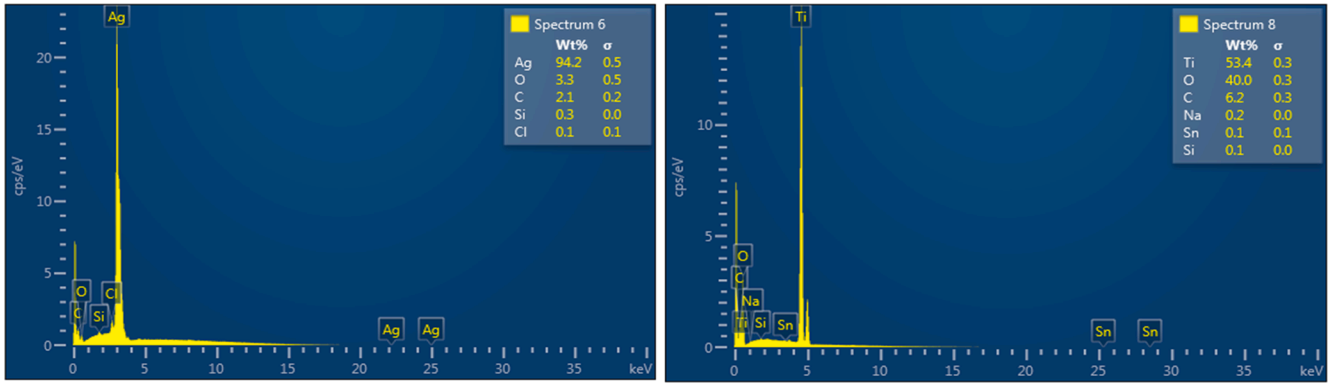


Fig. 11. EDX spectra of (a) Ag and (b) TiO_2 NPs.

size range of 50–76 nm showing that our substrate yielded lower particle sizes and therefore could be more efficient in applications.The TEM micrographs however also provided further insights into the morphology and size distribution the nanoparticles. The nature of the nanoparticles showed surface stability and capping effect due to the biomolecular coating from the protein and peptides of the micro-organism [20].

3.6. Antibacterial activities of the synthesized nanoparticles

The antibacterial evaluation of the synthesized nanoparticles were carried out using some selected gram-positive and gram-negative bacteria *Bacillus subtilis, Bacillus antiphrasis, Escherichia coli, and Staphylococcus aureus.* The agar-well diffusion method was used and the zone of inhibition of the nanoparticles at different concentration measured as shown in Tables 1 and 2. The inhibitory activities against these bacteria were very effective at the highest concentration of 1.0 mg/mL of both nanoparticles (Ag and TiO_2). At concentrations of 0.75 mg/mL, 0.5 mg/mL and 0.25 mg/mL, the inhibitory effect was seen to be less effective. This infers that the antibacterial activities is concentration-dependent.

At both Ag and TiO_2 NPs, the inhibition against Bacillus subtlis and Bacillus anthracis were greatest surpassing that of the control drug (Ciprofloxacin injection). However, TiO_2NPs showed outstandingly antibacterial properties better than the AgNPs.

The nanoparticles gave the least activity against the *E.coli* in the AgNPs but the least activity was seen against *S.auerus* in TiO_2NPs.

It can be seen that the metallic nanoparticles function to alter the metabolic activities of bacteria. Their mechanisms of action include making direct contact with microorganisms while destroying their membrane structure. The released metallic ions then binds to the membrane surface proteins and deactivates them forming antibiofilm which inhibits the establishment of biofilms by bacteria.

4. Conclusion

This research provides evidence for the synthesis of silver and titanium oxide nanoparticles using *Pseudomonas* spp as the substrate. The color change observed in AgNPs from light green to dark brown was supported by UV–Vis spectra showing absorption at 414 nm, while that of TiO_2 NPs was white with absorption wavelength of 390 nm. These peak wavelengths symbolizes strong coherent oscillations of electrons on the surface of metallic particles. The SEM image revealed porous structures with TEM giving particle sizes of 7.27 nm and 6.83 nm for Ag and TiO_2 NPs respectively. The application of the nanoparticles as antibacterial agents showed excellent results. There was great inhibition majorly against the Bacillus spp and the TiO_2 NPs was higher in activity more than the AgNPs. Therefore, our results have shown that some nanoparticles are more potent than some orthodox antibiotics eg (ciprofloxacin). It is thus recommended that research of this nature should continue in order to obtain wide spectrum antibacterial drugs.

Disclosure

The authors work smartly, hardly and friendly, and there is therefore no conflict of interest whatsoever in the course of the work.

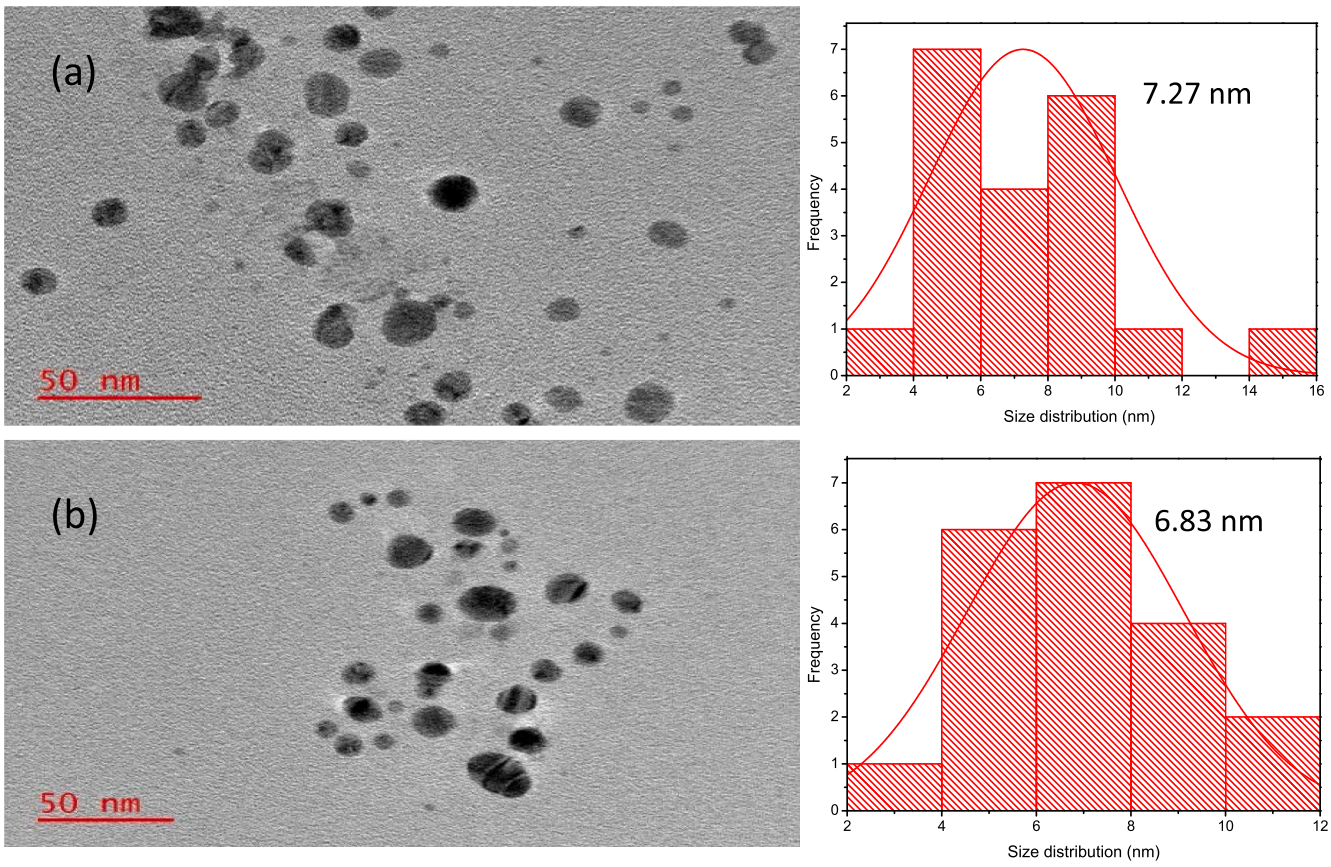


Fig. 12. TEM images and size distribution graphs for (a) AgNPs and (b) TiO_2 NPs.

Table 1
Antimicrobial Results for AgNPs.

Bacteria	Zone of Inhibition (mm)			
	1.0 mg/ml	0.75 mg/ml	0.5 mg/ml	0.25 mg/ml
Bacillus subtilis	20 ± 0.01	19 ± 0.01	18 ± 0.02	14.5 ± 0.001
Bacillus anthracis	16.5 ± 0.02	15 ± 0.01	13.5 ± 0.03	12.5 ± 0.01
Escherichia coli	8 ± 0.01	6 ± 0.02	6 ± 0.01	4 ± 0.01
Staphylococcus aureus	14.5 ± 0.02	12 ± 0.01	13 ± 0.01	11 ± 0.03
Control: Ciproflaxin injection	15 ± 0.02	14.5 ± 0.01	13 ± 0.03	12 ± 0.02

Table 2
Antimicrobial Results for TiO_2 NPs.

Bacteria	Zone of Inhibition (mm)			
	1.0 mg/ml	0.75 mg/ml	0.5 mg/ml	0.25 mg/ml
Bacillus subtilis	30 ± 0.03	25 ± 0.02	22 ± 0.01	20 ± 0.02
Bacillus anthrasis	26 ± 0.02	23 ± 0.01	20 ± 0.03	14.5 ± 0.01
Escherichia coli	6 ± 0.02	4 ± 0.01	4 ± 0.02	4 ± 0.02
Staphylococcus aureus	5 ± 0.01	3 ± 0.03	2.5 ± 0.03	2 ± 0.01
Control: Ciproflocaxin injection	15 ± 0.02	14.5 ± 0.01	13 ± 0.01	12 ± 0.03

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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