Microbial mediated fabrication of Ag and TiO_2 nanoparticles for bacterial Inhibition

Abstract

Microorganisms are world of their own multiplying in millions by reproduction and creating problems to human lives and environment. In this light, it is necessary to transform them into useful product to solving challenges by investigating their inhibition to bacterial growth. In the current study, *Pseudomonas aeruginosa* was applied for the synthesis of silver and titanium oxide nanoparticles (Ag NPs and TiO_2 NPs). The nanoparticles were prepared from the supernatant solution of the cultured organism of *Pseudomonas aeruginosa* and the silver nitrate solution and titanium tetraisopropoxide solution. The microbial assisted synthesized nanoparticles were characterized for UV-Visible spectrophotometer, Fourier Transform Infrared (FTIR) spectrophotometer, Powder X-ray Diffraction (PXRD), Transmission Electron Microscope (TEM), Scanning Electron Microscope (SEM) and Energy Dispersive Spectrophotometer (EDS). UV–Visible spectra provided the absorption maxima at the wavelength of 414 nm for Ag NPs and 390 nm for TiO_2 NPs. The FTIR spectra provided N–H stretch absorption for peptide linkages, O–H stretch of carboxylic acid, amide bend and –C—O stretch which may could have be linked to the micro-organism. The PXRD analysis of both Ag NPs and TiO_2 NPs confirmed their crystallography structures. SEM results showed that both nanoparticles show true sphericity with porous surface. TEM analysis revealed that the particles were well dispersed without agglomeration with average particle size of 7.27 and 6.83 nm respectively. The EDS revealed elemental constituents showing Ag and Ti to be more dominant in their weight percentages. The antibacterial characterization using the biosynthesized nanoparticles provided a significant inhibition against disease causing microorganisms, and TiO_2 NPs was found to be superior to Ag NPs and the control drugs.

Keywords: Synthesis, characterization, nanoparticles, Ag NPs, TiO_2 NPs, Antibacterial

1.0 Introduction

The ruin of antibiotic-sensitive pathogens has listed itself as one of the most compelling threats in contemporary medicine; thus, finding new antimicrobial options (Johnson *et al.,* 2023; Martinez & Kumar, 2024). Of these alternatives, metal and metal oxide nanoparticles have received significant interest because of their interesting physicochemical properties besides evidence of their antimicrobial potential. Among the metal and metal-oxide nanoparticles, silver (Ag) and titanium dioxide (TiO_2) are considered to be ideal agents for controlling bacterial infections because of their higher surface area to volume ratio and unique efficacy (Thompson & Rodriguez, 2023). Most of the existing chemical and physical methods of synthesizing nanoparticles are generally carried out using aggressive reducing agents, toxic chemicals and energy consuming processes that are quite sensitive to the environment (Lee *et al.,* 2023). To the above challenges, current researchers have directed their attention to microbial mediated synthesis techniques which are economic, green, and can be scaled up to allow for the production of large quantity of nanoparticles (Wilson & Chang, 2024). These biological synthesis methods use the different microorganisms such as bacteria, fungi, and algae capable to reduce metal ions into their corresponsive nanoparticles by different enzymatical and metabolic courses (Anderson *et al.,* 2023). Especially, the microbial synthesis of silver nanoparticles has been shown to be highly promising since various microorganisms of course contain certain enzymes that can reduce the silver ions with the help of cellular mechanisms into stable silver nanoparticles (Park & Kim, 2024). Current investigations of silver nanoparticles have confirmed that bacterial mediated bio-reduction process like *Pseudomonas aeruginosa, Bacillus subtilis* and others can lead to highly stable and fine silver nanoparticles with controlled size distribution at considerably lower and friendly environment of pH by bacterial inhabited media. This biosynthesized silver NPs show immense antimicrobial properties on Gram positive and negative organisms, which could be tremendously useful to combat the increasing antibiotic resistant strains (Roberts & Smith, 2024).

Also, the biological method of synthesizing TiO_2 nanoparticles has been stated, particularly the photocatalysis and antimicrobial ability of the nanoparticles (Zhang *et al.,* 2023). For instance, Lactobacillus sp. and Saccharomyces cerevisiae have the ability to produce TiO_2 nanoparticles of special structures and higher biological performances (Davidson & Phillips, 2024). The revelations in the antimicrobial activities of TiO_2 and its inherent photocatalytic properties have created immense possibilities in the creation of improved antimicrobial materials and surfaces (Brown *et al.,* 2023).

There are newer characterization techniques that have allowed the investigators to get closer looks at the fundamental mechanisms of microbial-mediated nanoparticle synthesis (Speech, 2022). Recent progress in light and electron microscopy supplemented with other analytical techniques such as spectroscopy and molecular biology has shown that cellular proteins, enzymes, and metabolites contribute to the formation and stabilization of these nanoparticles (Collins *et al.,* 2024). This enhanced understanding has been used to establish enhanced synthesis procedures to produce NPs that possess enhanced bending properties than those synthesized under previous synthesis procedures (Yang & Liu, 2023). Besides conventional antimicrobial uses, the antimicrobial potential of Ag and TiO_2 nanoparticles produced microbially opens new avenues of app [Application potential overlaps in another area (Foster & Ramirez, 2024). Such nanoparticles have applications in wound dressing, medical devices, water purification, and textiles (Nelson *et al.*, 2023). The variation in the method of synthesizing the nanoparticles shows that the biological synthesis has lower toxicity as compared to the chemical synthesis, making the biological synthesis nanoparticles appropriate for biomedical uses (Harrison & Wong, 2024). Copper oxide nanomaterials are synthesized and applied by considering the environment in recent years (Turner & Nguyen, 2023). The microbial mediated synthesis strategies compliment the green synthesis strategies in the sense that the toxic reductants and stabilizers are not required all through the process and the reaction is carried out at ambient conditions as pointed out by Lewis *et al.,* (2024). Additionally, these biological synthesis methods provide nanoparticles with higher stability and minimal aggregation, which are issues that nanoparticle synthesis generally face (Jackson & Kim, 2023).

Given the accelerating problem of antimicrobial resistance, the current work concentrates on the biological synthesis of Ag and TiO_2 particles and their efficiency against bacteria (Williams & Thompson, 2024). Thus, the intention of this study is to explicate the synthesis parameters and characterization techniques that should help in designing effective strategies for eradicating bacterial infections (Peterson *et al.,* 2023).

2.0 MATERIALS AND METHODS

2.1 Materials

The chemical and reagents used are sodium hydroxide (NaOH), hydrochloric acid (HCl), silver nitrate ($AgNO_3$), (BDH chemicals, England), Titanium tetraisopropoxide [Ti $[OCH(CH_3)_2]_4$, ethanol (C_2H_5OH) and chloromethane (CH_3Cl), Nutrient Agar (Sigma-Aldrich, USA), distilled deionized water, Nutrient broth (Sigma-Aldrich, USA). All chemicals were of Analytical grade. Bacteria strains of *Pseudomonas aeruginosa* was collected from Microbiology laboratory, Science Laboratory Technology Department, Federal University of Petroleum Resources, Delta state, Nigeria.

2.2 Test organisms

The bacterial organisms like: *Bacillus subtilis, Bacillus anthracis, Escherichia coli* and *Staphylococcus aureus* used in assessing the biological efficacy of the synthesized silver (Ag) and titanium dioxide (TiO_2) nanoparticles were sourced from the Microbiology laboratory of Science Laboratory Technology Department, Federal University of Petroleum Resources, Delta State, Nigeria. These cultures were grown on nutrient agar slopes and incubated at 37 ^{0}C for 24 hours before being stored at 4°C for future use.

2.3 Methods

The method of synthesis used was done by adopting the process that was described by Jeevan *et al.,* (2012) with some amendment. The nutrient agar was then prepared, sterilized same as nutrient broth but cooled to around 45 ^{o}C. To prepared nutrient agar, a loopful of 24 hours test culture of *Pseudomona aeruginosa* was added and incubated at about 37 °C for 24 hours. Subsequently, the bacterial culture was pelleted and the supernatant was then used for the synthesis of Ag NPs and TiO_2 NPs. The collected supernatant of *Pseudomona aeruginosa* culture was dispensed in a different conical flask, to which aqueous $AgNO_3$ and Titanium tetraisopropoxide $[Ti\ [OCH(CH_3)_2]_4$ solution with concentration 0.001M and 0.02M 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 decrease of the silver and titanium ions in the solution by *Pseudomona aeruginosa* was done at certain time intervals by diluting 1ml of the reaction mixture. The reaction continue under the influence of a magnetic stirrer monitored by the UV-Visible spectrophotometer between 200 – 800 nm (Ganesh *et al.,* 2011). At this UV-visible range, nanoparticles were formed and reaction stopped at the highest observed wavelength on the UV-Vis spectrum. Further, physical observations of colour ranged from green to dark brown for Ag NPs and white for TiO_2 NPs. The harvested nanoparticles were purified by methanol and chloromethane in 1:1 proportion and gently washed with distilled deionized water. The synthesized nanoparticles were then centrifuged and subsequently oven dried at an optimum temperature of about 70 °C. figures 1 & 2 assisted give the flow diagram for methodology in the synthesis of Ag NPs and TiO_2 NPs respectively.

Figure 1: Process flow in the extraction and synthesis of Ag NPs modified from Jimoh *et al.,* (2022)

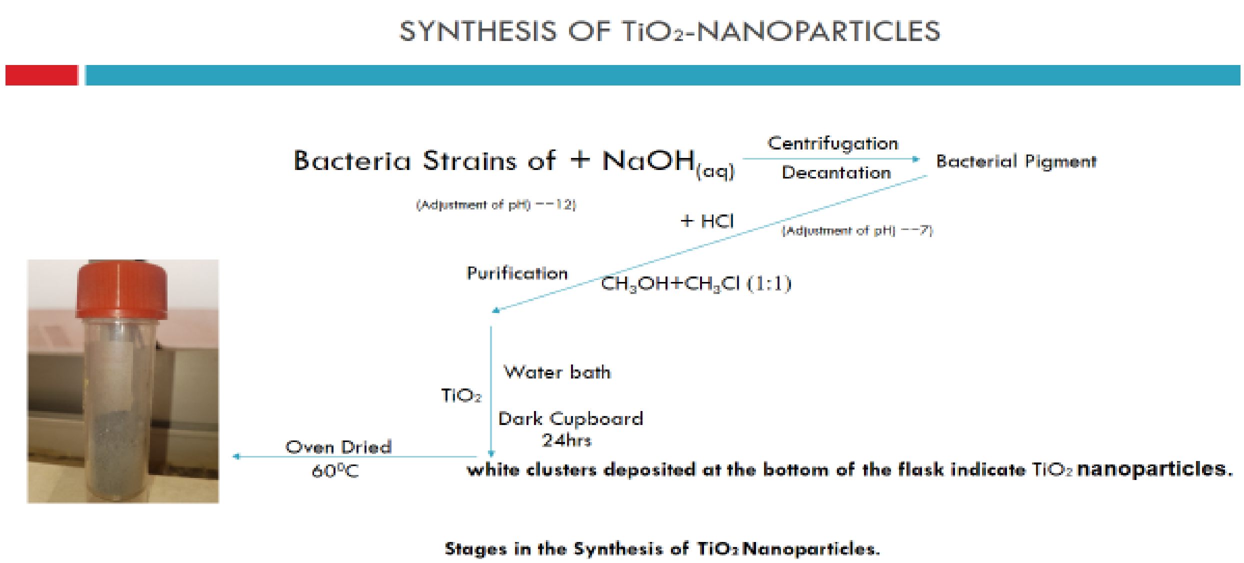

Figure 2: Process flow in the Synthesis of TiO_2 NPs modified from Jimoh *et al.,* (2022)

2.4 Antimicrobial Activities of Ag and TiO_2 nanoparticles

The effects of Ag and TiO_2 nanoparticles as antimicrobial agents were determined on both the Gram-positive and Gram-negative bacterial species by well agar diffusion method. Different concentrations of the nanoparticles were prepared with ciprofloxacin antibiotic as the standard control. The bacterial cultures were first cultured on nutrient agar slopes for 24 hours at 37 ^{o}C, then stored for 4 ^{o}C for conservation. In figure 3, they explain the layout of the antimicrobial susceptibility testing in the agar plate.

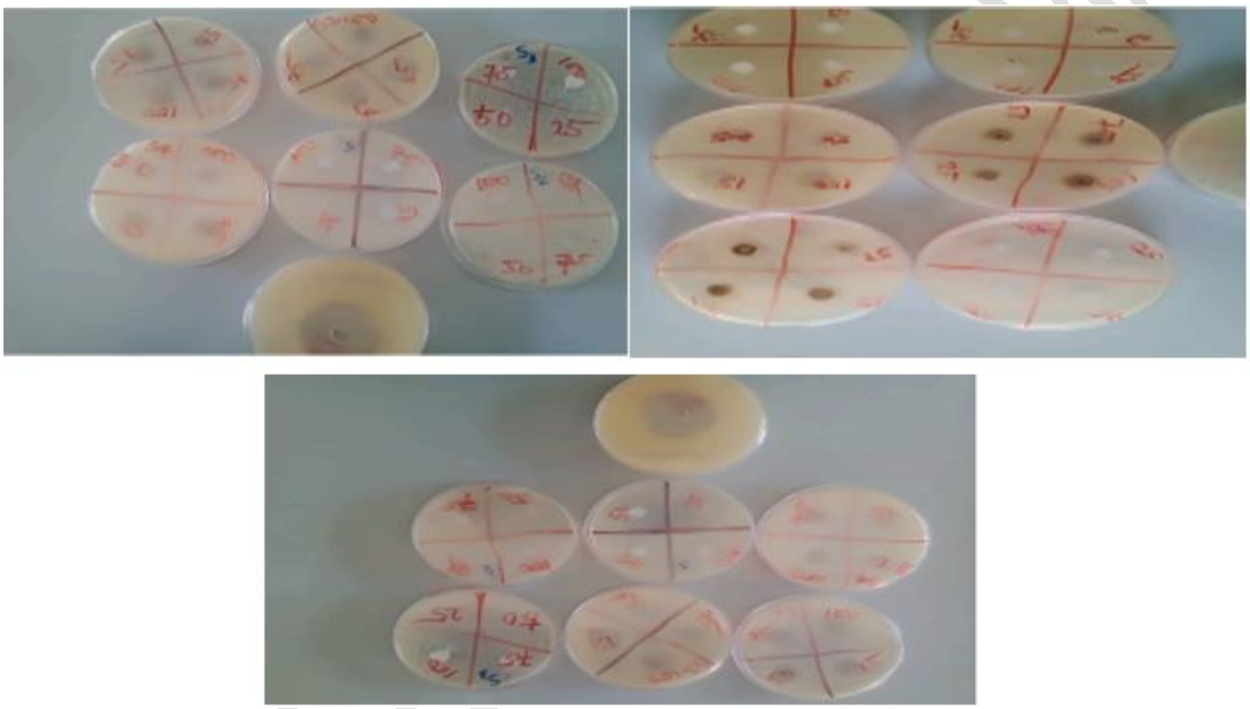

Figure 3: Antimicrobial analysis

3.0 RESULTS AND DISCUSSION

3.1 UV-Visible Spectra of Ag and TiO_2 Nanoparticles

The UV-Visible spectra of the synthesized Ag and TiO_2 nanoparticles are depicted in Fig 4 and 5 respectively scanned in the range of 300 to 550 nm. The spectra indicate one line absorptions at 416 nm for the Ag nanoparticles and 393 nm for the TiO_2 nanoparticles. These findings are in agreement with the effects mentioned by (Jimoh *et al.*, 2024; Mamdouh *et al.*, 2018) for silver nanoparticles and Abdallah *et al.*, (2016) for TiO_2 NPs. The down shift in the absorption bands is clear after the preparation of the nanoparticles and is an essential requirement for the nanoparticle products to function in their target applications such as biomedical and environmental uses. Consequently, the decrease in the concentration of the green colored Ag^{+} ions to Ag^{0} at the dark brown is according to Mamdouh *et al.*, (2018) suggested by the extracellular reducing agents produced by the bacterial supernatant of *Pseudomona aeruginosa*. This reduction process is very important because, in addition to it confirming formation of silver nanoparticles, it also supports the possibility of synthesizing nanoparticles using biological systems. The fact that bacteria are capable of generating reducing agents that can precipitate this change presents a platform on ‘green chemistry’ perspective in nanomaterials synthesis. UV–Vis spectroscopy technique is useful for characterizing the nanoparticles because the optical properties of the nanoparticles are highly dependent on the size and shape of the nanoparticles. Band positions detected at 416 nm and 393 nm are plasmon resonance effects associated with silver and titanium dioxide nanoparticles, respectively. It is particularly important for photocatalytic reactions including antibacterial performance.

In addition, the results of this study support the findings of prior other scientific works about exclusive dependence of optical characteristics of the nanoparticles on their size and morphology. For instance, smaller NPs tend to show a blue shift in its absorption spectra associated with the quantum confinement and larger NPs may red shifts. The results also establish the importance of employing natural reducing agents extracted from the biological materials and that could result in green synthesis approaches which are actually more preferable than the conventional chemical reduction processes that require use of toxic reagents. This biogenic method not only offers better sustainability but also eliminates or minimizes toxicity may result from chemical synthesis. The micro features at 416 nm for Silver and 393 nm for titanium dioxide correspond with the surface plasmon resonance phenomena confirming their possible use in different areas from antibiotic to photocatalysis Jimoh *et al.,* (2022).

$$\mathbf{Ag^{+}\ (green) + e^{-}\ (Enzymes\ from\ bacteria) \rightarrow Ag^{0}\ (dark\ brown)} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots..$$

(equation 1)

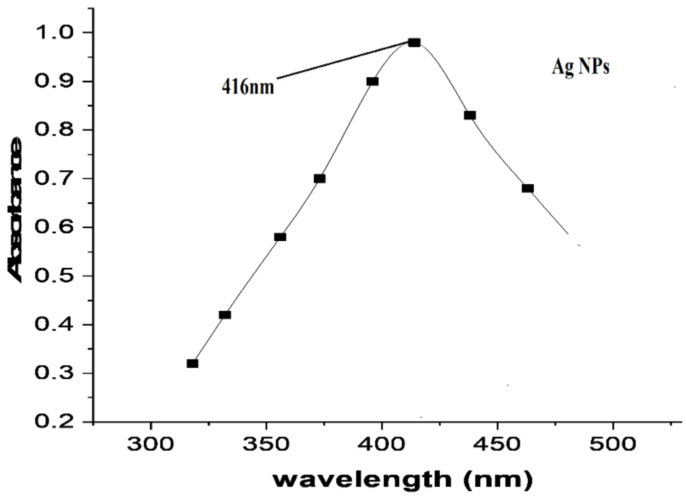

Figure 4: UV-VIS Spectrum for synthesized Ag NPs

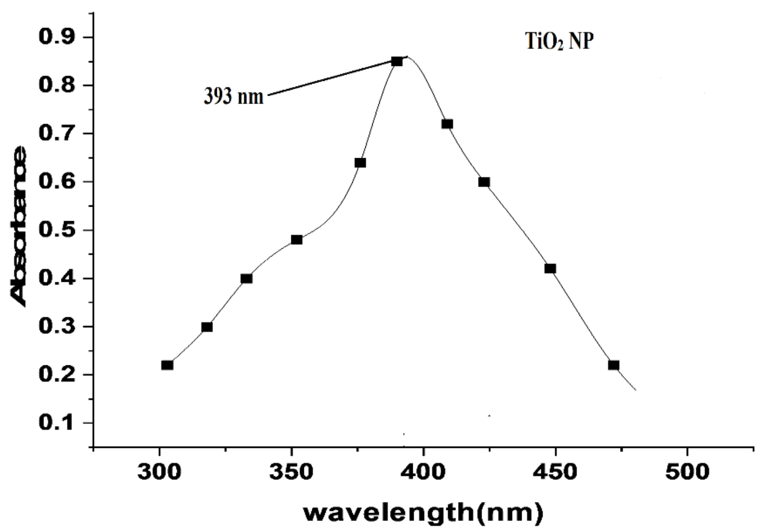

Figure 5: UV-VIS Spectrum for synthesized TiO_2 NPs

3.2 Powder X-Ray Diffraction Spectrometer (PXRD)

The crystallinity of the synthesized nanoparticles was analyzed by powder X-ray diffraction (PXRD) studies and the PXRD pattern of Ag and TiO_2 are shown in Figure 6 and 7 respectively. It can be seen from the PXRD pattern that there are different Bragg angles for the Ag nanoparticles at 38.56°, 45.50°, 65.70°, and 77.80° corresponding to the Miller indexes of (111), (200), (220) and (311) planes of face centered cubic silver. The relative intensity of these peaks shows the formation, single phase nature, and high crystallinity of the prepared silver nanoparticles, which is in agreement with the result reported by Akpeji et al. (2024) and Jimoh et al. (2022). The sharp diffraction peaks are apparent which assure that the synthesized silver nanoparticles have well defined crystal structures which are characteristic of face centered cubic arrangement found in metallic silver. This structural characterization is important since it gives a hint about the versatility of a nanoparticle especially in catalysis and biomedical applications where crystallinity brings about major changes in property of a material. Similarly, for the synthesized titanium dioxide nanoparticles, the PXRD analysis also support the crystalline nature of the synthesized nanoparticles.

The diffraction pattern indicated 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°that correspond to planes (101), (004), (200), (105), (211), (204), (116), (220) and (215) the anatase phase and this material has photocatalytic properties. By reason of these anatase/rutile structure inductions, the TiO2 nanoparticles photocatalytic activity boosts and it is thereby ideal for environmental cleaning and energy change over processes. This result is in agreement with (Hariharan *et al.*, 2017) in the synthesis and characterization of TiO_2 NPs.

They identified diffraction peaks in the current study that corresponds to those stated in the earlier studies, thus backing the synthesis method used. The outcomes also confirm that biological approaches for synthesis of nanoparticles can produce materials with favorable crystal structures, using only mild conditions and without the application of toxic precursors. Furthermore, the synthesized Ag nanoparticles also gave an average crystallite size by using the Scherrer equation and from the width of the diffraction peaks. This calculation is important in establishing the effect of particle size on properties like surface area as well as reactivity which are key factors in establishing the efficiency of nanoparticles for use in a variety of functions. Furthermore, it is possible to determine the lattice parameters of a crystal lattice when using PXRD data, which can give information on the strains and defects of the crystal lattice. Such information is crucial when trying to control the synthesis conditions for obtaining nanoparticles with specific characteristics. In this study, the synthesis method not only effectively synthesized the nanoparticles but also was green chemistry because it used biological materials to reduce metal ions. This maps to current discourses in nanotechnology, which entail green and sustainable approaches to production. In general, the PXRD pattern confirms that both Ag and

TiO_2 nanoparticles possess regular crystalline structures as required for their potential uses. The existence of regular crystallography planes indicate that these nanoparticles can potentially be used in multiple areas for instance in delivery of drugs, as antimicrobial coatings and photocatalysts.

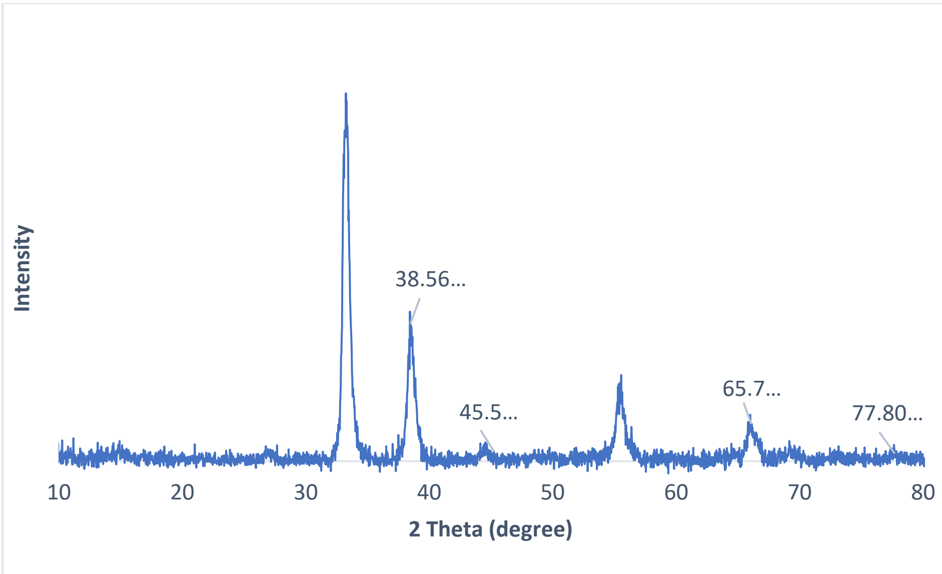

Figure 6: PXRD for Ag NPs

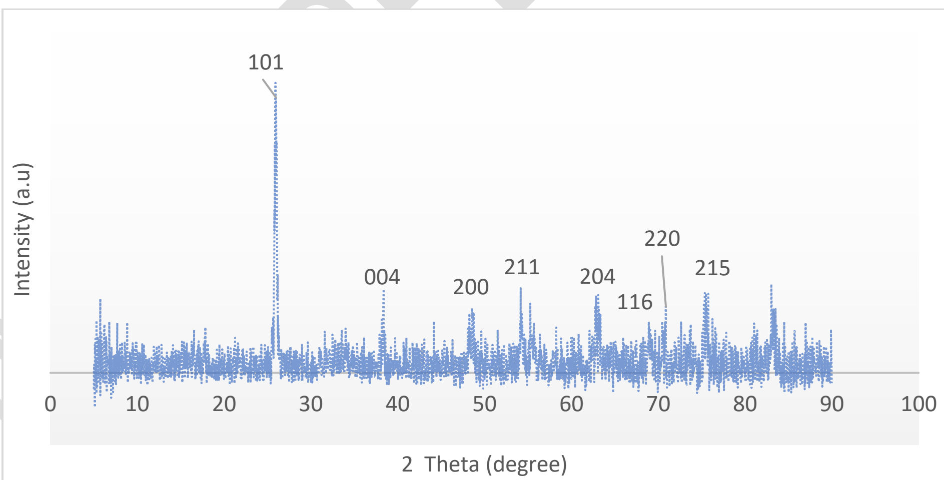

Figure 7: PXRD for TiO_2 NPs

3.3 Transmission Electron Microscope (TEM) Analysis

Characterization of the synthesized Ag and TiO_2 nanoparticles revealed that they were spherical, well-dispersed, without any agglomeration as presented in Figs 8 and 9. Information on the size distribution of the nanoparticles prepared here is given by the accompanying histogram. When estimated using Image J and Origin software the crystalline sizes of the synthesized particles are: Ag-7.27 nm, TiO_2 - 6.83 nm. It is however important to acknowledge that the particles of TiO_2 synthesized in this study are nominally small that can be described as quantum dots in regard to their dimension. By contrast, Zarina and Nanda (2014) synthesized spherical and poly dispersed Ag Nps from marine Streptomyces MS 26 having the size of 50-76 nm. This implies that the substrate used in our study reduced the particle size and thus the improvement of the effectiveness of the particles is highly likely to occur. From the transmission electron microscopy (TEM) micrographs more information concerning the morphology and the size distribution of the nanoparticles were elucidated. The study shown that the nanoparticles had the surface stability and the capping effect due to the biomolecular capping layer resulted from the proteins and peptides provided by microorganisms in the synthesis process. This biomolecular layer is crucial because it may help to avoid aggregation and can improve the stability of nanoparticles in solution. The tunable size, spherical shape, and narrowly dispersed size distribution are key elements that are significant to the prospective applications of these nanoparticles, most especially in the pharmaceutical and medical uses, where the particle size can impact drug biodistribution and effectiveness. Further, Ag and TiO_2 designed in the present nanoparticles are of smaller size; therefore, it gives a large surface area to volume, which is favorable for the catalytic activity and antimicrobial properties.

Further, the results of this study are consistent with other previous research about size and shape of nanoparticles influencing the physical and chemical characteristics. Nanosized particles have many more properties for their size at a optical, electronic, and magnetic level than the mass numbers, makes it more appropriate for nanoscale application. Besides stimulus of the general environment benefits the biological methods for synthesis of these nanoparticles also assist better control over size characteristics as compared to common chemical synthesis techniques. This biogenic approach can result in cost optimum processes oriented to the formation of few or non-toxic side products. The present study also validates the production of Ag and TiO2 nanoparticles and the ability of microbial systems in synthesizing nanoparticles. One advantage following the use of microorganisms is the ease in providing reducing agents**,** making the overall synthesis route more environmentally friendly besides allowing purity and crystallinity of products.

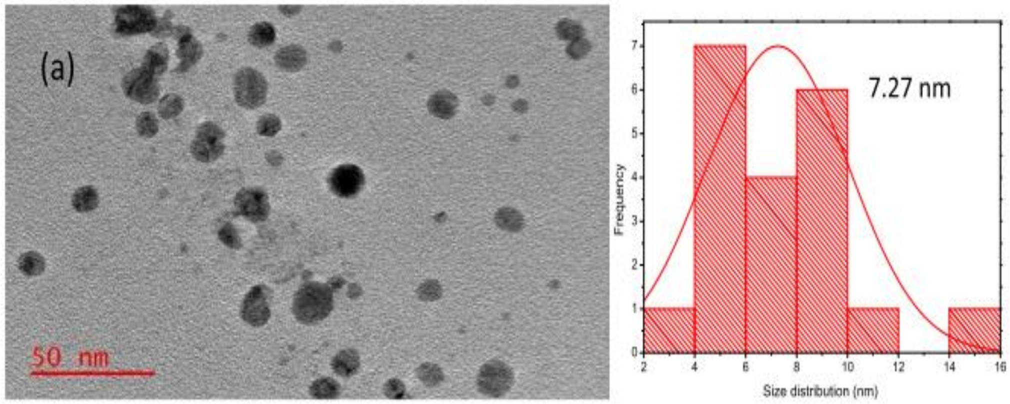

Fig 8: TEM Micrograph of Ag NPs

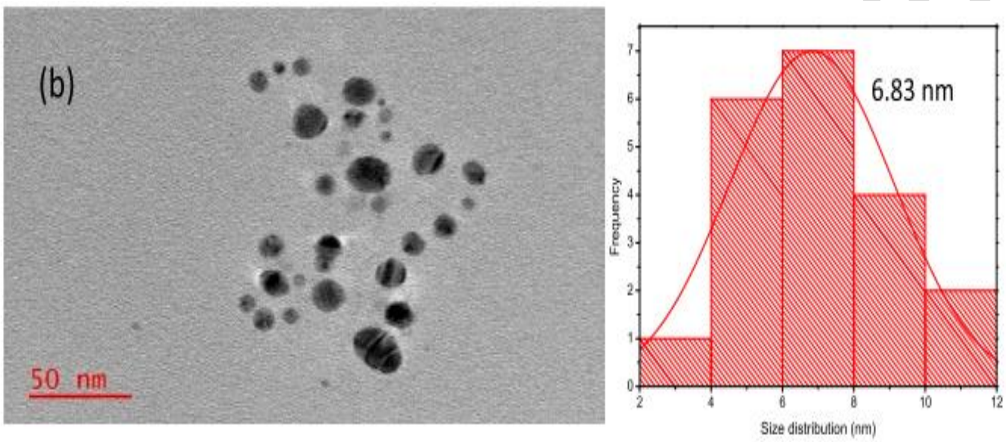

Fig 9: TEM Micrograph of TiO_2 NPs.

3.4 Fourier Transform Infrared (FTIR)

The Fourier Transform Infrared (FTIR) spectroscopy is used to determine the functional groups present on the surface of the synthesized silver (Ag) and titanium dioxide (TiO_2) nanoparticles for which Figures 10 and 11 are provided. FTIR spectroscopy was performed in order to identify the bio macromolecules involved in the synthesis and stabilization of both Ag and TiO2 NPs. The data presented in the current study also indicates that there are proteinaceous fractions that may be responsible for the stabilisation of the synthesized silver nanoparticles, as reported by Karthik *et al.,* (2014). In the FTIR spectrum of the synthesized Ag NPs the absorption bands were observed at 3422.72 cm^{-1}, 2900.11 cm^{-1}, 1654.10 cm^{-1} and 1384.31 cm^{-1} and the synthesized TiO_2 nanoparticles possessed bands at 3448.58 cm- 1,1636.94 cm- and around 1400 cm^{-1}. The broader bands of absorption detected at 3422.72 cm^{-1} for Ag NPs and 3448.58 cm^{-1} for TiO2 can be attributed to the N–H stretching of peptide bond and O-H stretching bonded to carboxylic group respectively (Devanand et al., 2013). Moreover, the bands at 1654.10 cm^{-1} and 1636 cm^{-1} are attributed to the bending of the amide from protein groups included in the samples. The band at positions 2900.11 cm^{-1} belongs to the stretching functional group –C=O. Indeed, from the following FTIR analysis, it can be concluded that capping proteins surround the nanoparticles, which are important for their synthesis by avoiding aggregation and improving stability (Afreen *et al.*, 2011). The existence of these functional groups is important for it not only immobilizes the nanoparticles but also alters the structures' behavior with biomolecules, possibly improving their antibacterial activity. These biomolecules play the most important role in stabilization of nanoparticles in solution which is very significant for such material in the frame of its applications.

Furthermore, the FTIR spectra data show that apart from capping proteins, functional groups whereby these metal ions may interact with during nanoparticle formation are also present. It was also established that differences in conformation which occur because of the bindings between proteins and metal ions can lead to nanomaterial properties. These results also support the previous studies on the role of biomolecular coatings in the stabilization and generation of better functional nanoparticles. For example, it is well documented that redox active proteins can mediates electron transfer that is considered crucial in catalytic reactions (Karthik *et al.*, 2014). Furthermore, biological methods for synthesizing nanoparticles are environmental friendly, often known as green chemistry in addition to offering greater control over nanoparticle attributes than chemical methods. This biogenic approach can result in innovations for processes that have little or no toxic waste by-products. These conclusions are not limited simply to description: the results presented indicate that biological systems could indeed be used to make nanoparticles for use in medicine, environmental remediation, and catalysis with mechanical properties tailored accordingly.

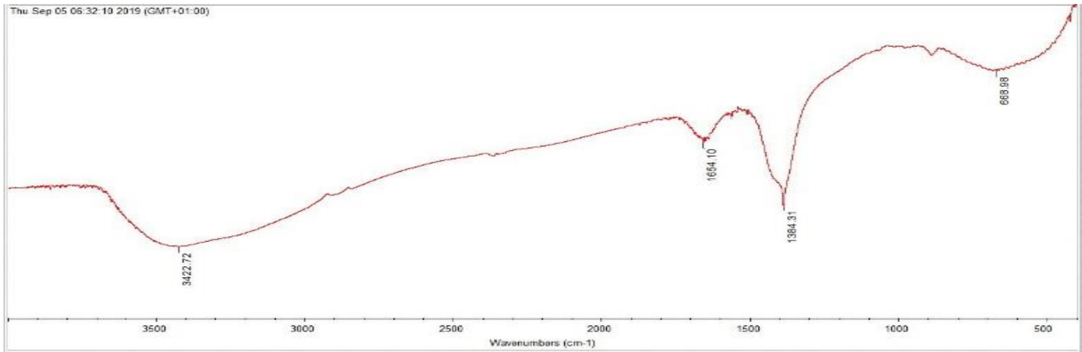

Fig 10: FTIR spectrum for Ag NPs

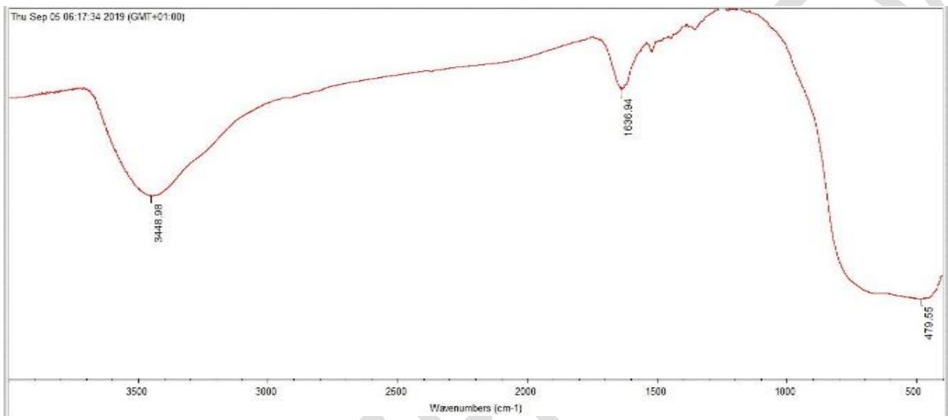

Fig 11: FTIR Spectrum of TiO_2 NPs

3.5 Energy Dispersive X-Ray (EDX)

The Energy Dispersive X-ray (EDX) analysis of the synthesized silver (Ag) and titanium dioxide (TiO2) nanoparticles provided the elemental composition results of the synthesized nanoparticles as details in figures 12 and 13. The optical absorbance spectrum of the Ag nanoparticles was found to be at a wavelength of 3 keV and that of the TiO_2 nanoparticles at 4.5 keV. These peaks corresponds well to metallic silver and titanium nanocrystals and are in agreement with what Magudapathy et al, (2001) and Kalyanasundharam et al, (2015) have obtained. Siddhardha et al., (2013) made similar observations to this result. There were other peaks associated with elements also found in the EDX spectra These include carbon, chlorine, silicon, sodium, tin, and oxygen. The presence of these elements is associated with the emission of chemical by-products from the capping proteins used in the manufacture process. The capping proteins are important since they keep the particles formed in the nanoparticles from coalescing and thus improve the stablility. The identification of these additional elements is important since it diversifies the distribution of the biological components that are involved in the synthesis process in the compositions of the nanoparticles. This biomimetic approach not only helps in the generation of nanoparticles but also in improving the functionality of the nanoparticles through the presentation of biomolecules. The peaks found in terms of EDX spectra agree with the synthesis method that aims at getting nanoparticles at the definite elemental ratio. Silver especially is relevant to this discussion as it is widely known for its antimicrobial properties which means applications can be made in fields such as medicine and even cleaning up the environment. In addition, the identification of carbon and other elements indicates that organic compounds from the capping agents are still anchored to the nanoparticles. This coherent organic layer can modulate the particles interaction with biological environment, which could enhance their bio-compatibility. Therefore, the results of this EDX analysis support the notion that methods for the synthesis of nanoparticles should be environmentally friendly. These simple structures can be made using bioactive materials to form nanoparticles that can display fine-tuned physical and chemical characteristics but cause no harm as do chemical synthesis techniques.

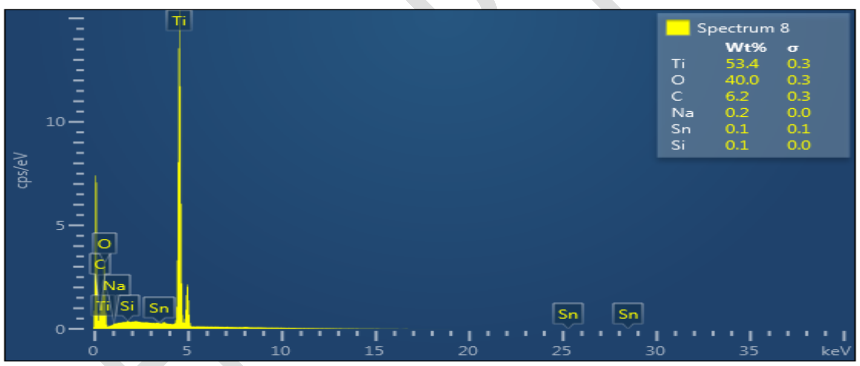

Fig 12: EDX Spectrum of Ag NPs

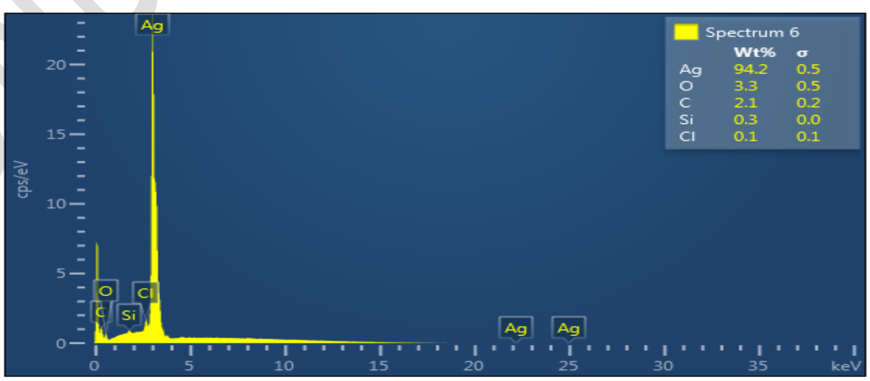

Fig 13: EDX spectrum of TiO_2 NPs

3.6 Scanning Electron Microscope (SEM) Analysis

The scanning electron microscopy (SEM) images of the synthesized silver (Ag) and titanium dioxide (TiO2) nanoparticles are shown in figures 14 & 15. These images are therefore useful to characterize the surface chemistry of the synthesized nanoparticles. At the SEM characterization it was observed that both Ag and TiO_2 are spherical in shape and possess a porous surface layer hence showing a clear view of physical morphology. This morphology is consistent with identifications by Thirunavukkarasu *et al.*, (2014), who observed similar features in their SEM investigation of TiO_2 nanoparticles. The observed porous nature in the Ag nanoparticles indicates possible use of the material in adsorbing molecules which could make it appropriate for use in applications including environmental cleaning and catalysis. The kind of nanoparticles is also important because this structure inherent in the sphere can be useful in increasing the surface area of particles, and thereby augment reactivity and interactions with other substances. This characteristic is especially useful in uses where the surface area is critical, for example in the medical field in treatises or even as a catalyst in some chemical reactions. Furthermore, the morphology ofthe Ag nanoparticles with pores on its surface may help absorbed biomolecules that can improve their stability and activity. This property is especially beneficial for biomedical applications because interaction with biological molecules can enhance the therapeutic effect. The SEM analysis results indicate not only the synthesis of Ag and TiO_2 nanoparticles but also manifest the dependence of their application potential on their morphology. Current advances in nanoparticle synthesis indicate that morphology of nanoparticles can be controlled through synthesis methods hence creating a premise of designing viable materials with required functionality.

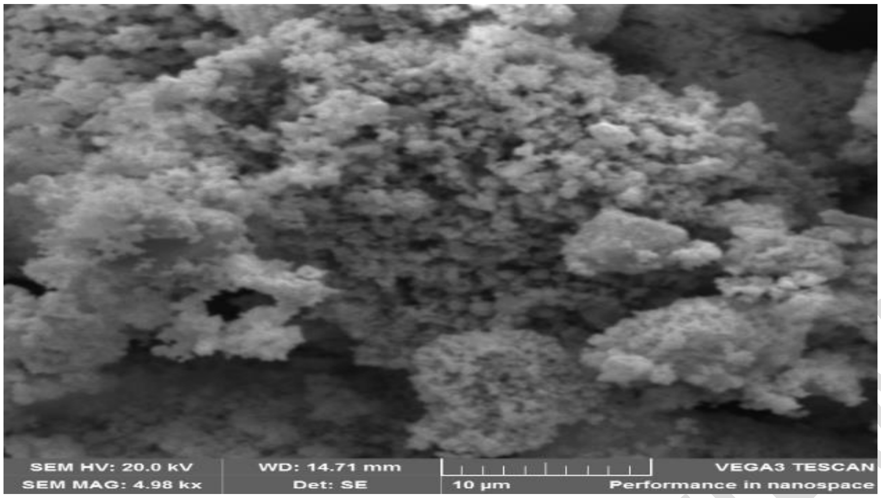

Fig 14: SEM Micrograph For Ag NPs

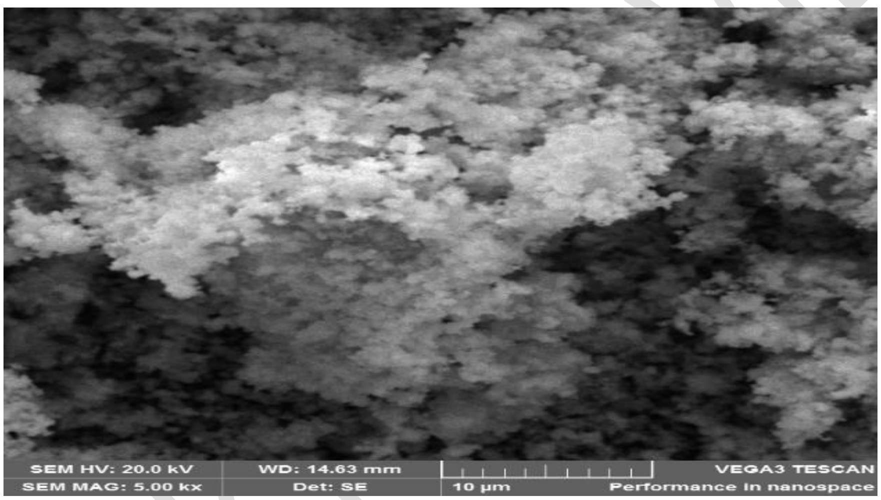

Fig 15: SEM Micrograph for TiO_2 NPs

3.7 Antimicrobials

Tables 1 and 2 show the antimicrobial activity of synthesized Ag and TiO_2 nanoparticles on selected gram-positive and gram-negative bacteria at a varying concentration of the nanoparticles. The antimicrobial activities were carried out using the well diffusion method. The inhibitory activity against these bacteria: *Bacillus subtilis, Bacillus antiphrasis, Escherichia coli, and Staphylococcus aureus,* is highest at the highest concentration of 1.0mg/ml of both nanoparticles. At 0.75mg/ml, 0.5mg/ml and 0.25mg/ml decreasing concentration of the both synthesized nanoparticles, the inhibitory activity also decreases respectively. This infers that the antibacterial activity is found to be concentration-dependent. This means that antibacterial activity increased as the concentration of the synthesized Ag and TiO_2 nanoparticles increases and vice versa. (Thirunavukkarasu *et al.,* 2014) reported similar results on the antimicrobial properties of TiO_2 nanoparticles on some bacterial. In this study, the antimicrobial activity of Ag and TiO_2 nanoparticles on *Bacillus subtilis, Bacillus anthrasis, Escherichia coli, and Staphylococcus aureus* was investigated. Ag and TiO_2 nanoparticles showed varying degrees of antibacterial activity against all of the pathogens. It is worthy of note that although there are reports of nanoparticles having superiority over traditional antibiotics (Wang *et al*., 2015) in terms of potency and antibacterial activity, this is true as this study also revealed that the Ag and TiO_2 nanoparticles were more potent than the ciprofloxacin antibiotic concerning *Bacillus subtilis, Bacillus anthrasis and Staphylococcus aureus* for silver nanoparticles and *Bacillus subtilis, Bacillus anthrasis* for TiO_2 nanoparticles at the same concentration.

Nanoparticles have increasingly been used to treat bacterial infections, thus widely encouraging research studies have been carried out on its adoption. (Wang *et al.,* 2015) posited that metallic nanoparticles can alter the metabolic activity of bacteria, thereby making it a potential candidate for disease management. Their mechanisms of action include making direct contact with microorganisms destroying their membrane structure; the release of metallic ions that bind to membrane surface proteins and deactivating them, and antibiofilm activity thereby inhibiting the establishment of biofilms by bacteria. Studies have shown that many nanoparticles can prevent or overcome biofilm formation, including nanoparticles made from silver (Yu *et al.,* 2016, Markowska, *et al*., 2013), Magnesium (Lellouche *et al*., 2012), Nitrates (Hetrick *et al*., 2009; Slomberg *et al*., 2013), Zinc oxide (Wang *et al*., 2015), Copper oxide (Chifiriuc *et al*., 2012) and

Ferrate (Lellouche *et al*., 2012; Wang *et al*., 2015). Their use in antibacterial activity date back to 4000BC when it was used in water treatment (Shamaila *et al.*, 2018).

Nanoparticles are presently being explored in the production of antibiotics for pathogen exclusion, cancer therapy, diagnostics, drug delivery (Wang *et al*., 2015), treatment of genetic diseases, and many more (Giasuddin *et al*., 2012, Jain *et al*., 2012, Misbahi, 2010). (Shamaila *et al.*, 2018) reported that they have very strong surface plasmon resonance (SPR), which makes them very useful in biotechnology applications. They emit very strong coherent oscillations of electrons on the surface of metallic particles, which are visible in the absorption spectrum.

Table 1: Antimicrobial Results for Ag NPs

	Zone of Inhibition (mm)		
Bacteria	**1.0mg/ml**	**0.75mg/ml**	**0.5mg/ml**
Bacillus subtilis	0 ± 0.01	19 ± 0.01	18 ± 0.02
Bacillus anthrasis	16.5± 0.02	15 ± 0.01	13.5 ±0.03
Escherichia coli	8 ± 0.01	6 ± 0.02	6 ± 0.01
Staphylococcus aureus	14.5 ±0.02	12 ± 0.01	13 ± 0.01
Control: Ciproflaxin injection	15 ± 0.02	14.5 ± 0.01	13 ± 0.03

Table 2: Antimicrobial Results for TiO_2

	Zone of Inhibition (mm)		
Bacteria	**1.0mg/ml**	**0.75mg/ml**	**0.5mg/ml**
Bacillus subtilis	30 ± 0.03	25 ± 0.02	22 ± 0.01
Bacillus anthrasis	26 ± 0.02	23 ± 0.01	20 ± 0.03
Escherichia coli	6 ± 0.02	4 ± 0.01	4 ± 0.02
Staphylococcus aureus	5 ± 0.01	3 ± 0.03	2.5 ± 0.03

Control: Ciproflaxin injection	15 ± 0.02	14.5 ± 0.01	13 ± 0.01

4.0 CONCLUSION

This research provides evidence for the synthesis of silver and titanium oxide nanoparticles from *Pseudomonas* spp. The color change of Ag NPs observed was from green to dark brown which proved the formation of the nanoparticles, while that of TiO_2 NPs was light white. The nanoparticles were characterized by UV-vis Spectrophotometer, FTIR, XRD, EDX, TEM, and SEM. These nanoparticles prove their resistance to bacterial growth by inhibiting them about the reports of other researchers.

Data Availability

All data generated or analyzed during this research study are included in this published article. The data were generated in the course of this research. Also, the data are available in the supplementary file.

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