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Green Synthesis of Titanium Oxide (TiO_2) Nanoparticles Using *Phyllanthus Niruri* and Assessment of Its Antibacterial Activity in Wastewater Treatment

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Abstract: *Given the existing and increasing need to reduce the volume of wastewater in the global society due to their potential threat to public health, this study was designed to synthesise titanium oxide nanoparticles (TiONPs) for the remediation of industrial wastewater contaminated by microorganisms. The TiONPs were synthesized using a green approach, which applied methanol extract Phyllanthus niruri as a precursor.The synthesized TiONPs were characterized by UV-Vis, FTIR, XRD, SEM, and TEM. The UV-Vis spectroscopy showed maximum absorption peaks at 401 nm and 569 nm resulting from the excitation of titanium oxide NPs. Fourier Transform Infra-Red (FT-IR) revealed the presence of alcohol, phenols and carboxylic acids and therefore showed possible interaction between the nanoparticles and the phytochemicals in the plant extract. the The O-Ti-O bonds vibrational band at 470 cm^{-1} due to anatase confirmed that the product of the synthesis is TiONPs. the The nanoparticles and also displayed a spherical shape and a tetragonal geometry with particle sizes ranging from 20-100 nm in diameter (The TiONPs showed a significant antimicrobial activity by decreasing the the colony forming unit (CFU) of Escherichia coli from 3CFU/50 ml to zero CFU/50 ml. The minimum inhibitory concentration (MIC) value of titanium oxide nanoparticles against bacteria was 12.50 µg/ml (Escherichia coli). The presence of the TiONPs correlated shifted the minimum bactericidal concentration (MBC) value to 25.00 µg/ml (Escherichia coli) The synthesized nanoparticles are therefore documented as an excellent antimicrobial agent against Escherichia coli, which is a known organism responsible for several health challenges through water.*

Keywords: *Environmental health, water, microorganism, remediation, TiONPs, photodegradation*

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1.0 Introduction

Water is one of the essentials of life, but due to exponential population growth and rapid development of industrialization, large volume of industrial and municipal wastewater are being generated daily, the resultant effect is continuous deterioration of quality water resources to plants and animals. Water is a key resource that must be protected and monitored because, according to the World Health Organization (WHO), poor water quality causes approximately 30% of all deaths worldwide (Belhassan, 2021). Globally, a major portion of drinking water sources are found to be contaminated with various toxins and pathogenic microbes, mostly due to the release of untreated man-made wastes or wastewater to the water sources (Priya *et al*., 2021). Water pollution has adverse effect on the environment, and can also be responsible for air pollution that impacts very negatively on human health. Water pollution also negates on the socio-economic growth and development of the affected society or country. Recently, A United Nation report stated that purified and freshwater availability is a global issue, and have remain a challenge in the twenty first century, because the survival of living creatures is guaranteed without these been addressed (Mishra and Hankankin, 2018).

Microbial contamination is the most common and widespread health risk associated with drinking water. When a pollutant such as microbes, gets into water body, most of the time it deteriorates and makes the water unhealthy and reduces the water's beneficial uses (Halder and Islam, 2015). Different types of treatment have been utilized to get rid of contaminations from wastewater before the discharge into the ecosystems (River *et al*., 2019).

Nanotechnology offers the potential for the development of alternative technologies for wastewater treatment (Eddy *et al*., 2023a-c. Nanotechnology involves the use of nanoscale materials that are generally between 1 and 100 nanometers in size (Khan *et al*., 2021). Nanotechnology, like microtechnology, involves materials defined by a size scale, but unlike micromaterials, nanomaterials are capable of possessing remarkable properties that deviate dramatically from the bulk of the parent material. Nanomaterials typically have a high reactivity and degree of functionalization, large specific surface area, and size dependent properties, etc., which makes them suitable for applications like wastewater treatment, as well as for water purification (Osagie *et al*., 2021). Greener approaches for production of nanomaterials are being adopted to minimize the use of unsafe reagents and maximize the efficiency of chemical processes (Gilbertson *et al*., 2015; Garg *et al*., 2022).

Titanium oxide (TiO_2) is one of the most important chemically stable nanoparticles that has received the most attention as a photocatalyst and adsorbent in the removal of pollutants from wastewater (Khashan *et al*., 2021; *Maiti,* et al*., 2019*). The superseding roles of some metal oxide nanoparticles such as Fe_3O_4, TiO_2, and ZnO in the purification of water contaminated by diverse contaminants have been investigated and reviewed in several works (Abbas , 2020; Odoemelam *et al*., 2023; Puri, and Gupta, 2023). Improvement has also been achieved through the use of composite titanium oxide materials such as Mn-TiO_2 (Stucchi *et al*., 2018), bentonite-TiO_2 (Sreekala *et al*., 2022) . As a general rule, a good adsorbent is characterized by a high surface area, high porosity, smaller pore diameter, thermal/chemical stability, re-usablity and other factors (Eddy *et* al, 2023a-b). Unfortunatly, most classical materials can meet one or more of the listed and other requirements but the fail to be an efficient adsorbent because of where they fail. It has been reported that the best set of adsorbents that are ecofriendly, relatively cheaper (when cost and advantages are compared) and accessible are the nanoparticles (Eddy *et* al., 2022a-b). TiONPs is one of the best known adsorbent for a variety of purposes. They are also useful as photocatalysts and are widely assumed to be the most common photocatalysts because of low toxicity and stability as well as high surface area and super paramagnetism (Singh *et al*., 2013: Patanjali *et al*., 2019). Photodegradation is one of the most cleaner technology in the processing of organic contaminants because it convert organic materials to harmless forms (Eddy *et al*., 2023a-b). The most interesting role of TiONPs as a catalyst for the phototdegration of microorganism has been reviewed and TiO_2NPs have shown strong tendency to completely eliminate microorganism in water becaue through the adsorption of light, it facilitates the generation of active species such as superoxide anion ((O_2^-) , hydrogen peroxide (H_2O_2), hydroxyl radicals (OH^-) and penhydroxyl radicals (HOO*). These species are capable of destroying the organic wall of microorganism (Han *et al*., 2016; Miller *et al*., 2012)

Green synthesized nanomaterials induce less detrimental effect on the environment and human health, compared to those by chemical methods. This approach has brought about tremendous applications of nanoparticles in various fields ranging from drug delivery in medicine and environmental remediation among others (Shaumbwa *et al*., 2021). The naturally occurring materials for biogenic synthesis include plant extracts and microorganisms (Anthony *et al*., 2017; Moghaddam *et al*., 2017)

2.0 Materials and Method

2.1 Collection and preparation of Phyllanthus niruri leaf extract

Fresh *Phyllanthus niruri* leaves were collected from (20 g) were collected from a garden at Sabon-gari Local Government Area, Kaduna State, Nigeria. The *Phyllanthus niruri* leaves were well-washed with water to remove the dust particles from their surface. The leaves were further rinsed with the deionized water followed by the drying process at room temperature. The leaves were kept at room temperature for 15 days and transferred to the grinder to crush into a fine powder. The powdered sample 2.00 g was carefully weighed using the analytical weighing balance and mixed with 100 cm^3 of methanol to obtain the hydro-alcoholic crude extract using an Erlenmeyer flask at room temperature for 3 days. After 72 hours, the filtrate was separated from the marc by using filter paper (Whatman No. 1). The obtained filtrate was stored for further analysis.

2.2 Collection of wastewater sample

The wastewater sample was collected from Sunseed Nigeria Limited, located at old Jos Road Dekace village Zaria, Kaduna State. The wastewater was collected on 25th January 2022 at about 10:00 a m in a 2000 ml sample bottle and was stored at 4^oC for further analyses.

2.3 Quality assurance

All reagents used are of analytical grade, including distilled water. All the glassware, and sample bottles were washed with liquid soap, rinsed with distilled water, soaked in 10% HNO_3 for 24 hours and rinsed thoroughly with distilled water and thereafter dried (Todorovi *et al*., 2001).

2.4 Green Synthesis of TiONPs

Green synthesis of the TiO_2 nanoparticles was achieved by following the method of Kalaiarasi *et al.* (2013) with some modifications. A 0.99 g amount of Titanium oxide was weighed and mixed with 250 cm^3 of distilled water. To the mixture, 25 cm^3 of the prepared extract of *Phyllanthus niruri* was added. After that, the mixture was stirred for four hours using a magnetic stirrer while stirring sodium hydroxide (1M) was added dropwise to adjust the pH to 7 and to increase the yield of titanium oxide nanoparticles, a colour change from light green to white was noted. The solution was then filtered using Whatman No 1 filter paper to separate the nano-sized particles from the suspension, the powder obtained was dried by using a hot air oven and stored for further analysis.

2.5 Characterization of the synthesized TiONPs

Fourier transforms infrared (FTIR) spectroscopy was used to examine the functional groups of the metabolites present on the surface of the nanoparticles, which might be responsible for the reduction and stabilization of nanoparticles. The optical absorption ability of green-treated synthesized titanium oxide nanoparticles was analyzed using UV–Vis. The crystal structure characterization of the synthesized titanium oxide nanoparticles was done through X-ray diffraction (XRD) spectrometry. The surface morphology and size of the synthesized TiO_2 NPs were examined using a Scanning Electron Microscope (SEM) at higher resolution with different magnifications and a Transmission electron microscope (TEM) was used for morphological observation of the synthesized TiO_2 nanoparticles.

2.8 Antimicrobial studies

This was done by the Kirby-Bauer method on Muller-Hinton agar plates (Merck-German) as recommended by the 2011 Clinical and Laboratory Standards Institute guidelines following the procedure of Pereira *et al*. (2009).

2.8.1 Microbiological analysis

Microbiological analysis of the wastewater samples to carry detection, isolation and enumeration of the bacteria was determined using heterotrophic plate count (HPC) by the spread plate method, at 37 °C for 24 h following the technique described by the American Public Health Association APHA (1989) and the procedures of Chigbu and Sobolev (2007) and Douterelo *et al.,* (2014) were adopted. All the plates were incubated at 37 °C for 18 - 24 hours. MacConkey agar which is a selective/differential medium was used to determine the lactose fermenting and non-lactose fermenting organisms. The media for isolation and characterization were prepared in conical flasks. The flasks were then corked with cotton wool and wrapped with aluminium foil and sterilized by autoclaving at 121 °C for 15 min.

2.8.2 Enumeration of bacteria by total viable cell count

The total viable cell count of the collected wastewater sample was determined using the spread plate method. The wastewater samples were serially diluted to thin out the microbial population density of bacteria. To obtain a stock solution of the wastewater sample, 0.1 cm^3 of the wastewater sample was measured and aseptically transferred onto a flask containing 0.9 cm^3 of sterile normal saline. The contents were thoroughly shaken, and the microorganisms were allowed to disperse on the sample with pre-sterilized pipettes before serial dilution to 10^5 from each 10^5 dilution. The dilution was then aseptically transferred onto a sterile nutrient agar plate, and the inoculum was spread on the surface of the culture media with a ben glass rod (hockey stick).

The inoculated plates were then incubated for 18-24 hours at 37 °C. Colonies were counted at the end of the incubation period and the result was expressed as colony forming unit per millilitre (cfu/ml), using the formula below:

$$Cfu/ml = \frac{total\ number\ of\ colonies\ counted \times dilution\ factor}{volume\ of\ inocula} \quad (1)$$

2.8.3 Gram staining

A loopful of culture was collected with a sterile wire loop and placed on a clean glass slide to be gram-stained, gram-positive and gram-negative organisms were detected, those that are decolourized and remain purple (the colour of the crystal violet) are known as gram-positive bacteria. Gram-negative bacteria are those that lose their purple color but retain their safranin color. Gram-positive organisms are purple, while gram-negative organisms are red, according to the observation. Motility, catalase, Voges-Proskauer (VP), and indole tests were also performed using standard methods at Ahmadu Bello University, Zaria's Microbiology Department

2.8.4 Evaluation of the microbial activity of synthesized TiONPs on waste water

Phyllanthus niruri-mediated titanium oxide nanoparticle was tested for photocatalytic purification of industrial wastewater contaminated with various bacteria. Membrane filtration and culturing in nutrient agar media were used for bacteria detection and enumeration. About 30 mg of the synthesized titanium oxide nanoparticles were dispersed into 150 cm^3 of the wastewater sample. The mixture was stirred gently with the aid of a magnetic stirrer under natural sunlight for three hours, and then of 50 cm^3 sample was drawn hourly then filtered through 0.45 µm membrane filters (47 mm diameter) under vacuum. The filtrate was then placed on a petri dish containing the culture media and was incubated for 24 hours at 37^{0}C. The colonies forming unit (CFU) were counted manually the procedure was as described by Wagutu *et al.* (2019) with slight modification.

2.8.5 Determination of inhibitory activity (sensitivity test) of the TiONPs using agar well diffusion method

The antimicrobial screening was carried out using the agar well-diffusion method as described by CLSI (2021) with slight modifications. The standardized inocula of the bacterial isolates were streaked on sterilized Mueller Hinton agar plates with the aid of a sterile swab stick. Four wells were punched on each inoculated agar plate with a sterile cork borer. The well was properly labelled according to different concentrations of the extract prepared, which were 50, 25, 12.5, 6.25 mg/ml respectively. Each well was filled up with approximately 0.2 cm^3 of the extract.

The inoculated plates having the extracts were allowed to stay on the bench for about one hour; this is to enable the extract to diffuse on the agar. The plates were then incubated at 37 ^{0}C for 24 hours. At the end of the incubation period, the plates were observed for any evidence of inhibition which appeared as a clear zone that was completely devoid of growth around the wells (zone of inhibition). The diameter of each zone was measured using a transparent ruler calibrator in millimeters and the result was recorded.

2.8.6 Determination of Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration of the extract was determined using the tube dilution method with the Muller Hinton broth used as diluent. The lowest concentration of the extract (7.50 µg/ml) showing inhibition for each organism when the extract was subjected to a sensitivity test was serially diluted in the test tubes containing Mueller Hinton broth. The standardized organisms were inoculated into each tube containing the broth and extract. The inoculated tubes were incubated at 37^{0}C for 24 hours.

At the end of the incubation period, each of the tubes was examined /observed for the presence or absence of growth using turbidity as a criterion; the lowest concentration in the series without visible sign of growth (turbidity) was considered to be the minimum inhibitory concentration (MIC).

2.8.7 Determination of minimum bactericidal concentration (MBC)

The result from the minimum inhibitory concentration (MIC) was used to determine the minimum bactericidal concentration (MBC) of the extract. A sterilized wire loop was dipped into the test tubes that did not show turbidity (clear) in the MIC test and a loopful was taken and streaked on sterile nutrient agar plates. The plates were incubated at 37^0C for 24 hours.

3.0 Results and Discussion

3.1 Characterization of the Synthesize TiONPs

Fig. 1 and 2 shows the FTIR and UV visible spectra of the synthesized TiONPS. The FTIR reveals three major peaks at 3356, 1034 and 470 cm^{-1}. FTIR analysis was used to find out the reduction of TiO_2 nanoparticles by biomacro molecules present in the plant extract. These biomacro molecules are responsible for the reduction and stabilization of TiO_2 nanoparticles. Figure 3.1 shows the FT-IR spectrum of *Phyllanthus niruri* mediated TiO_2 NPs. The band at 3355 is associated with hydroxyl (O-H) groups, and it was assumed that this hydroxyl group is responsible for the photocatalytic activity of nanoparticles. The band at 1034 is associated with the carbonyl (C=O) group (carbohydrate), and the bands associated with the vibration mode of O–Ti–O bonds of the anatase phase at the wave number of around 470 cm^{-1} confirmed the presence of TiO_2 nanoparticles. These characteristic absorption peaks suggested the presence of extract-derived phytochemicals (such as carbohydrates, phenols and flavonoids.) on the surface of the nanoparticles that were responsible for the capping and efficient stabilization of titanium oxide nanoparticles thus protecting them from aggregation.

Other studies show that the bands centred at 1621 cm^{-1} and 3354 cm^{-1} are the characteristics of surface-adsorbed water and hydroxyl groups (Mishra *et al.,* 2014). According to Dobruka (2017), a band at 1024 cm-1 indicates the presence of C-O stretching alcohols, carboxylic acids, esters, and ethers in biosynthesized *Echinacea purpurea* herb mediated with TiO_2 NPS.

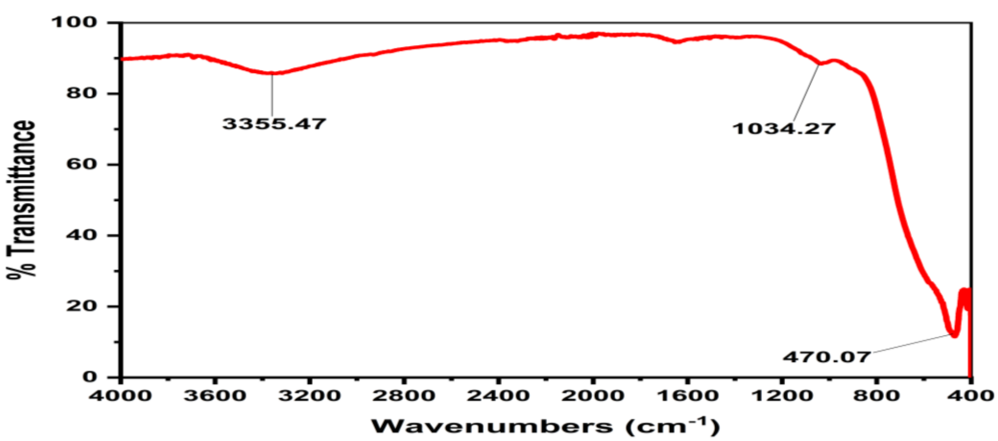

Fig.1: FTIR spectrum of the synthesized titanium oxide nanoparticles

he UV spectrum of the prepared biosynthesized TiONPs is shown in Fig. 2. The spectrum displays absorption within a wavelength range of 400 to 800 nm and reveals absorption maxima at 569 nm for the *Phyllanthus niruri* mediated titanium oxide nanoparticles which inferred that the *Phyllanthus niruri* mediated TiONPs have high absorbance peak observed due to the radiative recombination of self-trapped excitation and the capping of their surfaces with some phytochemicals from the plant extract (Abdullah *et al.,* 2018). This deduction is supported by observed literature providing information such as 384 nm (Al-Hamdani *et* *al*., 2016) and 324 nm (Durai, *et al*.m 2021). The results further demonstrate that the synthesized nanoparticles absorb maximally in the ultraviolet region, which is one of the requirements for a good photocatalysts.

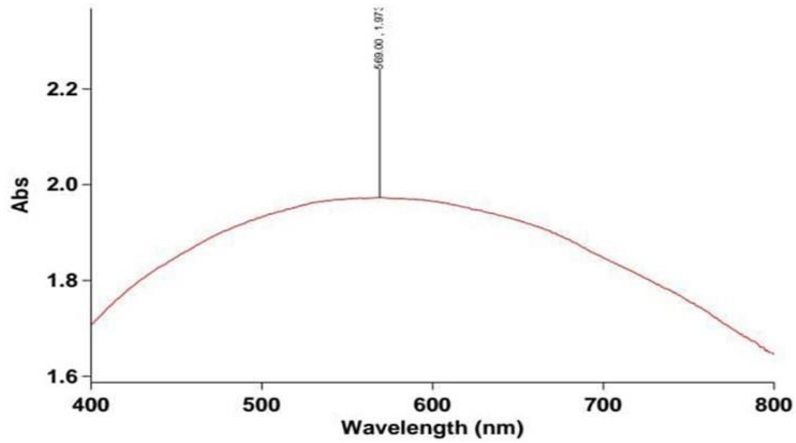

Fig. 2: UV visible spectrum of the synthesised TiONPs synthesised

The bandgap of the synthesised TiONPs was evaluated using Planck's equation (equation 2) (Ogoko *et al*., 2023)

$$E_{BG} = \frac{hc}{\lambda_{max}} \quad (2)$$

The numerical value of the Planck constant $(h = 6.63 \times 10^{-34}\, J.s^{-1})$, the speed of light $(c = 8.0 \times 10^{8}\, m.s^{-1})$ and the evaluated λ_{max} were inserted into equation 2 and the E_{BG} obtained (2.18 eV) shows an excellent semiconductor or conductor character that is also expected to function as an excellent photocatalyst. Also, when the evaluated bandgap is compared to literature values such as 3.23 eV (Na-Phattalung *et al*., 2022). Based on the research output published by Dette *et al*. (2014), normal TiONPs have a major challenge as a photocatalysts due to the larger band gap that does not allows it to absorbs in the visible spectrum. Consequently, green synthesis adopted in this study showed a significant enhancement in the photocatalytoc ability of the TiONPs

The UV spectra of the prepared biosynthesized TiO_2 nanoparticles and the commercial titanium oxide nanoparticle are represented in Figure 3.2, the results indicated that the UV spectra display maximum absorption in the vicinities of 400 -800 nm. The spectrum showed the formation of peak at wavelength of 569 nm for the *Phyllanthus niruri* mediated anium oxide nanoparticles. The absorption is 5.250. The UV-Vis spectra of plant extracts of *Phyllanthus niruri* mediated titanium oxide nanoparticles have high absorbance peak observed at 569 nm is due to the radiative recombination of self-trapped excitation (Abdullah *et al.*, 2018). It is a condition where the electron-hole pair loses the ability to move across the crystal lattice.

Fig. 3 shows the XRD pattern of synthesized titanium oxide nanoparticles mediated with *Phyllanthus niruri* leaf extract. The spectrum reveals distinct diffraction peaks, indicating the presence of a well-crystallized structure. The diffraction peaks were observed at 2θ angles of approximately 28.0^{o}, 36.1^{o}, 41.0^{o} and 55.0^{o} these peaks correspond to (110), (101), (111) and (211) crystallographic planes, respectively of the tetragonal rutile structure of TiO_2 NPS which is in agreement with JCPD (Joint Committee on Powder Diffraction Standards) card no:- 00-002-0494. This indicates that the synthesized nanoparticles possess a tetragonal rutile crystal structure, which is one of the most stable and widely studied phases of titanium oxide. The presence of this phase confirms the successful synthesis of titanium oxide nanoparticles using *Phyllanthus niruri* mediated green synthesis. Ganapathi *et al.*, (2015) synthesized TiO_2 NPS from orange fruit waste and discovered that the XRD pattern was tetragonal rutile structure.

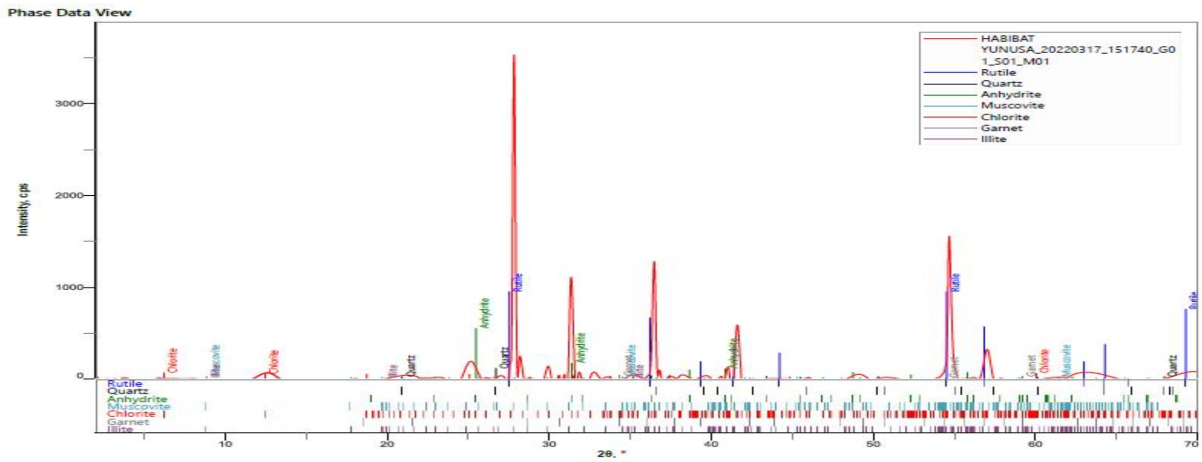

Fig. 3: XRD of the synthesized TiONPs

In Fig. 5, the scanning electrone micrograph of the synthesized nanoparticles is shown. The formation of spherical-shaped titanium oxide nanoparticles mediated by *Phyllanthus niruri* was is clearly manifested in the micrograph. There, it can be inferred that the reduction of TiO_2 by *Phyllanthus niruri* leaf extract resulted in the formation of spherical-shaped nanoparticles with particle sizes ranging from 20 to 100 nm. Previous reports have confirmed that phytochemical compounds (from plant extracts) that are trapped in nanoparticles are often disaggregated and and can lead to more stable with good dispersibility as observed in this study (Prathnaa *et al.*, 2010) Therefore, the leaf extract of the used plant contains phytochemicals that might have prevented aggregation.

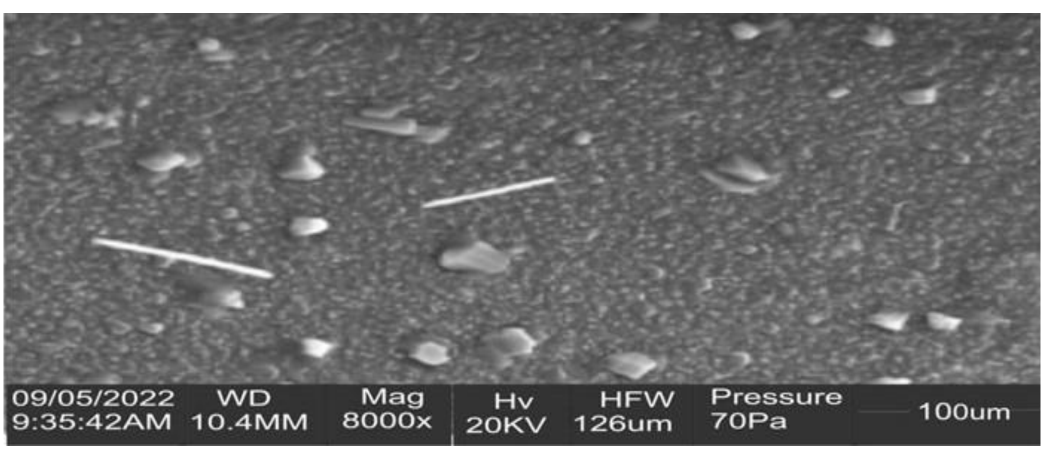

Fig. 4 Scanning electron micrograph of the synthesized titanium dioxide nanoparticles at magnification 100 µm

The formation of well-agglomerated *Phyllanthus niruri mediated* titanium oxide nanoparticles is shown in figure 3.7, 3.8 and 3.9. The TEM image reveals that most of the *Phyllanthus niruri mediated* titanium oxide nanoparticles were in spherical in shape with the particle size of about 20–100 nm. The particle size of *Phyllanthus niruri mediated* titanium oxide nanoparticles agreed well with the SEM micrograph. Ghulam *et al.* (2020) carried out green synthesis of TiO_2 NPS using lemon peel extract; their optical and photocatalytic properties. The TEM image showed a spherical shape with a particle size of 100 nm.

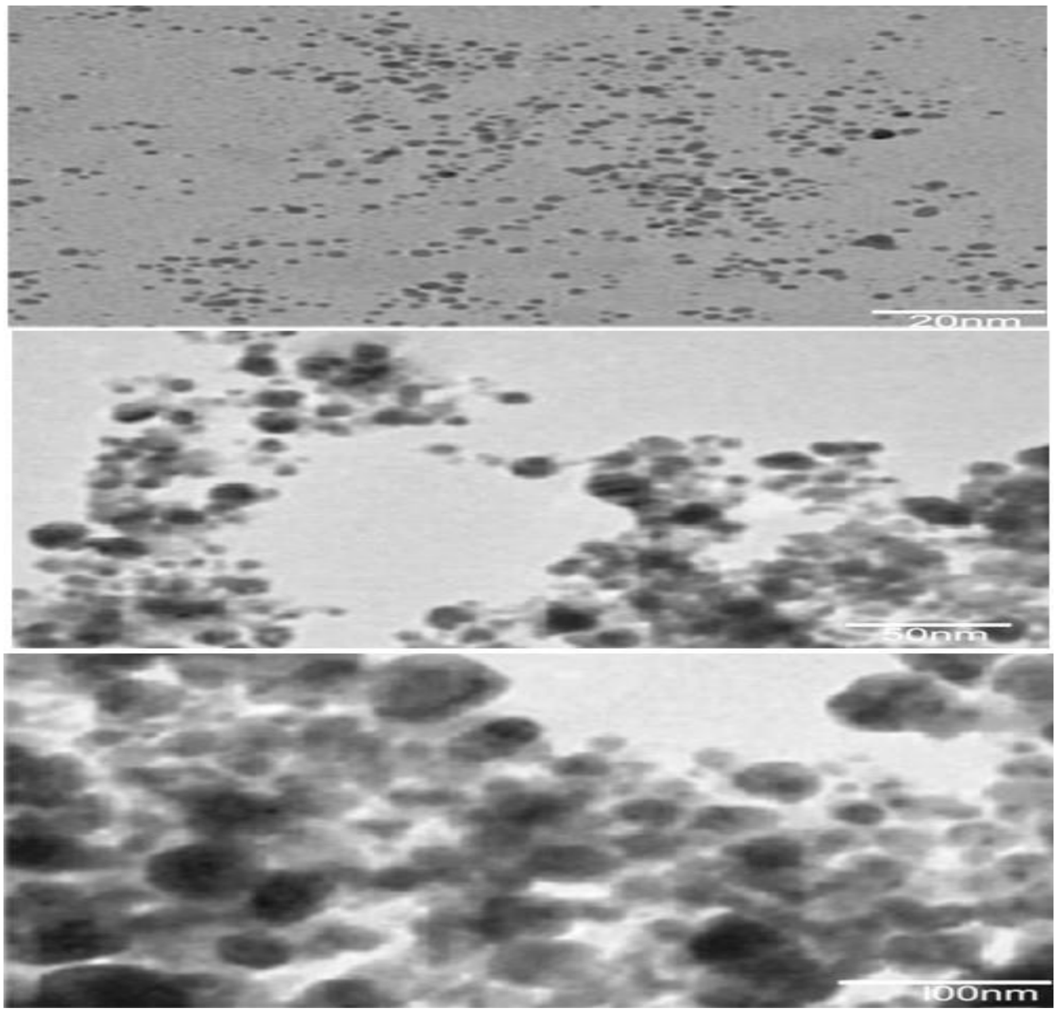

Fig. 5: Transmission electron micrography of the synthesized titanium dioxide nanoparticles at different magnifications (100, 500 and 1000 nm

3.2 Antimicrobial studies
3.2.1 Characterization of bacterial isolates

Table 1 shows the characteristics of the bacteria and coliform species. When cultured on nutrient agar plates, the wastewater sample displayed different morphological characteristics and reacted differently to the diagnostic biochemical tests. Gram positive bacteria (*Staphylococcus aureus* and *Bacillus Subtilis*) and gram negative bacteria (*Escherichia coli, Klebsiella,* and *Salmonella*) were isolated from wastewater.

Table 1: Characteristics of the bacteria and coliform species

Bacteria	**Shape**	**Gram stain**

Bacillus species	Long rod	+
Staphyloccocus species	Cocci	+
Klebsiella species	Short rod	-
Salmonella species	Short rod	+
Eschericha coli	Short rod	-

**** (+) = positive and (-) = negative**

3.2.2 Bacteriocidal analysis of the wastewater sample before and after treatment

The numerical estimates of bacteria from the primary culture revealed that the wastewater sample contains a high microbial load. The highest number of bacterial colonies 2.18×10^7 cfu/ml, could be attributed to rapid microorganism proliferation due to feeding on organic matter present in the effluent. The number of bacterial colonies with the lowest value was 4×10^7 cfu/ml. Fig. 6 the results of a heterotrophic plate count of primary culture after treatment with the synthesized titanium oxide nanoparticles, the result shows that no organism survived after three hours of stirring under natural sunlight, with colonies forming units (CFU) which reduced the bacterial colonies to zero cfu / 50 ml. The results showed that the synthesized TiO_2 NPs effectively inhibited the growth of *E. coli* in the wastewater. However, other microorganisms were present in the effluent at a low level. The observed sensitivity of the synthesized TiO_2 NPs in inhibiting the growth of *E. coli* can be attributed to their photocatalytic properties. When exposed to natural sunlight, the synthesized TiO_2 NPs produces reactive oxygen species (ROS), which have potent antimicrobial properties. ROS attack the bacterial cells, causing them to shut down. As a mediator, *Phyllanthus niruri* may improve the photocatalytic activity of the synthesized TiO_2 NPs and contribute to the observed antimicrobial efficacy.

The presence of *E. coli* in drinking water indicates faecal contamination and the possibility of dangerous pathogens such as salmonella, pseudomonas, viruses, and intestinal parasites (Harmel *et al.,* 2016).

Table 2 showed the formation of a Zone of Inhibition identified the sample's antimicrobial activity. The zone of inhibition on an agar plate is the area on the agar surface where an antibiotic prevents the growth of a control organism. If the test organism is sensitive to the antibiotic, it will not grow in the presence of the antibiotic. The size of the zone of inhibition is a measure of how effective the compound is; the larger the clear area around the antibiotic, the more effective the compound. By using the agar well diffusion method, the antimicrobial activity of the synthesized titanium oxide nanoparticles was compared to that of commercial TiO_2 nanoparticles against five microbial pathogens.

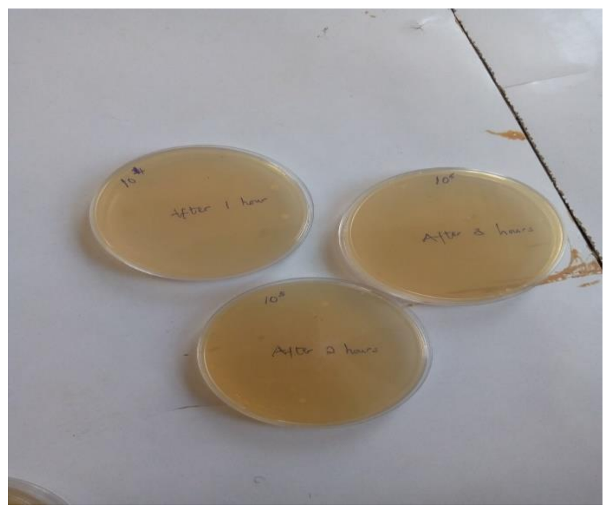

Fig. 6: Antimicrobial activities of the synthesised titanium oxide nanoparticles on the wastewater treatment

The antibacterial activity results showed that the biosynthesized TiO_2 nanoparticles were an effective antibacterial agent against gram negative bacteria (*E. coli*).

Table 2 shows that the zones of inhibition for *E. coli* are 17.90, 15.55, 14.00, and 12.00 mm, whereas commercial titanium oxide nanoparticles have no activity.

3.2.3 Antimicrobial activities

Nanoparticles adsorb on bacteria cells and dehydrate as a result of the respiration process that occurs at the cell membrane of bacteria. Because of the capping agent (phytochemicals) from the plant extract in the as-synthesized TiO_2 nanoparticles, the synthesized TiO_2 nanoparticles demonstrated efficacy against microbial activity when compared to commercial titanium oxide nanoparticles. These are plant metabolites with antimicrobial properties such as alkaloids, tannins, saponins, flavonoids, terpenoids, and so on.

3.2.4 Minimum inhibitory concentration (MIC)

The minimum inhibitory concentration (MIC) shown in Table 2. The minimum inhibitory concentration (MIC) of titanium oxide nanoparticles against *Escherichia coli* was determined after the antimicrobial activity of synthesized titanium oxide nanoparticles was confirmed using a well diffusion assay. The most commonly used technique for determining the MIC of antimicrobial agents against various microorganisms is the broth dilution method. Variability is determined by the bacterial strains.

Table 2: Antimicrobial activities of the microorganisms on the synthesized titanium oxide nanoparticles

Test organisms	**Zone of inhibition (mm) in concentrations (µg/ml)**				**control (ciprofloxacin)**	**MIC (µg/ml)**	**MBC (µg/ml)**
	50	25	12.5	6.25			
E.coli	17.90	15.55	14.00	12.00	50.00	12.50	25.00
Bacillus species	-	-	-	-	-	-	-
Klebsiella species	-	-	-	-	-	-	-
Salmonella species	-	-	-	-	-	-	-
Staphylococcus species	-	-	-	-	-	-	-
Commercial titanium	-	-	-	-	-	-	-

**** (-) No activity, MIC = minimum inhibitory concentration and minimum bactericidal concentration**

The result of the MIC determination revealed the antimicrobial activity of synthesized TiO_2 NPs against the tested microorganisms. The MIC value of the synthesized TiO_2 NPs was determined to be 12.50 µg/ml (*E. coli*). Ciprofloxacin, used as a control, displayed MIC values of 50.00 µg/ml. Comparative analysis demonstrated that synthesized TiO_2 NPs exhibited comparable antimicrobial efficacy compared to ciprofloxacin for the organism tested. The observed antimicrobial activity of TiO_2 NPS suggests their potential as an alternative to traditional antibiotics. The mechanism behind the antimicrobial action of synthesized TiO_2 NPs involves the generation of reactive oxygen species (ROS) upon exposure to light, which induces oxidative stress and damages microbial cells. The smaller size of nanoparticles allows for increased contact with microorganisms, leading to enhanced antimicrobial effects. These present findings reveal that synthesized TiO_2 NPs has promising antimicrobial properties. This is similar to the work carried out by Wafar *et al.* (2020) characterization of chistosan coated biologically synthesized TiO_2 NPs against *E. coli* of veterinary origin and found out that TiO_2 NPs were highly active in combating strain of *E.coli* than commercial TiO_2 NPs at 12.50 12.50 µg/ml minimum inhibitory concentration.

3.2.5 Minimum Bactericidal concentration

The minimum bactericidal concentration is shown in Table 2. The MBC of the synthesized titanium oxide nanoparticles was 25.00 µg/ml, indicating that this concentration completely inhibited the growth of the tested bacteria. In comparison, the MBC of ciprofloxacin, the control drug, was determined to be 50.00 µg/ml. However, the MBC of 25.00 µg/ml for synthesized TiO_2 NPs demonstrates their potent antimicrobial activity against the tested bacteria (*E. coli*). This concentration is lower than that of ciprofloxacin MBC, indicating that synthesized TiO_2 NPs has antimicrobial potential. Smaller nanoparticles with a larger surface area may interact better with bacterial cells, resulting in increased antimicrobial efficacy. Furthermore, TiO_2 NPs may aid in bactericidal activity by producing reactive oxygen species.

4.0 Conclusion

The characterization studies with UV-Vis, FTIR, XRD, SEM and TEM confirms the synthesis of TiO_2. The biological analysis revealed that the colony forming unit (CFU) of *E.coli* was decreased and eliminated to zero cfu/ml after treatment with the synthesized titanium oxide nanoparticles. The biosynthesized TiO_2 nanoparticles exhibited higher antibacterial activity against the gram negative bacteria (*Escherichia coli*) compared to commercial TiO_2 nanoparticles. Based on these result, it is concluded that the leaf extracts stabilized TiO_2 NPs have higher potential bioreduction applications when compared to the effect with the use of commercial TiO_2 nanoparticles.

5.0 References

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Compliance with Ethical Standards
Declarations

The authors declare that they have no conflict of interest.

Data availability

All data used in this study will be readily available to the public.

Consent for publication

Not Applicable

Availability of data and materials

The publisher has the right to make the data Public.

Competing interests

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Authors' contributions

Yunusa Habibat conducted the research, with Kehinde Isreal Omoniyi and Stephen E Abechi as the supervisory team. Aroh A.Oyibo, Owolabi A. Awwal and Imam Naziru assisted in the data analyses and provided access to the necessary equipment at a rebate.