Green Synthesis, Characterization and Antibacterial Activity of Zinc Oxide and Titanium Dioxide Nanoparticles Using *Terminalia Catappa* and *Cymbopogon Citratus* Leaf Extract

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Abstract: *This study synthesized zinc oxide and titanium oxide nanoparticles using green syntheses method mediated by Terminalia catappa and Cymbopogon citratus leaves extracts. Zinc nitrate hexahydrate Zn $(NO_3)_2$. $6H_20$ and TiO $(OH)_4$ were the precursors for the ZnO NPs and TiO $(OH)_4$ synthesis respectively. Results obtained revealed maximum wavelength of absorption for the ZnO-NP (330 nm) and TiO_4 (410 nm). Useful functional groups (that are typical for the presence of compounds known for their reducing properties) were found in the extracts) Synthesized nanoparticles were characterized using UV-visible spectrometer, FTIR, SEM and XRD. XRD pattern matching that of Joint Committee on Powder Diffraction Standards (JCPDS) card for ZnO confirmed the presence of hexagonal ZnO NPs with an average size of 76 nm while the results revealed the anatase and rutile form of TiO_2 with an average crystalline size of 79 nm Antimicrobial activities of the synthesized nanoparticles were established for some selected water-borne pathogens (Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa and Salmonella typhi).*

Keywords*: Nano-particles, inhibition, synthesis and pathogens*

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1.0 Introduction

Metal nanoparticles have attracted a lot of attention because of their unique characteristics such as catalytic, optical, magnetic and electrical properties (Garima *et al.,* 2011). The conventional chemical methods of synthesizing nanoparticles have been widely reported to be costlier and unfriendly with the quality of the environment (Geoprincy *et al.*, 2012). Researchers have created better approaches to prevent the use of harmful chemicals such as use of microorganisms like bacteria, fungi and yeast, which requires more steps in maintaining cell culture, intracellular synthesis and purification steps (Eddy and Ekop, 2007; Helan *et al.*, 2013). The second way is green or biogenic synthesis methods, which employs plant parts to reduced precursors to nano materials. The method has been considered as less toxic, less expensive and more environmentally friendly (Raja *et al.*, 2014; Sangeetha *et al.*, 2011;Vidya *et al.*, 2013).

The global water demands are still not met and this problem will increase with time (Hillie and Hlophe, 2007). There is an increase in demand for water as a result of deterioration of water quality, climate change and population growth (Ali and Aboul-Enein, 2004). Many countries are facing the challenge of poor access to clean and safe drinking water and this has put millions of people at risk of contracting water-borne diseases (Eddy and Garg, 2021; Garg *et* al., 2021; WHO, 2017). The application of nanotechnology for the treatment of waste water has proven to be a unique method (Amin *et al.,* 2014; Prachi *et al.,* 2013; Pandey *et al.,* 2011). This study is therefore targeted at synthesizing zinc oxide and titanium dioxide nanoparticles from *Terminalia catappa* and *Cymbopogon citratus* leaves extract and evaluation their antibacterial activity against selected waterborne pathogens.

Recently, due to their unique optical, magnetic and catalytic characteristics, metallic nanoparticles have gained much attention. Particle size, shape, monodispersity and morphology are crucial for the alignment of these properties (Sughanthy *et al.,* 2017). Various techniques of synthesis have been established for the formulation of such nanoparticles including chemical, physical and biological methods (Deyev *et al.,* 2017). The green synthesis of nanoparticles offers an alternative route using natural ingredients in plant extracts. A study of the method of metal bioaccumulation in plants has shown that metals are generally deposited as nanoparticles. It is therefore obvious that whole plants can be used to produce metal nanoparticles. Anderson *et al* (1999) conducted a study on the gold uptake by plants and reported the accumulation of gold in *Brassica juncea.* Qu *et al* (2011) reported that one of the highest accumulators of zinc is *Physalis alkekengi L.* Sharma *et al* (2007) revealed that *Sesbania drummondii* is a perennial shrub of medium size in the *fabaceae* family of legumes. The seedlings could absorb elevated quantities of gold (iii) ions, resulting in the formation of monodispersed spherical gold nanoparticles of size 6 – 20 nm in a plant cell or tissues. In another study by Krishnaraj *et al* (2010) the leaf extracts of *Acalypha indica* were used to produce silver nanoparticles of size between 20 – 30 nm in 30 mins.

Sundarrajan *et al* (2017) revealed rapid synthesis of platinum nanoparticles from leaf extract of *Ocimum sanctum* as a reducing agent. The synthesized platinum nanoparticles are of the size 23 nm and are irregular in shape. Platinum nanoparticles were also synthesized using *Diopyros kaki's* leaf extract by Song *et al* (2010). They revealed that at 95 ^{o} C more than 90 % of platinum ions were transformed to nanoparticles using 10% leaf biomass concentration and the synthesized nanoparticles ranged from 2 – 12 nm. The formation of palladium nanoparticles of size 3 – 5 nm was reported by Jia *et al* (2009) using an aqueous extract of *Gardenia jasminoids* and indicated that antioxidants such as geniposide, chlorogenic acid, crocin and crocetin played an important part in the reduction and stability of the nanoparticles. By using 0.5 % aqueous extract of *Jatropha curcas L* latex, Shriram Joglekar *et al.* (2011) reported a low cost and environmentally friendly path for fast synthesis of lead nanoparticles with size ranges from 10 – 12 nm. They also revealed titanium dioxide nanoparticles synthesis with 0.3% aqueous extract prepared from *Jatropha curcas* L latex with nanoparticle sizes ranging from 25 – 100 nm.

2.0 Materials and Methods

2.1 Materials

Fresh *Terminalia catappa* and *Cymbopogon citratus* leaf samples were collected from Magaji Farms in Chikun LGA, Kaduna State. Safety gloves were worn and the leaves were collected by detaching the leaves from the plant and removing the petioles. The leaves were placed in separate dry paper bags and labelled. Both leaves were identified and authenticated at the Herbarium Unit of the Department of Biology, Kaduna State University with voucher number 875 for *Terminalia catappa* and 334 for *Cymbopogon citratus*.

2.2 Methods

2.2.1 Preparation of the plant extract extracts

The *T. catappa* and *C. citratus* leaf samples were thoroughly washed with deionized water and each chopped into pieces and pounded using a mortar and pestle to get more of the extract. A hot water extract was prepared by boiling 5 g of each of the grounded leaf samples in distilled water (100 mL) in an Erlenmeyer flask for 5 min. The clear extract obtained in each case was filtered using the Whatman No 1 filter paper. (Oudhia *et al*., 2015).

2.2.2 Synthesis of Zinc oxide and titanium dioxide nanoparticles

The green synthesis of ZnO and TiO_2 nanoparticles was done according to the method -Aminuzzaman *et al*., 2018 and Dobrucka, 2017 respectively without any modification

2.3. Characterization

2.3.1 UV-Visible Spectra analysis

The maximum wavelength of absorption was studied using UV visible spectrophotometer (Spectrumlap 752s). The study was conducted by scanning the sample through different wavelengths and the maximum absorption wavelength was estimated from the plot of absorbance against wavelength.

The FT-IR analysis was carried out on the synthesized NPs to detect the presence of various functional groups. The model of the FTIR machine was Shimadzu 6000.

In XRD analysis, the powdered sample was smeared evenly on the sample holder made of aluminum material, with the aid of a smooth slide or any material with a smooth surface edge. The setting was between an angle of 2^o - 60^o as the bulk sample scanning range. The running rate (scanning speed) was set at 6 degrees per minute. The holder was carefully placed on the loading point of the movable goniometer arm that contain a clamp capable of gripping the sample firmly. The window indicated readiness after properly closed. The analysis commenced automatically. The pronounced Peaks or Diffractograms were displayed and they expressed the mineral's composition at various angles.

The surface morphology was determined using Scanning Electron Microscopy. The synthesized nanoparticles were initially converted into a dry powder and the powder was mounted on a sample holder followed by coating with a conductive metal. The particle was coated with a gold coating to have a good conductivity (Ghosh *et al.,* 2014).

2.4 The Antibacterial Activity of Synthesized NPs

2.4.1 Isolation of Bacteria from Water Sample

1 ml of water was extracted from the collected water sample and it was serially diluted with distilled water. The serial dilution was carried out up to a concentration of 10^{-5} . from each of the serially diluted samples, 0.1 ml of the sample was spread on the nutrient agar plate. The plates were incubated for 24 hr at 37^oC. On the nutrient agar plates, colonies appeared and they were sub-cultured. The subcultures were characterized and stored for further use (Thamidela *et al.*, 2017).

2.4.2 Antibacterial screening

The antibacterial activity of ZnO and TiO_2 NPs was determined according to the method Charannya *et al.,* 2018 each on pathogenic microorganisms isolated from a water source (River Kaduna) which includes *Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa* and *Salmonella typhi*.

2.4.3 Minimum inhibitory concentration (MIC)

The minimum inhibitory concentration of the synthesized NPs on the test organisms was determined using the broth dilution method as previously reported by Charannya *et al,* (2018).

2.4.4 Minimum bactericidal concentration (MBC)

The minimum bactericidal concentration was carried out to determine whether the test microbes were killed or only their growth was inhibited as reported by Charannya *et al*., 2018).

3.0 Results and Discussions

Green synthesis of metal-NPs generally involves oxidation-reduction reaction. Therefore, the role of the extracts from T. *catappa* and C. *citratus* leaves provided the constituents that acted as reducing agents in the synthesis. Most plants have been reported to contain several secondary metabolites such as amino acid, alkaloids, phenolic compounds and flavonoids that are capable of serving as capping agents or stabilizers for the NPs synthesis (Dar *et al.,* 2015). During the plant extracts mediated synthesis of ZnO-NP, the colour was observed to change from green to pale yellow, further application of heat changed the appearance to reddish from reddish to orange paste. Similarly, the synthesis of TiO2-NPs using C. citratus leaf (lemon grass), at room temperature resulted in color change from white to green indicating the formation of TiO_2-NPs. In each case, flavonoids, glycosides, proteins, and phenols played a vital role in the reduction and capping of synthesized zinc oxide. The elevated potential for bio-reduction has been attributed to the aliphatic alcohols and amines present in the leaf extract (Hashemi *et al.,* 2016). The synthesized NPs were characterized using FT-IR, SEM, XRD and UV-visible spectroscopy.

The UV-Vis spectrum of the synthesized ZnO-NPs gave a spectrum that indicated a maximum peak at 330 nm within a scanning wavelength range of 250- 400 nm. The identified wavelength is consistent with the observations made by others for ZnO-NP (Manokari *et al.,* 2016a; Sivakumar, 2004). However, the observed maximum peak for the TiO_2 nanoparticles at 410 nm and is also in harmony with the value reported by Sundrarajan *et al*. (2017) TiO_2 NPs

The FT-IR spectrum of the almond extract mediated ZnO nanoparticle is presented in Fig. 1. the spectrum of synthesize nanoparticles shows a C-F stretch at 1111.03 cm^{-1} and a corresponding ZnO-NPs at 941.29 - 840.99 cm^{-1}.

The FTI-R of ZnO-NP synthesized from lemon extract mediated synthesized indicated several functional groups but the prominent ones were 725.26 and 1388.79 cm^{-1} corresponds to the vibration of metal–oxygen and prominent peaks which may be due to Ti–O and Ti–O–O stretching vibrations, thereby confirming the formation of TiO_2 NPs (Rajakumar *et al*., 2015; Ali *et al*., 2015). The possible mechanism for the capping of the metal NPs in green synthesis has been established and this is due to the interaction between the phytochemical constituents of the plant extracts and the metal oxides. (Ali *et al*., 2015).

.Fig. 2 presents the micrograph of ZnO-NP synthesized from lemongrass. The micrograph suggests that there is a network formation that seems to present a macrosystem with a possible disjointed profile, that most likely depict poly dispersed system. Although we did not take the SEM image of the ZnO-NP synthesized by other method and that of the ZnO before syntheses, it is certain that image is not strange for ZnO-NP obtained from green synthesis when compared to the information obtained elsewhere (Rajendran and Sengodan, 2017).

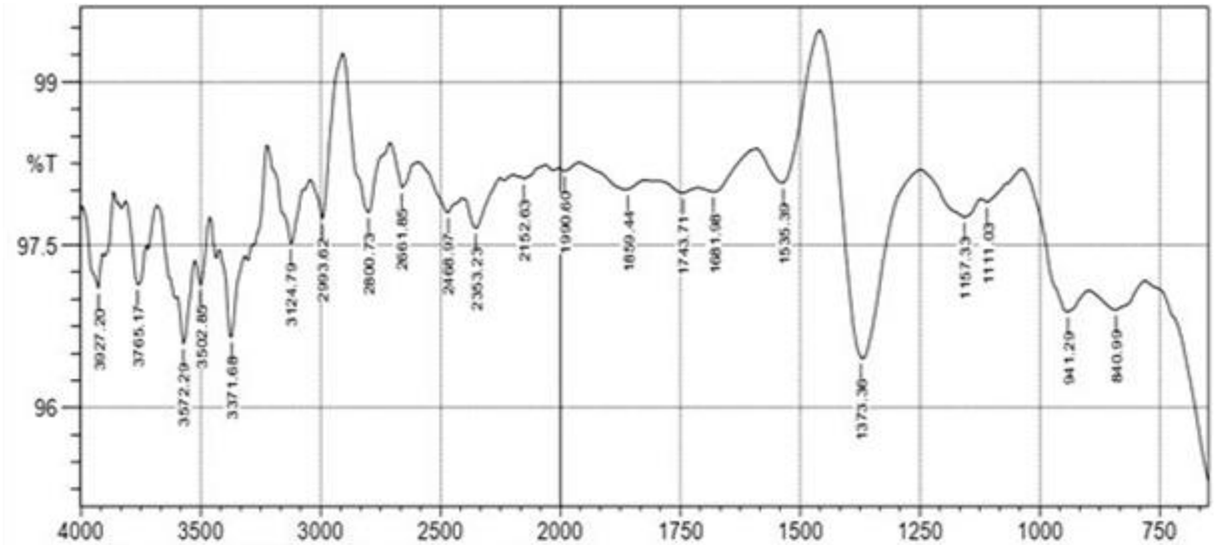

Fig. 1: FT-IR Spectrum of *T. catappa* (Almond) extract

Also, image for the SEM for Lemongrass mediated synthesized TiO_2 NPs is shown in Fig. 6, which reveals the presence of neatly but irregular sizes in the nanomaterials. Evidence of differential capping is indicative when the two micrographs are compared. This is due to the difference in composition (including identity and concentration) of the phytochemical in the respective plant leaf.

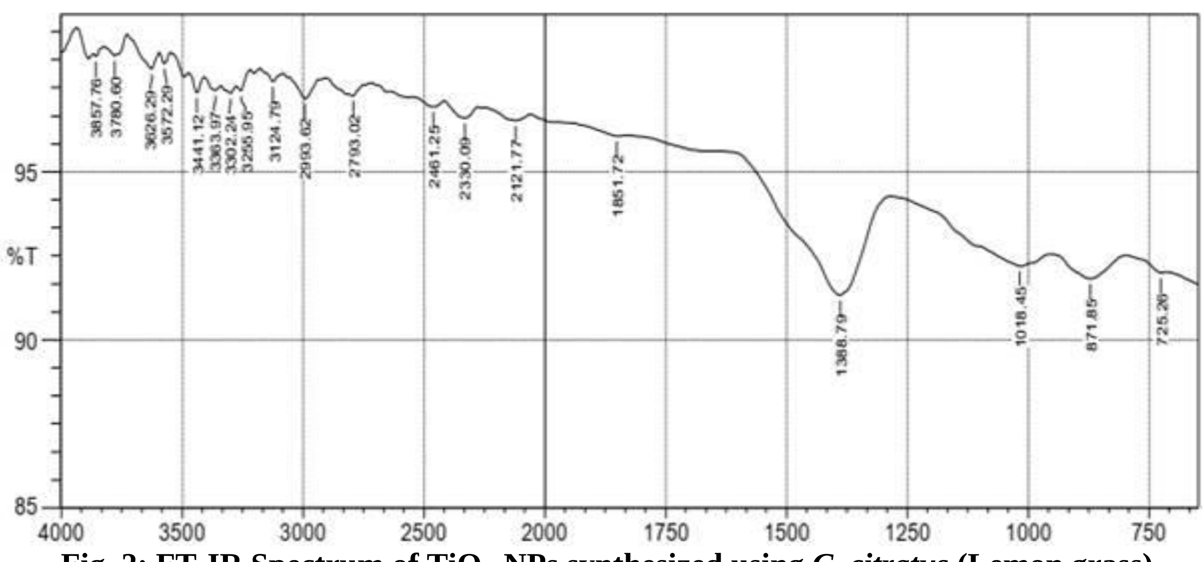

Fig. 2: FT-IR Spectrum of TiO_2-NPs synthesized using *C. citratus* (Lemon grass)

The tendency of the synthesized NPs to inhibit bacterial growths as studied and the results were aligned in terms of zone of inhibition as shown in Table 1The ZnO-NPs demonstrated effective activity ranging from 25-30 mm, whereas the TiO_2-NPs showed inhibition that ranged from 25-29 mm against the test organisms. This indicates that ZnO-NP produced from the two sources have almost similar inhibition zones. ZnO-NPs exhibited exhibited significant activity on a Gram-negative bacterium, that is *P. aeruginosa* (30 mm) than the drug (ciprofloxacin, which exhibited 28 mm) that was used as a Also, the susceptibility of Gram-positive bacteria such as *S. aureus* also indicated better potency for ZnO-NPs TiO_2-NPs compared with the control drug.. These results are consistent with previous findings of Brayner *et al* (2006) plant-mediated the synthesis of ZnO nanoparticles.

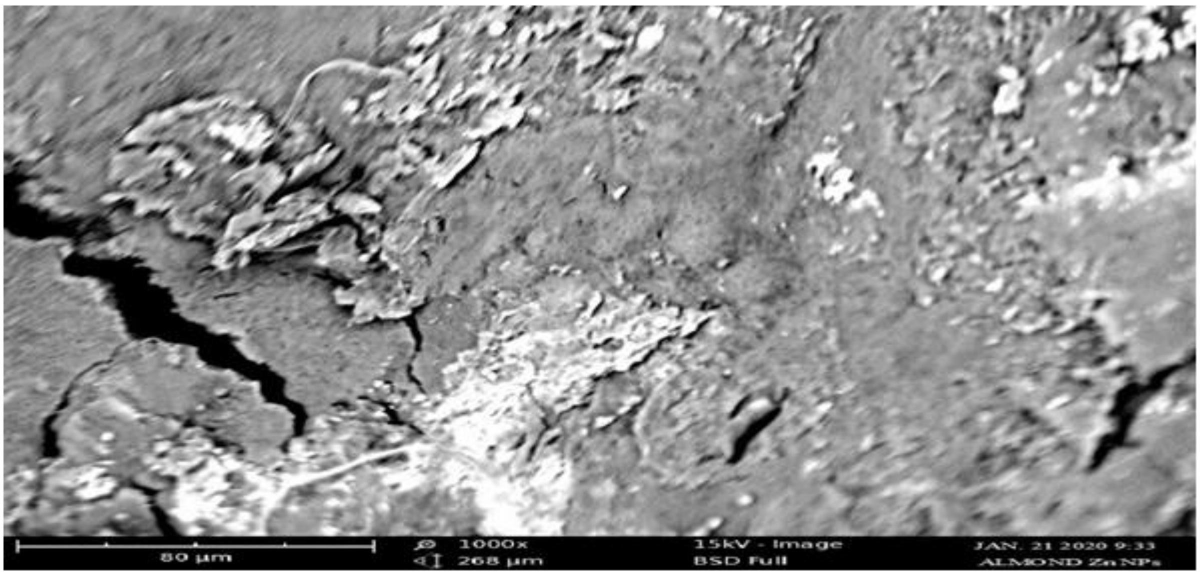

Fig. 3: SEM image of ZnO-NPs synthesized using *T. catappa* (Almond)

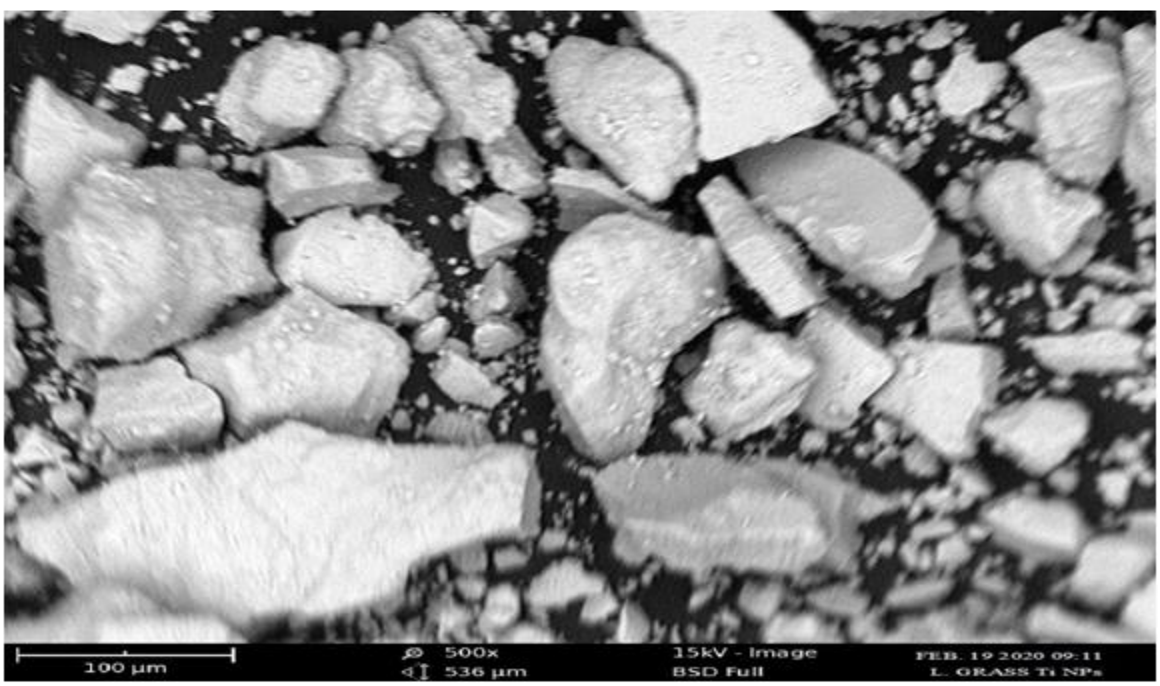

Fig. 4: SEM image of TiO_2-NPs synthesized using *C. citratus* (Lemon grass)

Table 1 Antibacterial activity of the nanoparticles and control

	Zone of inhibition (mm)		
Test organisms	**ZnO-NPs (µg/mL)**	**TiO_2-NPs (µg/mL)**	**Ciprofloxacin (µg/mL)**
Staphylococcus aureus	29	25	0
Bacillus subtilis	0	0	0
Escherichia coli	25	27	37
Pseudomonas aeruginosa	30	26	28
Salmonella typhi	26	29	41

The TiO_2-NPs showed significant activities against gram-negative bacteria, namely, *S. typhi* (29 mm) and *E. coli* (27 mm) but were not too effective compared to the control drug Ciprofloxacin (28 mm). This may be due to the inability of the TiO_2-NP to overcome the barrier set up by the respective bacterial cell walls (Kumar *et al.*,2014). These findings are also in line with the report of similar work presented by Jayaseelan *et al.* (2013) in a published document.

The Minimum Inhibitory Concentration (MIC) i s the lowest concentration of the nanoparticle in sterile broth which shows no turbidity, which is the concentration of the NPs that can inhibit the growth of the bacteria without killing it (as shown in Table 2). The recorded results reveal that ZnO-NPs demonstrated the lowest MIC on *P. aeruginosa* indicating the viability to be used as a drug candidate. The minimum inhibitory concentration of synthesized TiO_2-NPs was also found to exhibit the least for *E. coli* (25 µg/mL)

The Minimum bactericidal concentration (MBC) is also an informative index for the assessment of the population of microorganisms that are killed and those with growth inhibition. Table 2 also contain information on the minimum bactericidal concentration of the nanoparticles against tested microorganisms. The results reveal better inhibition potency for the two nanoparticles ZnO-NPs and TiO_2-NPs (compared to the MIC values), except for *P aeruginosa* which were completely terminated.

Table 2 MIC and MBC of the synthesized NPs against test microorganisms

	ZnO-NPs (µg/mL)		**TiO_2-NPs (µg/mL)**	
Test organisms	MIC	MBC	MIC	MBC
Staphylococcus aureus	25	50	50	100
Bacillus subtilis	NT	NT	NT	NT
Escherichia coli	50	100	25	100
Pseudomonas aeruginosa	12.5	50	50	100
Salmonella typhi	50	100	25	50

****NT=not tested**

4.0 Conclusion

Zinc and titanium oxides nanoparticles has been synthesized using green synthesis that were mediated by leaves extracts of *Terminalia catappa* and *Cymbopogon citratus* respectively. The nanomaterials have some activities against some gram-negative microorganisms. These nanoparticles may also be useful in other application areas such as adsorption removal of environmental contaminants, etc.

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Conflict of Interest

The authors declared no conflict of interest