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Antibacterial Effect of Nanoparticles Synthesized from *Ficus exaseprata* (Sandpaper Leaves)

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The eco-friendly, cost effectiveness, and the less toxic nature of synthesized green nanoparticles to the environment have become a global attraction to many studies. The study aim to synthesize Silver (Ag) nanoparticles from *Ficus exasperata* (Sandpaper leave) leaves and to determine its antimicrobial effect on bacteria isolates. Silver (Ag) nanoparticles were synthesized from the plant extracts using standard extracting techniques and their presence was verified and confirmed using an ultraviolet-visible (UV-Vis) Spectrophotometer. A prepared series of silver nitrate (AgNO3) solutions were mixed with the plant extracts at a ratio of 1:1 (v/v) to a total volume of 20ml in a text-tube. The test tubes were rapped with aluminum foil and heated in a water bath at 60OC for 3 hours and allowed to cool and analyzed using UV-Vis Spectrophotometer. The UV-Vis spectra confirmed the different concentrations of silver nitrate (AgNO3) that produced Ag nanoparticles and their average size was more than 50nm and less than 100nm. The mixture of the leave extract was

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tested for its antimicrobial activity against gram-positive and gram-negative organisms such as Staphylococcus aureus Bacillus cereus and Escherichia coli respectively. The results showed that the growth of the different bacteria was inhibited by the extracts containing silver nitrate on Staphylococcus aureus and Escherichia coli and not on Bacillus cereus using *Ficus exaseprata* nanoparticles. Statistical evaluation showed that zones of inhibition on the three bacteria produced by the aqueous leave extracts containing different concentration of silver nitrate (AgNO3¬¬) precursor was significantly different from the silver nitrate (AgNO3) precursor. It were observed that the higher the concentration of the silver nitrate (AgNO3) the greater the zone of inhibition. It can be concluded that *Ficus exaseprata* leave extracts dope with silver nanoparticles, can produce antimicrobial effects on some microorganisms.

Keywords: Nanoparticles; silver nitrate; Ficus exasperate; antimicrobiall; sandpaper leaves.

1. INTRODUCTION

The hazard-free nature of nanoparticles have placed it in the climax of global recognition due to its eco-friendly and less-toxic nature. Understanding the composition, morphology, application and control of nanoparticles has given nanotechnology attention from all fields of science [1]. Nanomaterials are so small that analytical method is applied to determine its scale, which was recorded to be one (1) billionth of a meter (10^{-9} 1nm), that possess different physical and chemical properties with unique features that are effective in various industrial and fascinating pharmaceutical application. [2]. Nanoparticles can be synthesized using different methods and include the following methods, Cosmological [3], Geological [4], Metrological [5]) and Biological [6,7] processes. Nikalje et al, [8] explained that the study of nanoparticles is as "the study of extremely small structure of matters", but nanoparticles are viewed, and defined depending very much on its specific application.

The research, examination, development, improvement, application and revolution of nanoparticles have effectively contributed to the overall growth of all sectors of life, such as; Information Technology [9]), Security [10]; Medicine [8]; Transportation [11]); Energy [12]; Food [13]; Safety [9]; Environmental Science [14], among many others. The application of nanoparticles in health science (medicine) has open the medium to improve the synthesis of therapeutic medication to produce precise solutions to disease prevention, diagnosis and treatment to better the standard of health in the society.

Employing the use of green synthesized nanoparticles using plants such as *Ficus exaseprata* (sandpaper leaves) will be a great achievement. *Ficus exasperate* (sandpaper leave) is a small size tree, it grows up to 15-30 meters. It is a medicinal plant and the extracts are used as anti-ulcer, and to treat hypotensive, analgesic and anti-inflammatory activities [15]. Researchers has proven that various part of *Ficus exasperate* have medicinal activity. Enogieru et al., [16], recorded that the extract of *Ficus exasperate* contains flavonoid and saponin compound. Amed et al., [17] accounted that the extracts of *Ficus exasperate* is said to contain active components such as phenolics and tannins, and also concluded that *Ficus exasperate* have therapeutic potential with some degree of toxicity.

2. MATERIALS AND METHODS

2.1 Study Area

This study was carried out in the Department of Chemistry, Faculty of Science, Rivers State University, Nkpolu Oroworukwo, Port Harcourt.

2.2 Chemical Preparation / Equipment

The chemical used was silver nitrate ($AgNO_3$) which was of analytical grade and had purity up to 99%. The reagent was all prepared in the laboratory before use.

The equipment used were G-force centrifuge for centrifuging, autoclave for sterilization and Scanning Electron Microscope for examination of the generated particle size and they were properly cleaned, protected and used at the recommended temperature and conditions.

Plant material: Fresh *Ficus exasperata* (sandpaper leaves) were collected from Rivers State University, Nkpolu Oroworukwu in Rivers State.

2.3 Preparation of Leave Extract

The leaves *Ficus exasperata* were washed separately under running water to remove dirt and later with distilled water to eliminate all forms of impurities. The fresh leaves were cut and labeled properly and were were blended to form a paste. Ten (10g) gram of paste was weighed into a 250ml capacity beaker, while 100ml of distilled water was added into the 250ml capacity beaker with paste and were mixed thoroughly using a stirrer. The mixtures were heated at 60^{O}C for 20minutes while stirring occasionally and allowed to cool at room temperature. The mixtures were filtered using Whatman Filter Paper and centrifuged for 10minutes. The extracts were used for the of synthesis of silver nanoparticles from silver nitrate ($AgNO_3$) concentrations.

2.4 Synthesis of Silver (Ag) Nanoparticles

A zero point four hundred and twenty-five (0.425g) grams of silver nitrate ($AgNO_3$) was dissolved in 250ml of distilled water to prepare a 10mM silver nitrate ($AgNO_3$) stock solution from which a series of 1mM, 2mM, 3mM, 4mM and 5mM silver nitrate ($AgNO_3$) solutions were prepared. The series of silver nitrate ($AgNO_3$) solutions were mixed with the plant extracts at a ratio of 1:1 (v/v) to a total volume of 20ml in a text-tube. The tubes were wrapped with aluminum foil and heated in a water bath at 60^{O}C for 3hours. Then, the mixtures were stored in the refrigerator for the antibacterial activity test and further analyzed by using UV-Vis Spectrophotometer.

2.5 Assay for Antimicrobial Activity of Silver Nanoparticles against Microorganisms

All equipment and growth media were sterilized by autoclaving at 121^{O}C and 15psi for 15mintues. The antimicrobial activity was investigated against *Staphylococcus aureus*, *Bacillus cereus* and *Escherichia coli* for gram-positive and gram-negative bacteria respectively. The antimicrobial activity was evaluated by the disc diffusion (culture) method, preparation of the bacteria stock was done to reproduce and rejuvenate bacteria. This was done by inoculating each bacteria using a sterile wire loop on three differently identified dry nutrient agar plates and then incubated at 37^{O}C for 24hours in the incubator to produce a pure culture.

Nutrient agar plate was prepared by weighing out the amount required by the manufacturers' instruction and twenty milliliter (20ml) was poured into petri dish after sterilization and allowed to solidify. Zero- point one milliliter (0.1ml) of broth culture of *Staphylococcus aureus* which served as gram positive cocci, *Bacillus cereus* which served as gram positive bacilli and *Escherichia coli* gram-negative bacilli were each spread separately on nutrient agar using a glass spreader. The two plant extracts and the silver nitrate ($AgNO_3$) precursor were then placed on each cultured plate separately and served as the control. Thereafter, the different concentrations of the silver nanoparticles 1mM, 2mM, 3mM, 4mM and 5mM were applied on the different bacteria plates. The inhibition method was used to evaluate the antibacterial activity of the synthesized nanoparticles. Thereafter, the positive and the negative controls were also placed separately on the culture plate. Then, it was incubated at 37^{O}C for 24hours to observe if the plant extracts and the silver nitrate ($AgNO_3$) precursor will inhibit the bacteria growth and to compare the effectiveness of the plant extracts and silver nitrate ($AgNO_3$) precursor.

3. RESULTS AND DISCUSSION

3.1 UV-Vis Spectrometry of Synthesized Nanoparticles

The silver nitrate ($AgNO_3$) solution was analyzed using UV-Vis spectroscopy. This was done to determine the wavelength peak spectrum of the different silver nitrate ($AgNO_3$) concentrations (1mM-5mM). The graph (Fig. 1) below shows the wavelength and absorbance of each concentration of the silver nitrate precursor.

At a wavelength of 450nm and 600nm there was absorbance showing the interaction of the radiation with Ag+ and a diminishing peak to wavelength of 650nm.

3.2 UV-Vis Spectrometry of Synthesized Plant Extract with Nanoparticles

The characteristics of silver nanoparticles ranged at a wavelength of 400nm - 650nm. UV-Visible spectra absorption band increased with increasing silver nitrate ($AgNO_3$) concentration. The 1mM, 2mM, 3mM, 4mM and 5mM concentrations of silver nanoparticles synthesized using *Ficus exasperata* aqueous

extract evince a shift, the plant extract with the concentration has a trend of wavelength but with different concentrations which shows the presence of the silver nitrate ($AgNO_3$) concentration in the nanoparticles are as shown Fig. 2.

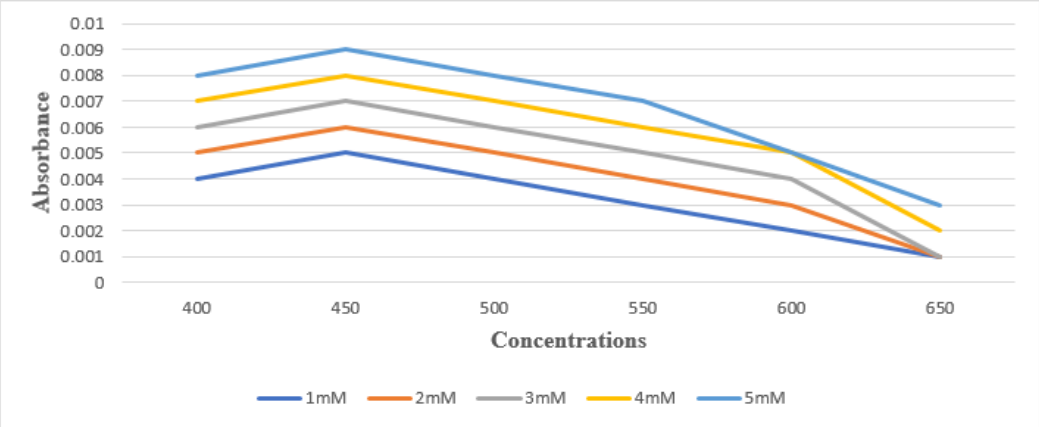

Fig. 1. Absorbance of silver nitrate at different wavelengths

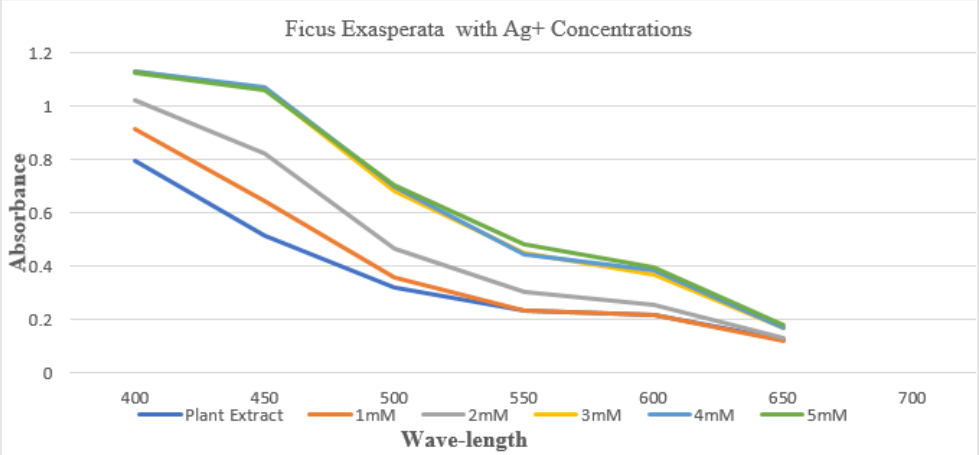

Fig. 2. Absorbance of *Ficus exasperata* with Ag+ at different wavelength

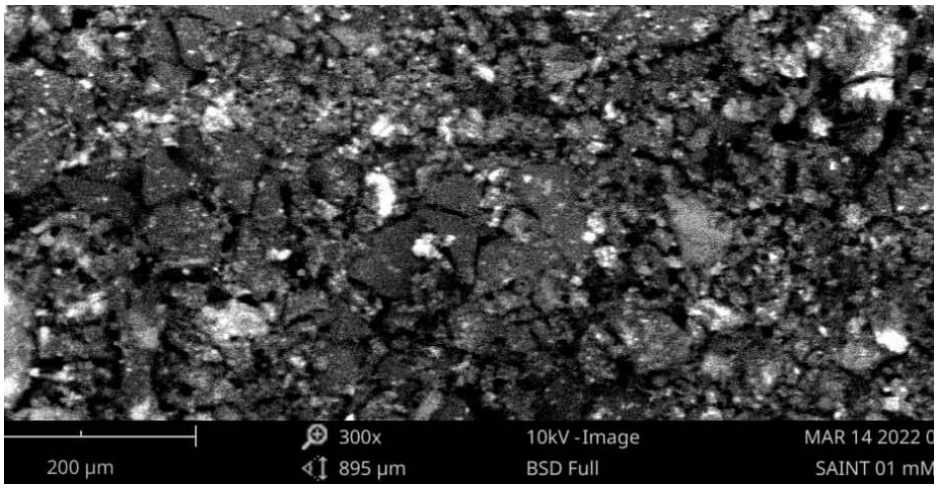

Plate 1. 0.1mM of *Ficus exasperata* (Sandpaper Leave) nanoparticles

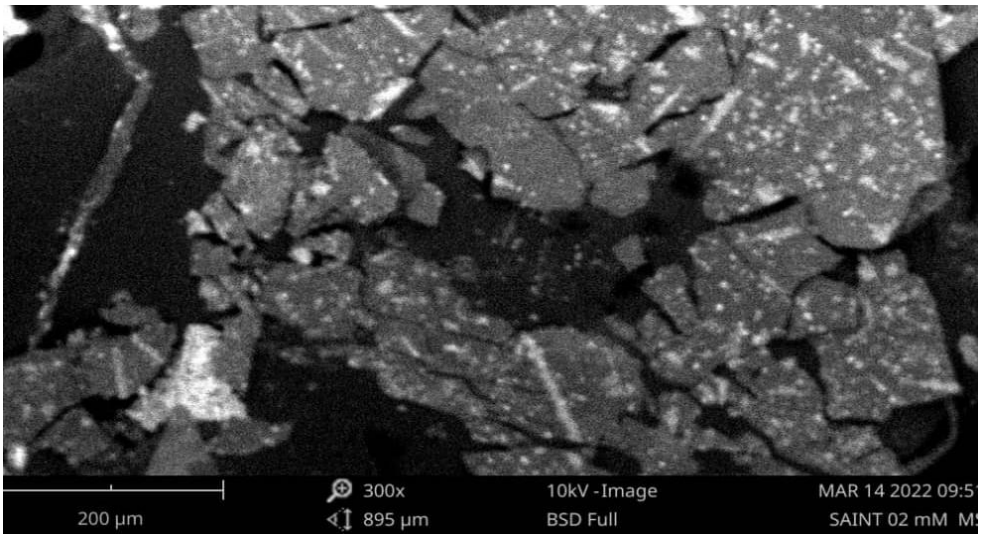

Plate 2. 0.2mM of *Ficus exasperata* (Sandpaper Leave) nanoparticles

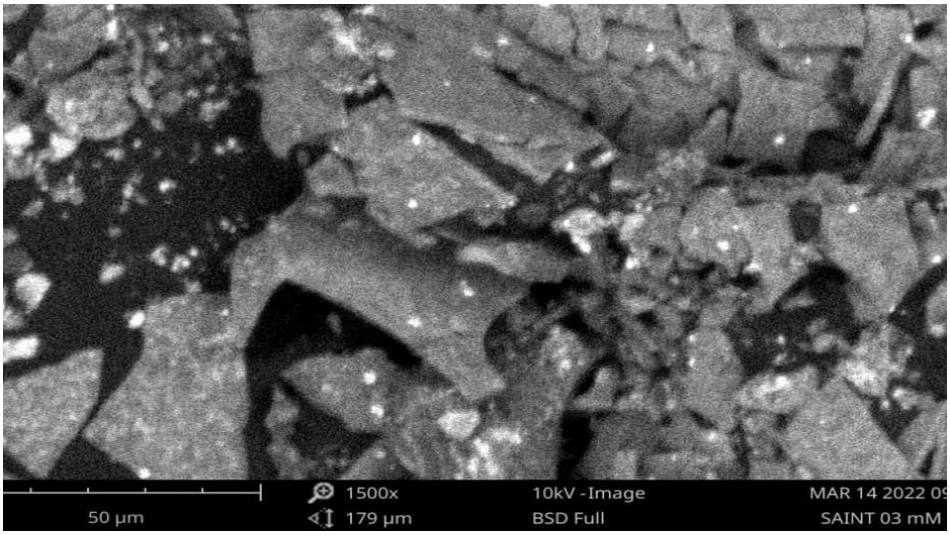

Plate 3. 0.3mM of *Ficus exasperata* (Sandpaper Leave) nanoparticles

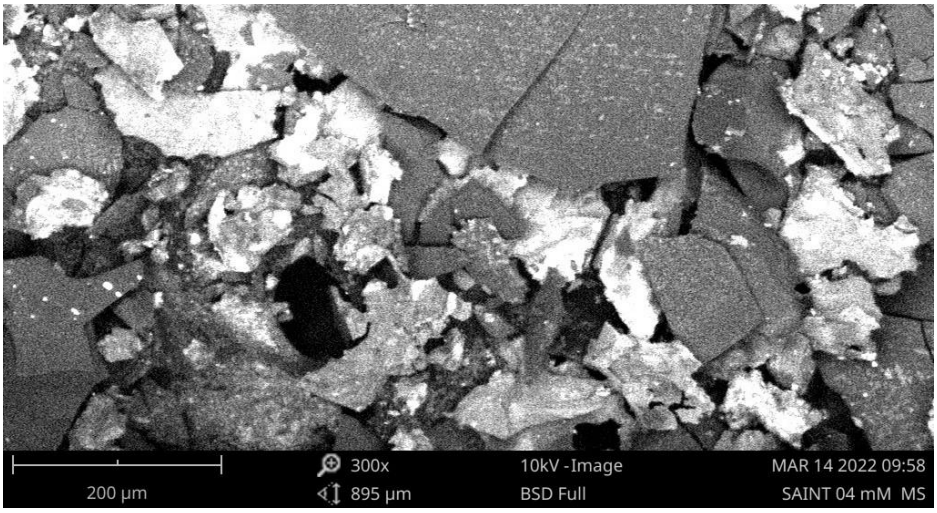

Plate 4. 0.4mM of *Ficus exasperata* (Sandpaper Leave) nanoparticles

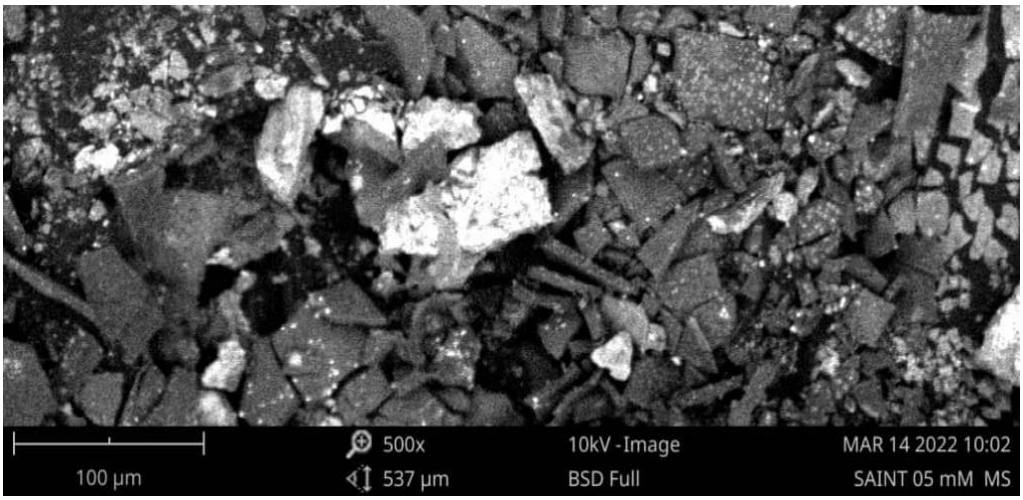

Plate 5. 0.5mM of *Ficus exasperata* (Sandpaper Leave) nanoparticles

3.3 Scanning Electron Microscope (S.E.M.) Analysis

The size and morphology of the nanoparticles synthesized using aqueous extract of fresh leaves of *Ficus exaseprata* have been evaluated using SEM analysis. The obtained SEM images of the Silver Nanoparticles prepared by each leave extract from *Ficus exasperata* (Sandpaper Leaves).

Using a magnifying lens at 300X at 10kv-image, the nanoparticles ranged from 50-100 nm (nanometers). The nanoparticles are quite poly-dispersed and a layer of the organic material surrounding the synthesized silver nanoparticles could explain the good dispersion of the nanoparticles in solution. The size of the nanoparticles increases as the concentration of the silver nitrate ($AgNO_3$) increases.

Generally, the silver nanoparticles synthesized using aqueous extracts were well dispersed and some of them appear agglomerated. The majority of the particles in the SEM images are not in physical contact with each other but appear separated by the organic layer, which they coating of silver nanoparticles with an organic layer.

Antibacterial activity studies: The present study revealed that the tested leave extracts of *Ficus exaseprata* showed potent antibacterial activity against two bacterial strains: gram-positives *S. aureus* and *Bacillus* and gram-negative *E. coli* at a low outcome. Aqueous extracts containing silver nanoparticles showed activity in all concentration against the bacteria as shown in Fig. 3.

Antibacterial activity shows an inhibition zone which was characterized by a clear zone between the wall (containing plant samples) and a certain distance. The inhibition zone around the walls showed bacteria sensitivity to silver nitrate bulk (10mM) and plants extracts (used as the controls). The control is used to compare the diameter of inhibition zone formed by the plant extracts at different concentrations. The *Ficus exasperata* extracts containing silver nanoparticles (plant extract with Ag+ bulk solution) gave the highest antibacterial activity against S. aureus followed by *E. coli* and the Bacillus had poor zone of inhibition.

The diameter of inhibition zones in millimeters formed by each of the concentrations of silver nanoparticle precursor added to the aqueous extracts are presented in Fig. 4. The figure shows the resistant effect of silver nitrate ($AgNO_3$) bulk precursor inhibitor on *S. aureus*, *E. coli,* and *Bacillus cereus* at different concentrations, where it was observed that all the particles had poor zones of inhibition (ie < 5mm) at the different concentrations used. Only S. aureus had moderate zone of inhibition (6mm) using the nanoparticle alone as control.

The Fig. 5 show a typical zone of inhibition of *Ficus exaseprata* nanoparticles. The zone of inhibition of *Ficus exasperata* nanoparticles increased with increase in concentration for all three organisms which may be due to its medicinal properties. From graph, (Fig. 5) *Ficus*

exasperata has considerably high (7 and 9mm at 4 and 5MM respectively) inhibitive effect on *S. aureus*; moderate (5mm at 5MM) on *E. coli* and low (<5mm) on *Bacillus cereus.* Silver nitrate on its own, has no inhibitive activity on *Bacillus* but on mixing with *Ficus exasperata* to synthesize nanoparticles, the zone of inhibition increased as the concentration increases and this may be as a result of its therapeutic potential and the presence of phenolics in *Ficus exasperate* according to Mohammed et al, [18]. The size of the nanoparticles due to the concentration of the silver contributed effectively to the its antibacterial activity on the organisms, exposing the surface area of the plant compounds to more action.

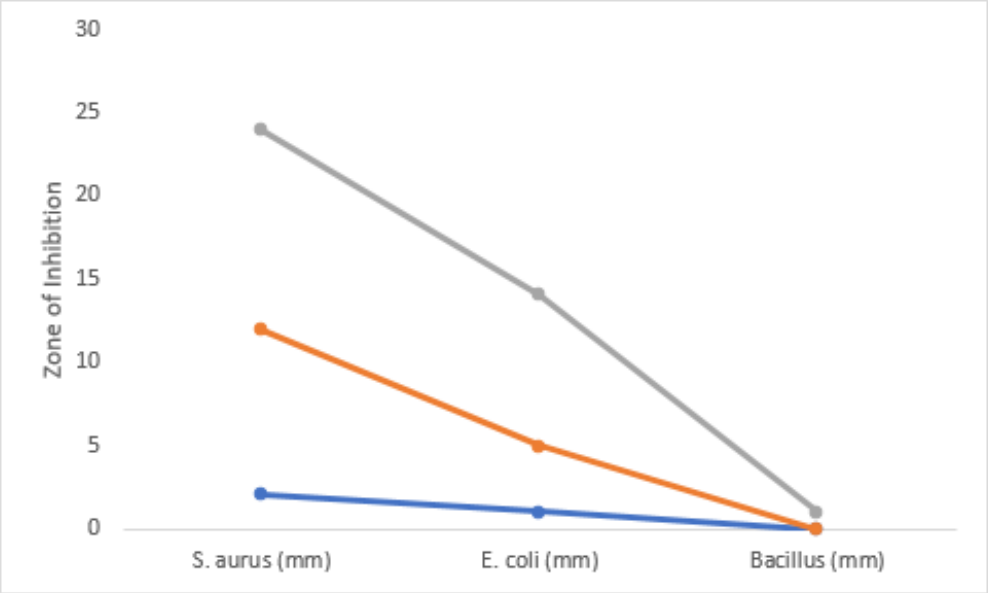

Fig. 3. Zone of inhibition of control (plant extract), Ag+ bulk and plant extract with Ag+ bulk solution

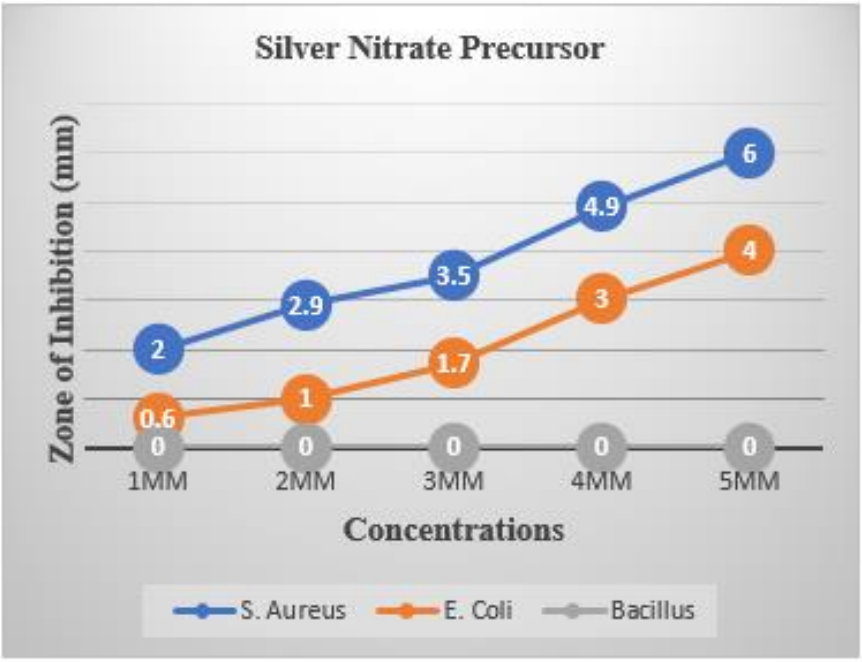

Fig. 4. Zones of inhibition of nanoparticles

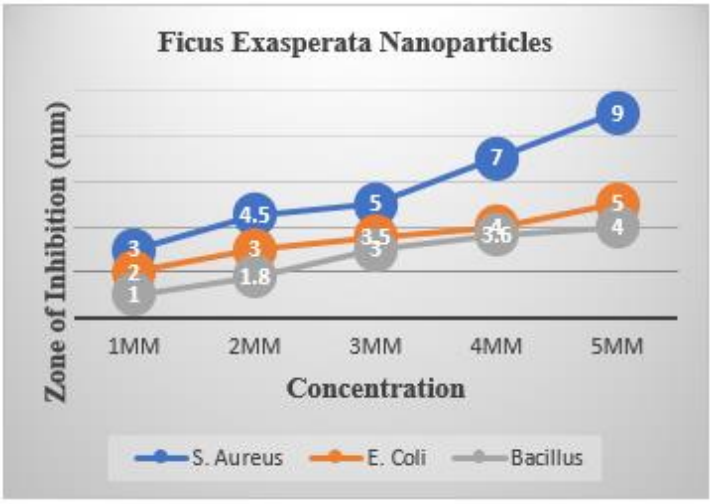

Fig. 5. Zones of inhibition of fiscus exasperate nanoparticles

4. CONCLUSION

The effect of green synthesis of nanoparticles of silver nitrate plant extracts of *Ficus exasperata* leaves has shown improved zone of inhibition on *S. aureus*, *E. coli* except *Bacillus cereus*. Antibacterial activity was shown by the characterized clear zone between the wells containing samples. This is to conclude that the plant extract on its own, did not show good zone of inhibition but on addition of silver nanoparticles improve the zone of inhibition of the plant extracts. Using silver nitrate to synthesize therapeutic substances against *S. aureus* and *E. coli* but not *Bacillus cereus* will improve the antibacterial effect on the organisms and this can be used as a potential synthetic procedure for antibacterial drugs.

5. RECOMMENDATION

Further in-vivo studies can be done using these synthesized nanoparticles and toxicity of nanoparticles produced can be investigated in future studies.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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