

Antibacterial and Antioxidant Activity of Biosynthesized Silver Nanoparticle using *Cassia Occidentalis*

Musa G.M.¹, Modibbo A.A.¹ and Tanko M. M.²

¹Department of Chemical Science Technology,

Federal Polytechnic, Mubi, Nigeria

²Department of Biomedical and Pharmaceutical Technology,

Federal Polytechnic, Mubi, Nigeria

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ABSTRACT

Nanotechnology is a fascinating field of research that has applications in various areas such as material science, medical science, life science, physical and chemical sciences. A study was conducted to determine the antioxidant and antimicrobial properties of silver nanoparticles. The silver nanoparticles were biosynthesized from the leaves of *Cassia occidentalis*. The antimicrobial effectiveness of the biosynthesized silver nanoparticles was tested against *Staphylococcus aureus* and *Pseudomonas Stutzeri*, and *in vitro* antioxidant activities were measured using DPPH, FRAP, and Hydrogen peroxide. The microorganisms were treated with 10 and 20 µg/mL of the silver nanoparticles and 20 µg/mL of ciprofloxacin for 72 hours. After incubation, the silver nanoparticles significantly inhibited the growth of *Staphylococcus aureus* (17.00 ± 2.50), which was higher than the inhibition observed with ciprofloxacin (15.00 ± 0.05). The zone of inhibition of *Pseudomonas Stutzeri* was 15.00 ± 3.00 , which was inhibited by the silver nanoparticles compared to ciprofloxacin (22.00 ± 2.00). The silver nanoparticles also showed significant antioxidant activity compared to the standard ascorbic acid. In conclusion, the study found that the biosynthesized silver nanoparticles exhibited antimicrobial and antioxidant properties against the tested microorganisms.

Keywords: Anti-microbial, silver nanoparticle, *Cassia occidentalis*, antioxidant,

INTRODUCTION

Nanotechnology deals with the manufacturing and applications of materials with Nano scale of 1–100 nm in size (Bhushan, 2017). Nanoparticles (NPs) have shown distinctive properties, which can be rightfully manipulated for several preferred applications due to their high surface area to volume ratio (Chadha *et al.*, 2022). Nanoparticles (NPs) have wide range of applications in areas such as health care, cosmetics, food and feed, environmental health, biomedical sciences etc (Osama *et al.*, 2020).

In general, NPs can be classified into metal, metal and metalloid oxides (inorganic) NPs (e.g. gold, silver, iron oxide and silicon dioxide), carbon-based NPs (e.g. carbon nanotubes and nanofibers) and organic NPs (e.g. dendrimers, liposomes and micelles) (Joudeh & Linke, 2022).

Silver nanoparticles (AgNPs) are applicable nanomaterial in medicine that are used as an antimicrobial production that shows antiseptic activity owing to silver toxicity that induces cell death by making changes in the enzymatic system, and cell permeation (Salleh *et al.*, 2020). Several studies have shown the toxic effects of AgNP on numerous cell lines such as macrophages, embryonic kidney cells, skin keratinocytes, hepatocytes, neuroblastoma cells, etc (Glinski *et al.*, 2021, Milić *et al.*, 2015, Gao *et al.*, 2021 and Zhang *et al.*, 2016). Moreover, animal studies have demonstrated that AgNP administered to animals by different methods such as inhalation, ingestion, or Injections, these nanoparticles detect presence in the blood, and finally lead to toxicity of multiple organs such as the lungs, liver, kidneys, intestines, and brain (Ferdous & Nemmar, 2020, Bergin & Witzmann, 2013, and Nayek *et al.*, 2021).

Silver nanoparticles (SNP) bind to the bacterial cell wall surface, which has a negative charge and cause stability interruption and permeability alteration in the envelope of the cell wall and the flow of silver ions inside the cell (Hassanien & Shaker, 2020). It has been reported that interaction of nanoparticles with cell protein and DNA results in interference of cell division and cell death (Sharmin *et al.*, 2021).

This study was aim at determining the antioxidant and antimicrobial of silver nanoparticles

METHODOLOGY

Collection of *Cassia occidentalis* leaf

Fresh leaves of *Cassia occidentalis* was collected from Adamawa State University (ADSU) Mubi Botanical garden. *Cassia occidentalis* plant was botanically identified using the standard morphological characteristic features.

Aqueous extraction of *Cassia occidentalis* leaves

The fresh leaves of *Cassia occidentalis* was shade dried at room temperature for 11 days. The shade dried leaves of *Cassia occidentalis* was grounded into powder using a mortar and pestle (Raghasudha, 2016). The powdered leaves of *Cassia occidentalis* was stored in well labelled airtight container. Twenty grams of powdered leaves was weighed and transferred into 500 mL beaker containing 200 mL of deionized water. The mixture was boiled for 10 minutes allowed to cool and filtered through Whatman No.1 filter paper (Kumar *et al.*, 2014).

Biosynthesis of silver nanoparticle:

Using the method of Keshari *et al.* (2016), 10 mL extract was added to the 180 mL silver nitrate solution (1mM) in 90 mL and stirred for 30 min using a magnetic stirrer at room temperature. The change in colour of the solution indicate the reduction of silver nitrate into the biosynthesized AgNPs. The precipitate formed was centrifuged and will behed with distilled water 3 times and be subjected to characterization as follows.

Separation and Purification of Silver Nanoparticles

After desired reaction period, the mixture containing product will be centrifuged at 110 X 100 rpm for 20 min. The process of centrifugation and re-dispersion in *deionized water* was repeated three times to ensure better removal of unreacted phytochemicals from the AgNPs (Premasudha *et al.*, 2015).

Characterization of Synthesized Nanoparticles

The biosynthesized nanoparticles were characterized using the method according to Ghosh *et al.* (2015) UV

visible spectrophotometer and Fourier Transmission Infrared (FTIR) were used in characterizing the nanoparticle.

The Antimicrobial Activity of AgNPs

Agar well diffusion method was followed for the determination of Antimicrobial activity. The isolates were grown in Nutrient agar medium at 37 °C for 24 hrs. Bacterial suspension (inoculum) was diluted with a sterile physiological solution to 10^8 cfu mL⁻¹ with reference to the McFarland turbidometry. The bacterial suspension was added to each plate containing Muller Hinton Agar (MHA) by sterile cotton swab and allowed to remain in contact for 1 min. The AgNPs were dissolved by using Dimethyl sulphur oxide (DMSO) at concentration of 10 and 20 µg/mL, wells were made on the plates using a core borer with a diameter of 8 mm, these wells filled by addition 100 µL of each AgNPs concentration with the use of DMSO and Ciprofloxacin as a control. The plates were incubated at 37 °C for 24 hours the inhibition zone around each well was measured in mm (Barapatre *et al.*, 2016)

Free Radical-Scavenging Activity Using 2,2-Diphenyl-1-picrylhydrazyl (DPPH)

The antioxidant activity was monitored according to the DPPH method described by Martins *et al.* (2015) with modifications. This method was performed by using a SPECTRO star Nano microplate reader (BMG Labtech, Offenburg, Germany). The reaction mixture in each of the 96-wells consisted of 30 µL of sample solution (in an appropriated dilution, according to the reference range of the calibration curve) and a 0.004% methanolic solution of DPPH. The mixture was further incubated for 30 min in the dark, and the reduction of the DPPH radical was determined by measuring the absorption of the sample at 515 nm. Trolox was used as a standard reference and the results were expressed as Trolox equivalents (TE) per g of dry weight (mg TE/g dw TS).

Reducing power assay

The ferric reducing power of the *Cassia occidentalis* biosynthesized AgNPs were determined (Alavi and Karimi, 2017). The concentrations were used in the range of 1 – 5 µg/mL and the standard used as ascorbic acid. Based on the absorbance value, the ferric reducing power was expressed. IC₅₀ values were calculated using the linear regression curve for all the antioxidant assays.

Hydrogen peroxide scavenging assay

The free radical scavenging activity was determined by hydrogen peroxide assay. Hydrogen peroxide (40 mM) solution was prepared in phosphate buffered saline (0.1 M, pH 7.4). 1 ml of the AgNPs containing samples of different concentration will be rapidly mixed with 600 µL of hydrogen peroxide solution. The absorbance was measured at 230 nm after 10 min of incubation at room temperature against a blank (without hydrogen peroxide). The percentage of scavenging of hydrogen peroxide will be calculated using the following formula. Quercetin was used as a positive control (Venkatesan *et al.*, 2016).

Percentage scavenging H₂O₂ = $(A_0 - A_1) / A_0 \times 100$.

A₀ – Absorbance of control; A₁ – Absorbance of sample.

Statistical Analysis

Statistical analysis was performed using SPSS version 16 software (SPSS Inc., Chicago, IL) and the results were evaluated by one way ANOVA. Values were presented as mean ± S.E.M of the three replicates of each experiment. The results with $p < 0.05$ were considered to be statistically significant

RESULTS

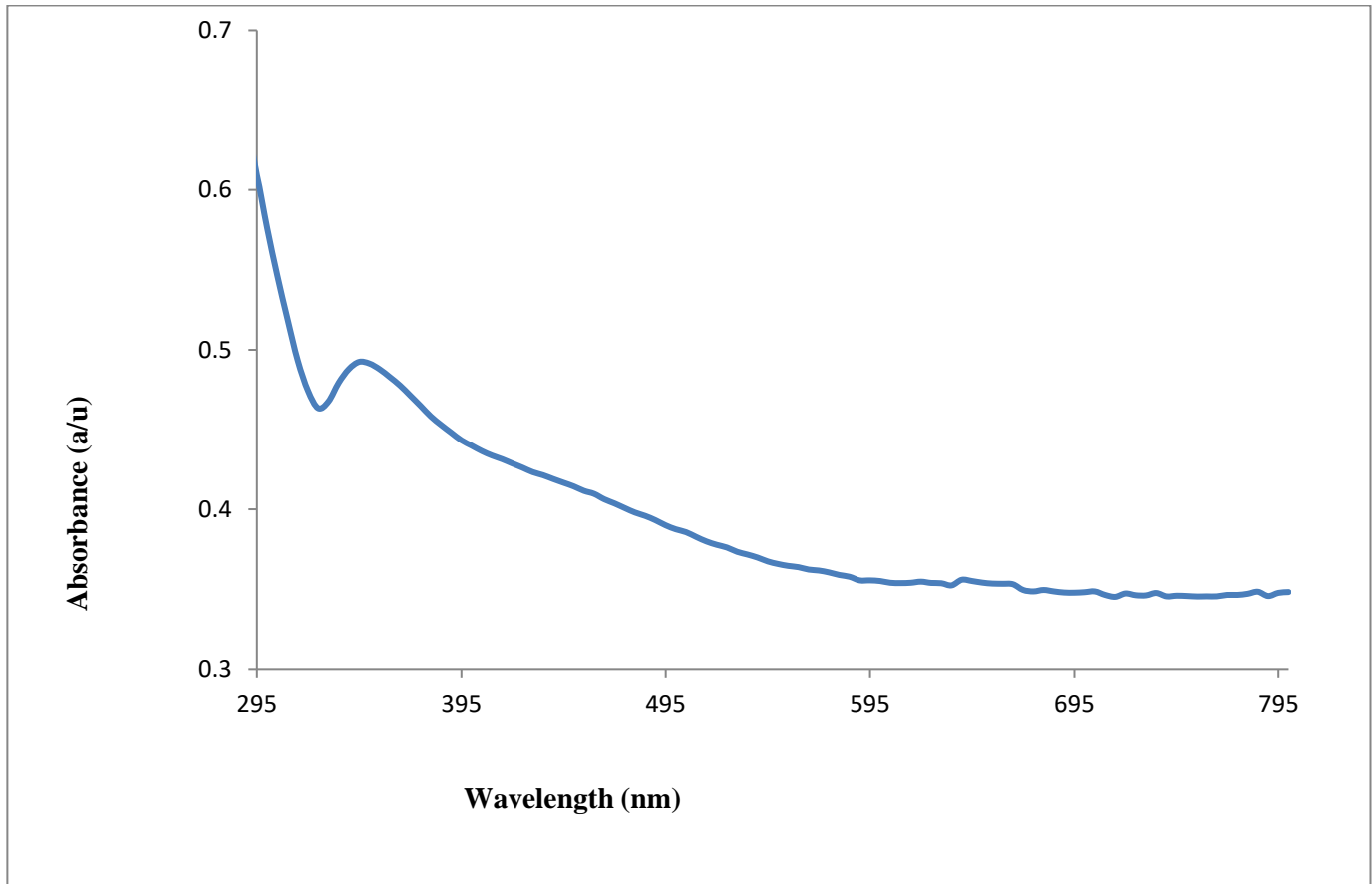


Figure 1: UV Visible spectrum of silver nanoparticle with absorption maximum at 350 nm

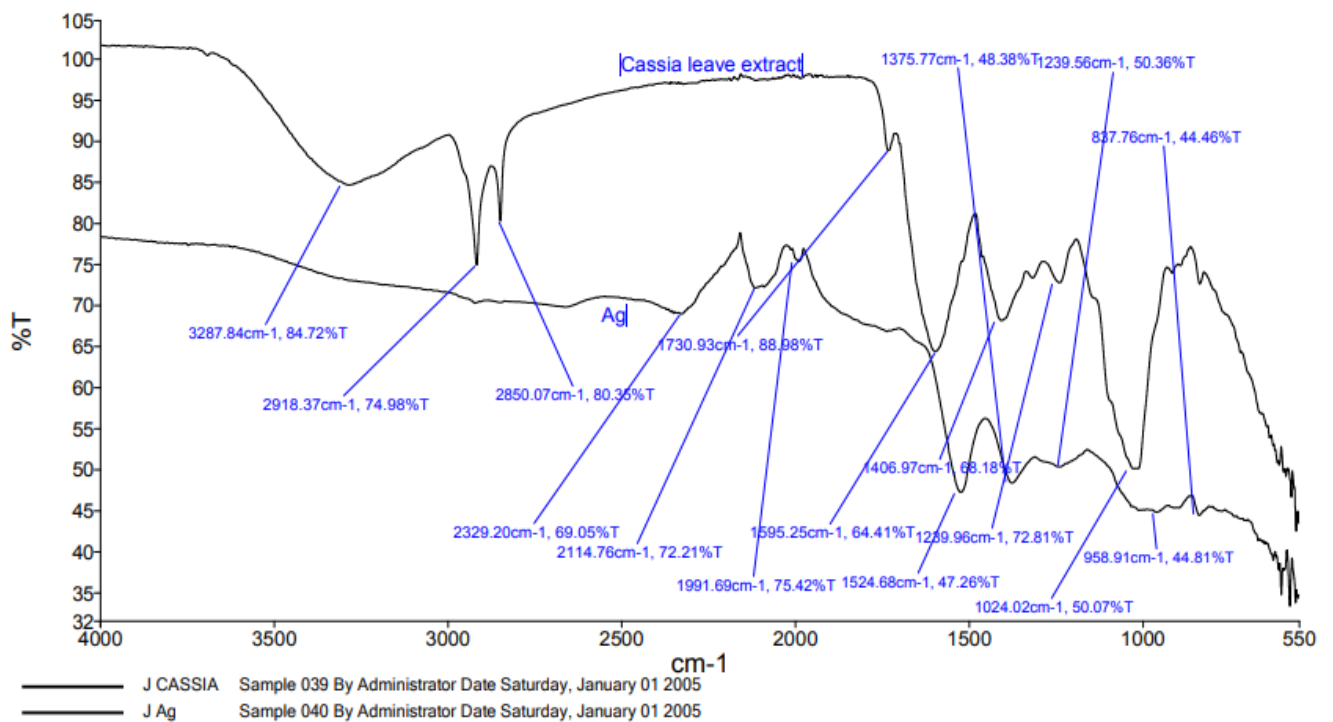


Figure 2: FTIR spectra of both silver nanoparticle and cassia occidentalis plant aqueous extract

Table 1: Zone of inhibition (mm) of antimicrobial activity silver nanoparticles of *Cassia occidentalis* leaf against *Staphylococcus aureus* and *Pseudomonas stutzeri*

Culture	AgNPs (10 µg/mL)	AgNPs (20 µg/mL)	Ciprofloxacin (20 µg/mL)
<i>Staphylococcus aureus</i>	10.00 ± 1.00	17.50 ± 2.50	15.00 ± 3.00
<i>Pseudomonas stutzeri</i>	11.00 ± 1.00	15.00 ± 0.05	22.00 ± 2.00

All values are mean ± SD of three replicates

Table 2: Antioxidant activity of DPPH on silver nanoparticles and ascorbic acids

S/No	Conc. (mg/mL)	AgNPs (%)	Ascorbic acid (%)
1	5	76.5 ± 0.21	89 ± 0.12
2	4	44.4 ± 0.98	86 ± 0.53
3	3	42.3 ± 0.34	83 ± 1.01
4	2	33.0 ± 0.48	82 ± 0.56
5	1	21.6 ± 0.87	77 ± 0.78
IC ₅₀		4.36	0.15

The values are mean ± SEM of three determinants

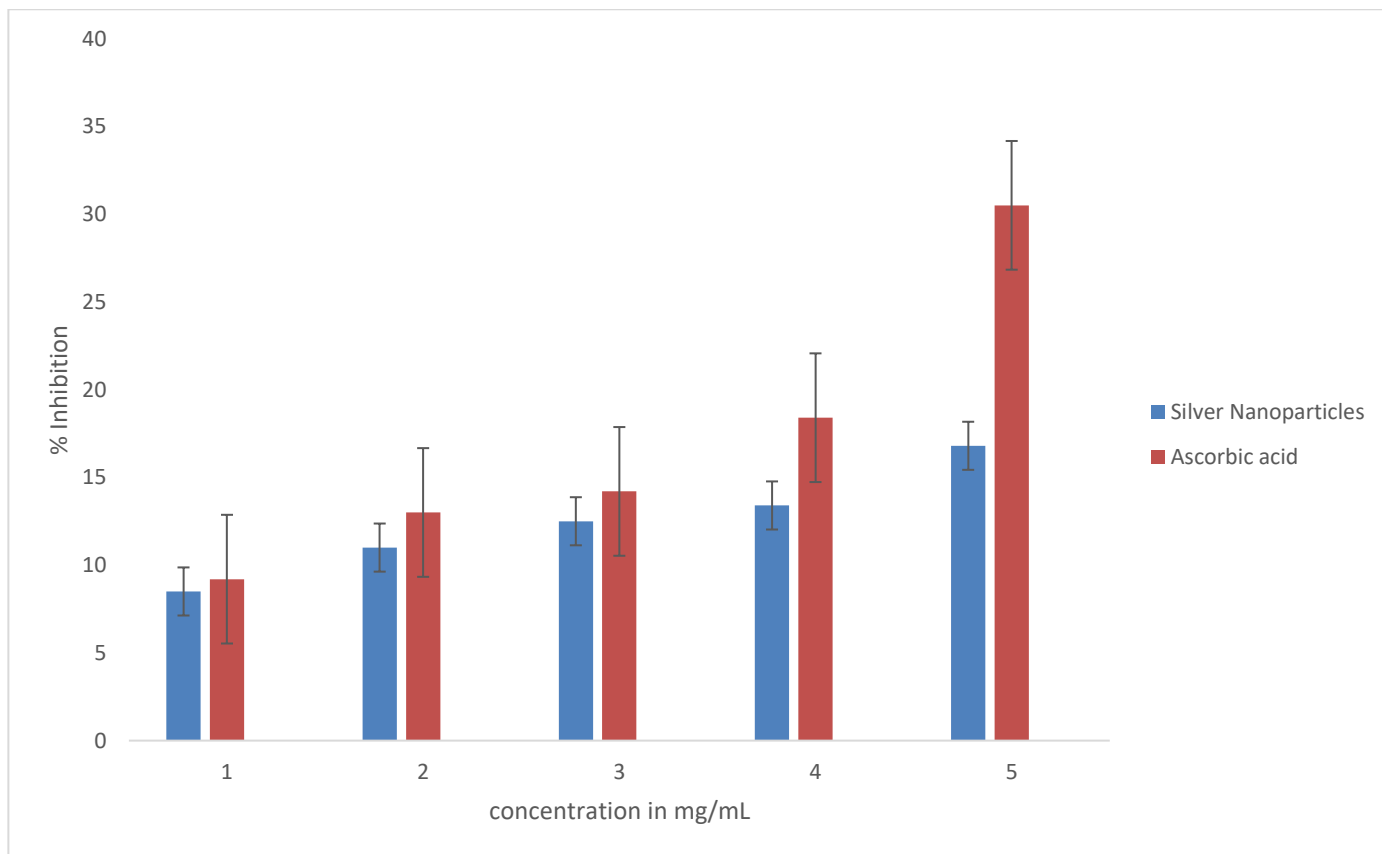


Figure 3: Antioxidant activity of AgNPs biosynthesized from *Cassia occidentalis* using FRAP

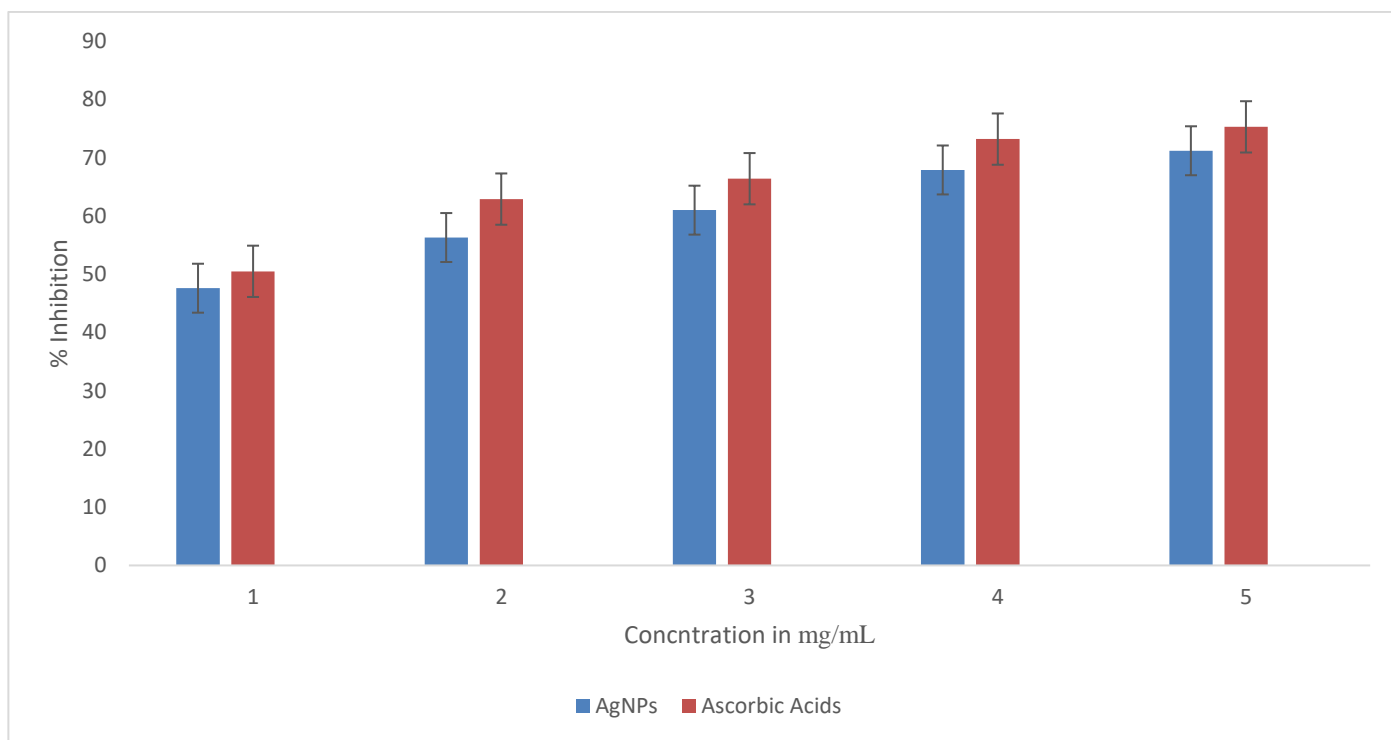


Figure 4: Antioxidant activity of AgNPs biosynthesized from *Cassia occidentalis* using H₂O₂

DISCUSSION

The UV visible spectroscopy analysis of the silver nanoparticle gave a sharp absorption maximum at 350 nm (figure 1) which is common to silver nanoparticles (Singh *et al.*, 2012; Nuraqeelah *et al.*, 2018). The narrow nature of the band and its singleness is an indication that the particles are same size, mono disperse and well aggregated (Tira *et al.*, 2020). There has been report of silver nanoparticles with a wavelength maximum of 335.9 nm (Anith *et al.*, 2022). Synthesized zinc oxide and observed that the UV-Vis absorbance spectrum shows strong absorption peaks occurring in the wavelength range of 220-380 nm for the unannealed samples and 190-235 nm for the annealed samples (Zikalala *et al.*, 2022).

FTIR are employed in determining organic functional group present in organic compound or other substance. Eight different absorption peaks were observed in the plant extract as shown in Figure 2 at wavenumbers which corresponds to a particular organic functional group. The broad band observed at 3287.84 cm⁻¹ corresponds to H-bonded O-H stretch alcohols. The peak at 2918.37 cm⁻¹ is assign to C-H stretch of alkane. The absorption band at 2850.07 cm⁻¹ corresponding to C-H stretch of alkane. The band at 1730.93 cm⁻¹ was given CHO of saturated amide. The strong band at 1592.31 cm⁻¹ reveals the presence of N-H bending of amide groups. Another absorption band was observed at 1406.97 cm⁻¹ it was assigned to C-H bending of saturated aliphatic. The stretched absorption band at 1239.96 cm⁻¹ indicate the C-O of ether while the absorption peak of 1024.02 cm⁻¹ correspond to C-O stretched alcohol. This is an indication that the aqueous leaf extract of *Cassia occidentalis* may contain bioactive molecules like carboxylic acids, proteins, alcohol, alkaloid, flavonoid, etc. There is a shift in these absorption band when compared to the FTIR of the nanoparticle as seen in Figure 2. The band at 2918.37 cm⁻¹ to 2329.20 cm⁻¹, 2850.07 cm⁻¹ to 2114.76 cm⁻¹, 1592.25 cm⁻¹ shifted to 1524.68 cm⁻¹, 1406.97 cm⁻¹ to 1375.77 cm⁻¹, 1730.93 cm⁻¹ to 1991.69 cm⁻¹, 1239.96 cm⁻¹ to 1239.56 cm⁻¹ and 1024.02 cm⁻¹ to 958.91 cm⁻¹ while the peak at 837.76 cm⁻¹ presents in nanoparticle but absent in the plant extract was assigned to silver. Mohammad and Aneela, (2016) reported that during the biosynthesis of nanoparticle there is a shift in the peak position.

The bactericidal effect of metal nanoparticles has been attributed to their' small size and high surface to

volume ratio, which allows them to interact closely with microbial membranes and also due to the release of metal ions in solution (Gold *et al.*, 2018). A cell wall is present around the outside of the bacterial cell membrane and it is essential for the survival of bacteria (Egan *et al.*, 2017). The cell membrane is called peptidoglycan made up of polysaccharides and peptides (Garde *et al.*, 2021). These can be thick cell wall containing many layers of peptidoglycan as in Gram positive or thin cell wall consisting of a few layers of peptidoglycan as in gram negative bacteria (Rohde, 2019). Surfaces of AgNPs interact directly with the bacterial outer membrane causing the membrane to rupture and killing bacteria (More *et al.*, 2023).

Antimicrobial activity of the biosynthesized AgNPs based on size of zones of inhibition in millimetre (mm) is shown in table 1. Result revealed that the prepared nanoparticles Possessed antibacterial properties against both *Staphylococcus aureus* and *Pseudomonas stutzeri*. The screened nanoparticles exhibited higher activities of 17.54 ± 2.5 mm on *Staphylococcus aureus* test organisms at a concentration of 2.0 u/ml as compared with *Pseudomonas stutzeri* in which lower activity of 15.00 ± 0.01 mm was recorded. The result of *Staphylococcus aureus* in this study seem to be similar to that obtained by Taglialeghna *et al.* (2019) but in contrast to their study, the inhibitory activity of the nanoparticles on *Staphylococcus aureus* was found to decrease as the concentration of the nanoparticles increases whereas in this study the inhibition activity increases as the concentration of the nanoparticles increases. This could possibly be due to the nature of the plant used in the synthesis of the nanoparticles (Jain & Mehata, 2017).

The antimicrobial activity of the nanoparticles could be due to the smaller size of the particles which leads to tightly adsorbed on the surface of the bacterial cells so as to disrupting the membrane which would lead to the leakage of intracellular component, thereby killing the bacterial cells (Häffner & Malmsten, 2017). Another possible mechanism involves the association of AgNPs nanoparticles with oxygen and its reaction with sulfhydryl (-S-H) groups on the cell wall to form R-S-S-R bonds, thereby blocking respiration and causing cell death (Ezeuko *et al.*, 2021). In this study Ciprofloxacin 20 ug/ml was used as positives control which has high zone of inhibition as compare with the nanoparticles in *Pseudomonas stutzeri*.

The antioxidant activity of *Cassia occidentalis* biosynthesized AgNPs was determined by using DPPH free radical and FRAP assay. DPPH is a stable compound which can be reduced by accepting the hydrogen or electrons and has been widely used to evaluate the antioxidant activity (Ivanova *et al.*, 2020). The lower IC₅₀ value indicates a stronger ability of the substance to act as a DPPH scavenger, while the higher IC₅₀ value indicates a lower scavenging activity (Rahman *et al.*, 2015). The results revealed that the synthesized AgNPs are free radical scavengers. The DPPH activity of the AgNPs was found to increase as the concentration increases ranging from 21.6 % to 76.5 % at concentrations 5 mg/mL, AgNPs showed a scavenging activity of 76.5 % with IC₅₀ value 4.36. The antioxidant activity was far greater than that of standard ascorbic acid which is 0.15 at 5 mg/mL with highest inhibition of 89 %. In FRAP assay, the ability of AgNPs to reduce Fe³⁺ to Fe²⁺ was also found to increase as the concentration of the AgNPs in the analysis increases, again antioxidant activities of the nanoparticles were less than that of standard ascorbic acid (Dridi *et al.*, 2022).

In conclusion, the AgNPs biosynthesised from *Cassia occidentalis* have the ability to inhibit the growth of bacteria.

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