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Room temperature phytosynthesis of silver nanoparticles using leaf extract of *Momordica charantia*: optical and antimicrobial properties

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Abstract. Nigeria is endowed with rich diversity of medicinal plants whose potential as “green” reducing agents are under-utilized. As the world is advocating for safe environment, plant-mediated synthesis of nanoparticles is considered as an eco-friendly and sustainable synthetic route instead of using toxic chemicals. The method is fast, easy and cheaper compared with other conventional techniques. In this study, phytochemicals present in the leaf extract of indigenous *Momordica charantia* served as reducing, capping/stabilizing agents. The synthesized silver nanoparticles were characterized with Uv-vis spectrophotometer, photoluminescence (PL) and energy-dispersive x-ray spectrometer (EDX). Antimicrobial activities of the synthesized nanosilver were investigated on isolated *Streptococcus pyogenes*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* *Candida albicans* and *Trichophyton rubrum*. Optical measurement showed surface plasmon resonance with broad absorption peaks (400-450 nm). Significant growth inhibitions were also found at $P < 0.05$ by means of analysis of variance SPSS tool. The leaf influenced nanosilver displayed highest activity on *S. aureus*, *S. pyogenes* and *E. coli* with MIC and MBC value of 12.5 mg/mL. Least activity was detected against *P. aeruginosa* (50 mg/mL MIC and 100 mg/mL MBC). From this work, the biogenic nature and optical properties displayed by the as-prepared nanosilver strongly suggest its applications as candidate for therapeutic drugs, diagnostic and medical imaging.

Keywords: *Momordica charantia*, antimicrobial, optical properties, nanosilver

1. Introduction

Colloidal science has distinguished nanoparticles (particles within 1-100 nm range) and colloidal particles (particles between 2-200 nm range) [1], as this forms backbone of nanotechnology [2]. Particles in



nanoscale are widely applied as a result of their special biological, electrical and optical properties [3]. They found applications for instance, in drilling mud formulation [4], solar cells [5], optical spectroscopy like surface-enhanced Raman scattering, catalysis, imaging, biosensing, drug delivery [6], mild steel as anti-corrosion using nano-additives agent [7], among others. Nanoparticles of silver, gold and copper are known for their unique plasmonic characteristics which qualify them to sense label free and low-level analytes in biological media [8]. Silver nanoparticles (Ag NPs) are used in biomedical, food packaging industry and pharmaceutical fields. Their exceptional properties like large surface area to reserve silver ion, thermal stability, among others and antimicrobial activities against bacteria make them suitable as potential candidate against drug resisting bacteria [9]. Ag NPs have been applied as antimicrobial agent in catheters, cosmetics, bandages, water purification etc [10].

Biological methods of synthesizing Ag NPs include the use of microorganisms, enzymes and plant extracts [11-13]. These methods are of great advantage as they are environmentally friendly, cost effective and save time compared with the physical and chemical methods. *Momordica charantia* Linn., is a member of Cucurbitaceae family, it is a climber with tendrils, globous and lobed palmate leaves. It is commonly known as African cucumber. Some tribes in Nigeria name it *Ejinrin were* (Yoruba), *Alo-ose* (Ibo), *Kakayi* (Hausa). *M. charantia* is used to treat diabetes, piles, convulsions, jaundice, sore, nervous disorders, diabetic recipe, emetic, night blindness, aphrodisiac and dysmenorrhoea in traditional medicine. The plant also possesses antifungal properties [14]. Ethnobotanical use of African cucumber includes antimicrobial, treatment of amenorrhoea and gonorrhoea [15, 16].

Green mediated synthesis of Ag NPs is gaining recognition by researchers. Leaf extracts of *Nigella arevensis* [17], *Garcinia indica* [18], *Thuja occidentalis* [19], *Givotia moluccana* [20], etc. were utilized for the synthesis of Ag NPs. Phytochemicals and anti-oxidants present in plants have been reported to act as capping and reducing agents [21, 22]. Our previous work has shown synthesis of Ag NPs at an elevated temperature by leaf extract of *Momordica charantia* [23]. It is worth mentioning that there is an environmental concern due to toxic chemicals used in physical and chemical syntheses of nanoparticles. Also, drug resistance by the disease-causing microbes has become an issue in health industry which needs to be addressed. Therefore, in view of the sustainability, eco-friendliness and low cost of this route, we report the optical and antimicrobial properties of phyto-synthesized nanosilver at room temperature using biomass as bio-reducing agent and stabilizer.

2. Experimental work

2.1. Materials and reagents

Pure grade of silver nitrate (99.99%) for this experiment was procured from Sigma–Aldrich Chemical Limited, used without extra purification. Other materials include Whatman no. 1 filter paper, double distilled (d-d) water, fresh leaves of *Momordica charantia* Linn.

2.2. Preparation of aqueous leaf extract

Fresh plant of *Momordica charantia* Linn. (Fig. 1) was collected from Atan, Ogun State, Nigeria (Fig. 2). It was identified and authenticated at Forest Research Institute of Nigeria (FRIN). Voucher sample (FHI No. 109926) was deposited at FRIN herbarium headquarters. Leaf parts were thoroughly rinsed with tap water after which d-d water was used to remove any other impurities from the surface. The leaves (40 g) were cut into small pieces and homogenized using clean blender. Double-distilled water (200 mL) was added to the slurry formed and then filtered through Whatman no. 1 filter paper. The filtrate was stored at 4°C for phytochemical screening and nanoparticles synthesis.



Figure 1. Image of *Momordica charantia* Linn. (FHI No. 109926).

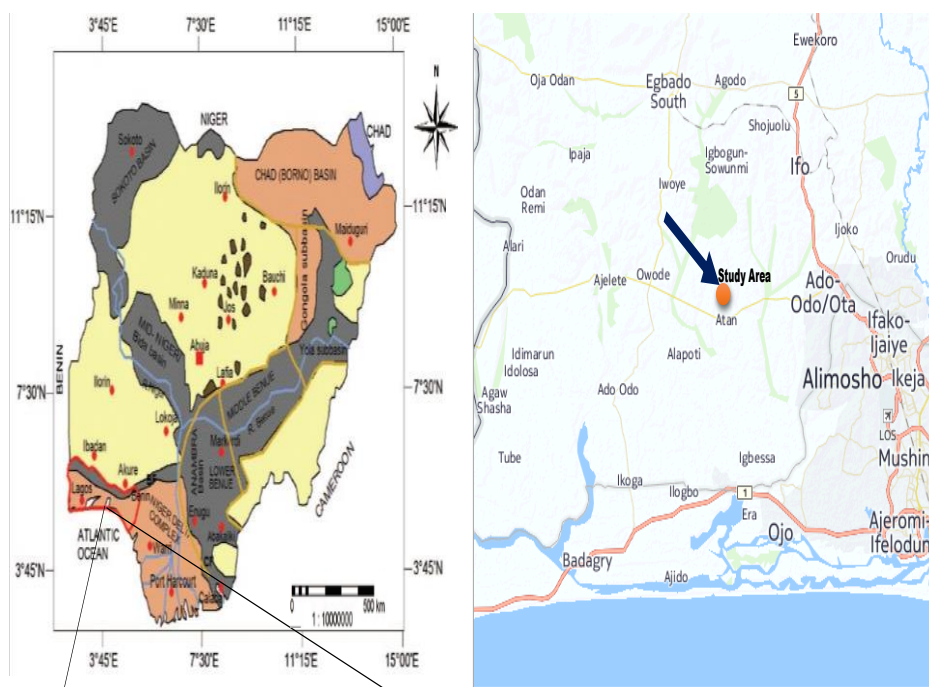


Figure 2. Map of Nigeria with arrow showing plant collection site.

2.3. Phytochemical Screening of *M. charantia* Plant

The leaf extract of *M. charantia* was screened to identify the phytochemicals present using standard procedure by Ciulei [24].

2.4. Synthesis silver nanoparticles (Ag NPs) at room temperature

Synthesis of Ag NPs was carried out via plant-reduction of metal source with variation to earlier study [3, 25]. Varying AgNO_3 concentrations: 0.5, 1.0, 2.0 and 3.0 mM were prepared, then to 400 mL of each metal precursor concentration, 40 mL of *M. charantia* extract was added in a 500 mL conical flask. Reaction mixture was stirred vigorously and left at 25°C, and the initial colour of the solution was noted. Absorbance of the sample was checked by taking aliquot sample at different times for 2 hours, until noticeable colour change and appearance of surface plasmon band (SPB) were achieved.

2.5. Isolation of Ag NPs

Formation of Ag NPs was hinted by appearance of surface plasmon band (SPB). Nanocluster was isolated from the reaction mixtures by centrifugation using thermo fisher scientific centrifuge (Thermo Electron LED) at 10,000 rates per minute (rpm) for 40 minutes. Resulting suspension from each solution concentration was re-dispersed separately in d-d water to remove any solvated materials. This was repeated severally to ensure purification from organics. Suspension obtained from each reduced metal precursor concentration was air-dried and collected in Eppendorf tubes for further characterizations and antimicrobial study.

2.6. Characterization

2.6.1. Optical characterization. Optical absorption spectra of the synthesized Ag NPs were detected with Scientific GENESYS 10S model Uv-visible spectrophotometer between 280 nm and 800 nm. Maximum absorbance was measured by placing each aliquot sample in quartz cuvette, operated at a resolution of 1 nm. Photoluminescence (PL) emission of the nanoparticles was also measured with a Perkin-Elmer 55 spectrophotometer at room temperature.

2.6.2. EDX study. Data of the element components of Ag NPs were recorded with Technai G2 transmission electron microscope (TEM) attached with an energy-dispersive X-ray spectrometer (EDX), operated at an accelerating voltage of 200 KeV and 20 μ A current. Ag NPs suspensions were drop-coated on carbon-coated copper TEM grids and allowed to dry before taking any measurements.

2.7. Antimicrobial study

2.7.1. Test microorganisms. Freshly cultured clinical isolates were collected from the Department of Medical Microbiology and Parasitology, Sacred Heart Hospital, Lantoro, Abeokuta in Nigeria. The organisms used were: Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*), Gram-positive bacteria (*Staphylococcus aureus* and *Streptococcus pyogenes*) and fungi—(*Candida albicans* and *Trichophyton rubrum*).

2.7.2. Sensitivity of test organisms. Sensitivity testing was carried out on the biosynthesized Ag NPs to investigate their antimicrobial properties. Sterile agar slant was used to collect the test organisms and incubated at 37°C for 24 hours. Sugar fermentation, citrate utilization, oxidase reaction, voges-proskauer, methyl red, capsule staining, spore staining, motility, indole test, urease test, hydrogen sulphide test, gelatin liquefaction and gram staining tests were evaluated on bacteria test organisms. *Candida albicans* (fungus) was identified by Gram staining, germ tube test, sugar fermentation and assimilation tests. *Trichophyton rubrum* (fungus) was identified macroscopically and microscopically using lactophenol cotton blue stains. These were kept and served as stock culture at 4°C. The procedure is agreement with endorsed standards of National Committee for Clinical Laboratory Standards (NCCLS) [26].

2.7.3. Agar well diffusion method. In this study, the Ag NPs synthesized using *M. charantia* leaf extract were evaluated for their antimicrobial activities on clinical isolates using well plate agar diffusion method [25]. The microbial cultures were adjusted to 0.5 McFarland turbidity standards, which was then used for the inoculation on Mueller Hinton agar plate (diameter 9 cm). The plate was filled and swirled with 1 mL of each standardized test organisms, followed by decantation of superfluous inoculum. Well of 6 mm diameter was made on the agar plates using sterile cork borer, then 0.1 mL aliquot of the Ag NPs dilution was reconstituted in 50%, 100 mg/mL DMSO. It was then applied on each well in the earlier inoculated culture plates containing the test organisms. The experiment was carried out in duplicate as 0.1 mL of 5 μ g/mL ciprofloxacin and fluconazole served as positive controls for bacteria and fungi respectively. Proper dilution of the Ag NPs was ensured by leaving the experiment on the bench for a duration of 1 hour. Thereafter, incubation of the bacteria at 37°C, 24 hours and yeast (*T. rubrum*) at 28°C for 72 hours. Antimicrobial activity of Ag NPs was determined by measuring zone of inhibition around each well (without the diameter of the well) for Ag NPs. Duplicate tests were conducted against each organism [26].

2.7.4. Minimum inhibitory concentration (MIC) by tube dilution method. Sterilized test tubes (12) were arranged in a rack, afterwards 1 mL of sterile nutrient broth was added to tube labelled 2 to 10. Known nutrients broth concentration (1 mL) was added to tubes 1 and 2. Serial doubling dilution from tube 2 to tube 10 was made, while the remaining 1 mL was discarded. 1 mL of ciprofloxacin was added to tube 11 (positive control); and water to tube 12 (negative control). 1 mL of 0.5 McFarland was added overnight and broth culture to all the tubes and then covered. The experiment was incubated overnight at 37°C and observed for the highest dilution showing no turbidity. The zone of inhibition was then verified and interpreted according to CLSI guidelines (2006) and the MIC was determined.

2.7.5. Minimum bactericidal concentration (MBC). MBC was determined by using modified method by Doughari *et al.* [27] where 0.5 mL of the sample was removed from those tubes from MIC which did not show any visible sign of growth, it was then inoculated on sterile Mueller Hinton agar by streaking. The plates were then incubated at 37°C for 24 hours. The concentration at which no visible growth was seen was recorded as the minimum bactericidal concentration [27].

2.7.6. Minimum fungicidal concentration (MFC). The method described by Doughari *et al.* [27] was used to determine MFC. 0.5 mL of the sample which showed no visible sign of growth during MIC screening was taken from the test tubes and inoculated on sterile potato dextrose agar by streaking. The plates were then incubated at 37°C for 24 hours. The concentration at which no visible growth was seen was recorded as the minimum fungicidal concentration (MFC) [27].

3. Results and Discussion

3.1. Qualitative analysis

The phytochemical screening carried out on the leaf extract of *Momordica charantia* in this study indicated the presence of biomolecules which were responsible for the reduction of metal ions and capping of the newly formed nanoparticles (table 1). Choice of water as the extraction solvent limited the number of phytochemicals in water extract compared with alcohol solvent medium. This result is similar to previous study [28].

Table 1. Qualitative analysis of *Momordica charantia* aqueous leaf extract.

Phytochemicals	Water extract	Methanolic extract
Tannins	-	++
Phenols	-	++
Flavonoids	-	+
Saponins	++	+
Glycosides	+	++
Anthraquinones	-	-
Alkaloids	-	++
Carbohydrates	-	-

(+) = Weak presence of phytochemical; (++) = Strong presence of phytochemical; (-) = Absence of phytochemical

3.2. Optical properties of the Ag NPs

Optical absorption spectra of the bio-inspired Ag NPs are shown in Fig. 3. Reduction of Ag⁺ to Ag⁰ was possible due to the presence of suitable biomolecules present in the leaf extract of *M. charantia* (table 1). There was excitation of surface plasmon vibrations in the energy levels of the Ag NPs, as evident in

the observed colour change (visual evidence of Ag NPs formation), displayed in the reaction media from light brown to dark brown at room temperature [29].

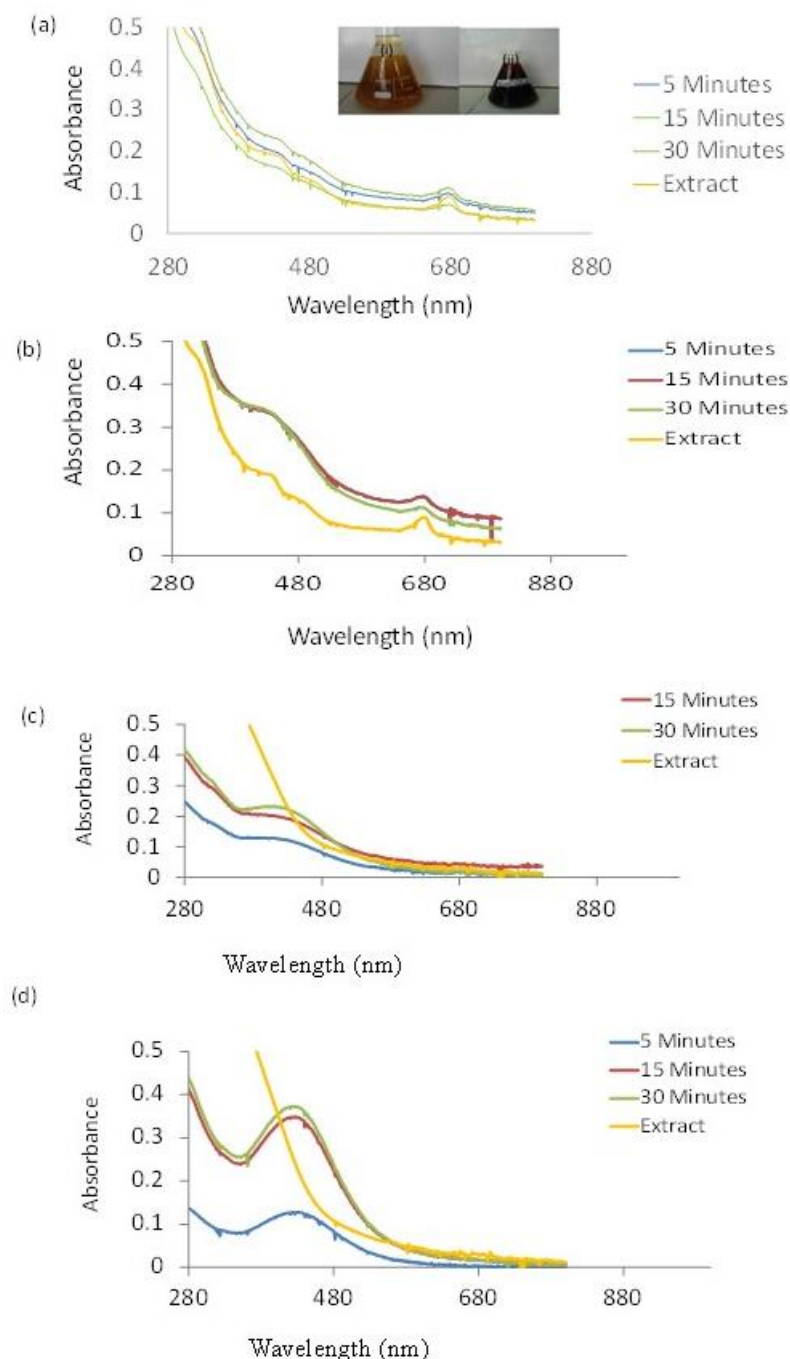


Figure 3. Room temperature time-resolved uv-vis spectra of Ag NPs prepared from (a) 0.5 mM (b) 1.0 mM (c) 2.0 mM (d) 3.0 mM AgNO_3 with the leaf. Inset: Colour dispersion before (i) and after reduction (ii).

Nucleation and onset growth took longer time (1 hour) in 0.5 mM precursor solution as indicated in the SPR peaks broadening which informed larger particle sizes as synthesis of nanoparticles is time

dependent [30]. This could plausibly be the result of strong interaction between phytochemicals: saponins and glycosides in the extract and the growing particles. At higher metal precursor concentrations, 1.0-3.0 mM, rapid formation of Ag NPs took place within 5 minutes of the reaction. This was noticed with a change in colour from light brown to dark brown (Inset in Fig. 3). Overlap in the spectra signified reaction completion between 15th and 30th minute of the reaction time. Narrow SPR peaks due to confinement of electrons and highest intensity of absorption (0.367 a.u.) which increased in intensity as a function of reaction time between 15 and 30 minutes occurred at peak 435 nm in the reduced 3.0 mM precursor solution, while broad peaks (400-450 nm), indicating polydispersed nanoparticles were observed at lower precursor solutions concentrations (Fig. 3d). Growth comparison in the varied precursor solution concentrations is depicted in Fig. 4. The observed Ag NPs spectra is also in agreement with previous work [31].

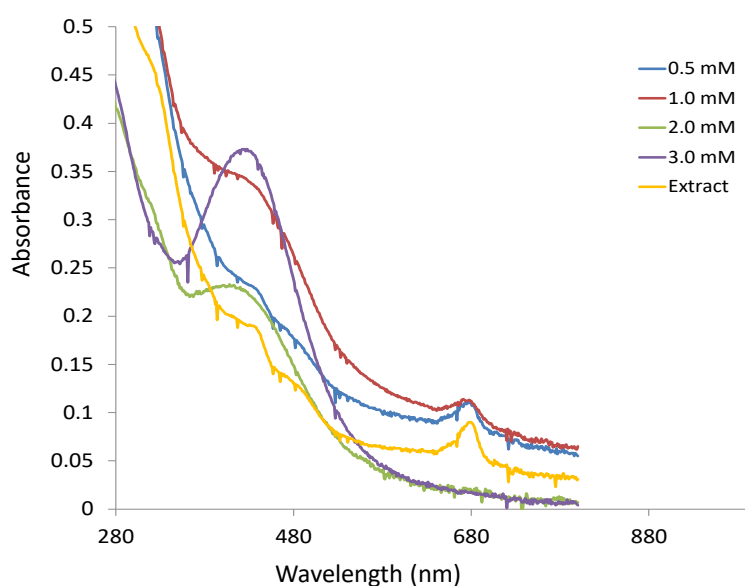


Figure 4. Comparison of onset growth in Ag NPs prepared from varied precursor solution concentrations at room temperature for 30 minutes.

3.3. EDX study

EDX study showed element constituents of Ag NPs and capping groups by the phytochemicals, which provided stability of the nanoparticles indicated the weight composition of Ag (31.5%). Oxygen (20.80%) and carbon (14.99%) (Fig. 5).

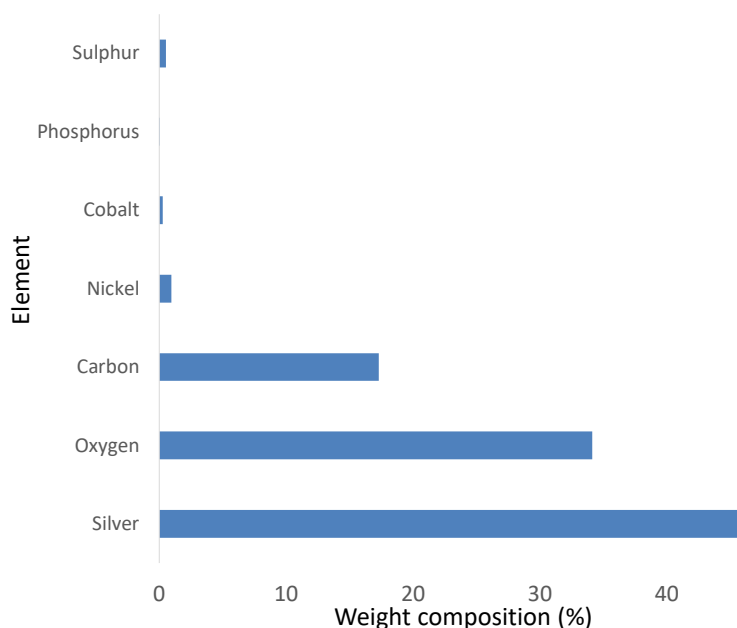


Figure 5. EDX showing the weight composition of elements.

3.4. Antimicrobial activities

Test showed that the activity of Ag NPs was based on the size of zones of inhibition in millimetre (mm) (Fig. 6). From agar diffusion test, Ag NPs possessed antibacterial and antifungal properties, as they displayed zones of inhibition to a reasonable level. Interestingly, the as-prepared Ag NPs exhibited highest activities on the organisms at 3.0 mM concentration by increasing the inhibition zones on all the organisms considered, except *P. aeruginosa* in which low activity was recorded. This agrees with literature as they demonstrated concentration-dependent inhibitory effects [32].

However, ANOVA and SPSS statistical tools indicated significant difference among varied concentrations of Ag NPs on the growth inhibition against the microbes; as $p < 0.05$. This indicated different activity at all concentrations of the nanoparticles compared with the standard-ciprofloxacin (Bacteria) and fluconazole (Fungi).

Furthermore, the zones of inhibition recorded in agar well diffusion test led to the conduction of Minimum Inhibitory Concentration (MIC), Minimum Bactericidal Concentration (MBC) and Minimum Fungicidal Concentration (MFC) tests. The results showed the activities of nanoparticle on *S. aureus*, *S. pyogenes*, *E. coli*, *P. aeruginosa*, *C. albicans* and *T. rubrum* (table 2). From the MIC testing on Gram negative bacteria (*E. coli* and *P. aeruginosa*) and the Gram-positive bacteria (*S. aureus* and *S. pyogenes*), the nanoparticles inhibited the growth of *S. aureus*, *S. pyogenes*, *E. coli* at the same MIC and MBC values of 12.5 mg/mL and 25 mg/mL respectively. There was no activity on *E. coli* at 2.0 mM precursor concentration. However, the growth of *C. albicans* was inhibited at the values of 12.5 mg/mL (MIC) and 12.5 (MFC) while the same MIC and MFC value of 50 mg/mL was observed on the inhibition of *C. albicans* and *T. rubrum*. ANOVA SPSS statistical tool indicated significant difference with the highest activity being $P < 0.05$ among different concentrations considered. Remarkably, increase in the zone of inhibition is directly proportional to the increase in concentration of the bio nanoparticles; yet, their activities on the pathogens were slightly weaker compared with the antibiotics ciprofloxacin and fluconazole (standards).

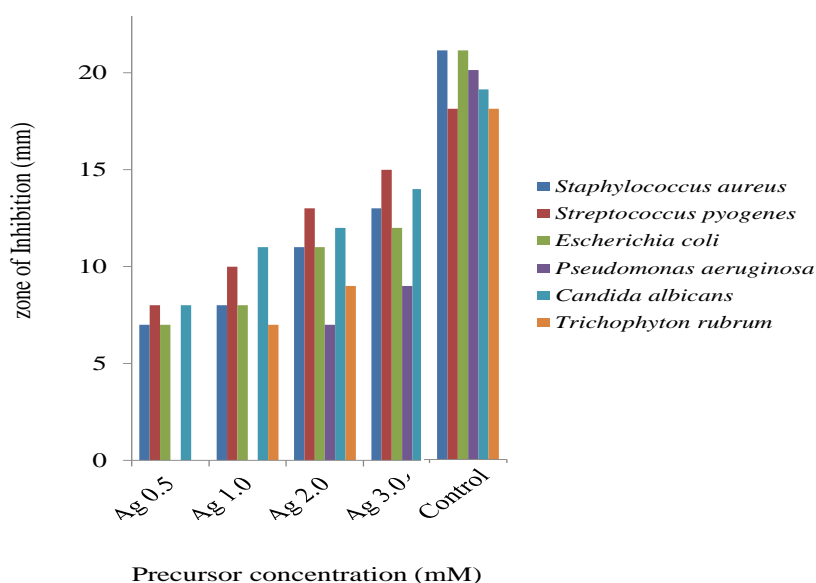


Figure 6. Comparison of inhibition zones between Ag NPs synthesized at varied AgNO_3 concentrations.

Table 2. Minimum inhibitory concentration (MIC), Minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC).

Organisms Nano particles	Organisms/MIC, MBC & MFC (mg/mL)					
	<i>Staphylococcus aureus</i> MIC, MBC	<i>Streptococcus pyogenes</i> MIC, MBC	<i>Escherichia coli</i> MIC, MBC	<i>Pseudomonas aeruginosa</i> MIC, MBC	<i>Candida albicans</i> MIC, MFC	<i>Trichophyton rubrum</i> MIC, MFC
Ag 0.5	100, 100	100, 100	100, 100	100, 100	100, 100	100, 100
Ag 1.0	100, 100	25, 50	100, 100	100, 100	25, 50	100, 100
Ag 2.0	12.5, 25	12.5, 12.5	12.5, 25	100, 100	12.5, 25	50, 100
Ag 3.0	12.5, 12.5	12.5, 12.5	12.5, 25	50, 100	12.5, 12.5	50, 50
STATISTICS	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$
Control	3.13	6.25	6.25	6.25	6.25	6.25
STATISTICS	Control vs Aa, Ba, Ca, Da, Ea, A, B, C, E, F-value 34.06, $P < 0.05$					

Control-Ciprofloxacin (Bacteria) and Fluconazole (Fungi)

4. Conclusion

In this present study, Ag NPs was successfully synthesized at room temperature using phyto-reduction method that is inexpensive and eco-friendly. *Momordica charantia* served as the reducing agent instead of toxic chemicals. The nanoparticles are optically active as they display excitation of SPR. The colour change, elemental composition and the absorption peak between 400-450 nm was an evidence of Ag NPs formation. Possession of surface plasmon resonance indicated them as potential enhancer in optical material. Furthermore, the antimicrobial activity of Ag NPs qualifies them as possible antibacterial against *S. aureus*, *S. pyogenes* and *E. coli*.

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