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Green synthesis of copper nanoparticles using *Musa acuminata* aqueous extract and their antibacterial activity

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Abstract. *Muhammad A, Umar A, Birnin-Yauri AU, Sanni HA, Elinge CM, Ige AR, Ambursa MM. 2023. Green synthesis of copper nanoparticles using* Musa acuminata *aqueous extract and their antibacterial activity. Asian J Trop Biotechnol 20: 10-16.* Green synthesis is a convenient and affordable alternative to the synthesis of copper nanoparticles. The objective of the present study was to synthesize copper nanoparticles via the green method, using *Musa acuminata* (banana) fruit extracts as a reducing agent with copper sulfate serving as a precursor. The antibacterial activities of the synthesized CuNps were also tested. Fruits extracts of *M. acuminata* were used to synthesize with $CuSO_4$(aq) as a precursor. The synthesized nanoparticles were characterized using UV-Vis, FTIR, XRD, SEM, and EDX techniques. The synthesis results showed that the nanoparticles were of Face-Centered Cubic (FCC) structure with high stability. The average diameter of the synthesized copper nanoparticles is 18nm. UV-Vis, scanning electron microscope, X-ray diffraction, energy disperses X-ray, and Fourier transforms infrared spectroscopy established that the formed nanoparticles are copper nanoparticles. The antibacterial activity of the synthesized nanoparticles was also tested using pathogenic bacteria *Escherichia coli* and *Staphylococcus aureus*. The results showed that copper nanoparticles were promising antibacterial agents.

Keywords: Antibacterial activity, banana, copper nanoparticles, $CuSO_4$ solution, *Musa acuminata* extract

INTRODUCTION

Green synthesis can be defined as the derivation of materials from green or eco-friendly resources by using solvents, excellent reducing agents, and harm less material for stabilization (Bonde 2011: Sable et al. 2012; Jadoun et al. 2021). Furthermore, this synthesis route is simple, economical, reliable, sustainable, and relatively reproducible and provides more stable compounds. Therefore, researchers are interested in developing various nanomaterials through this biosynthesis pathway, including metal/metal oxide nanoparticles, hybrid materials, and biologically inspired materials. As a result, green synthesis is widely regarded as a necessary tool to reduce the negative effects of conventional nanoparticle synthesis methods used in laboratories and industry (Yedurkar et al. 2017).

With numerous reports and studies, this green synthesis process already produces a large number of metal/metal oxide nanoparticles such as silver (Ag), gold (Au), selenium (Se), platinum (Pt), zinc oxide, etc. (Chakraborty et al. 2022). Furthermore, the studies also reported that some metallic nanoparticles have a variety of biological and biochemical activities, but CuNPs have recently received a lot of attention. Copper plays a variety of roles in humans health, including the production of neuropeptides, regulation of cell signaling pathways, antioxidant defense, and co-factoring of many enzymes involved in immune cell function (Georgopoulos et al. 2001; Ghaderian and Ravandi 2012).

Copper Nanoparticles (CuNPs) attract positive global attention because of their low-cost and novel optical, mechanical, catalytic, electrical and thermal conduction properties, which differ from that of bulk metals (Brust and Kiely 2002). Copper nanoparticles have been considered alternatives for noble metals in many applications, such as heat transfer and microelectronics. However, the synthesis of copper nanoparticles poses a challenge due to the high tendency for oxidation. Unlike gold and silver, copper is extremely sensitive to air, and the oxide phases are thermodynamically stable (Jeong et al. 2008).

Plants produce many biologically active chemicals. Because very small amounts of these heavy metals are hazardous even at very low concentrations, plants have shown extraordinary potential in detoxifying and accumulating heavy metals, which may allow them to outrun environmental pollutants (Chakraborty et al. 2022). In addition, the synthesis of nanoparticles using plant extracts is superior to other biological synthesis methods, such as microorganisms. That is because the rate of synthesis of metallic nanoparticles using plant extracts is more sustained (Saif et al. 2016), significantly faster and more monodisperse than other biological methods (Sarkar et al. 2020).

Copper nanoparticles have been synthesized using extracts from various plants found all over the globe (Murthy et al. 2020). From this note, many plant parts, fruits, or whole plants have been used for the green synthesis of CuNPs due to the presence of a large number of bioactive compounds (Murthy et al. 2020). The Synthesis of CuNPs has been successfully extracted from various parts of *Punica granatum* (Kaur et al. 2016), *Zingiber officinale* stem (Delma and Rajan 2016), *Citrus medica* juice (Shende et al. 2015), *Ziziphus spina-christi* fruit (Khani et al. 2018), *Asparagus adscendens* roots and leaves (Thakur et al. 2018); leaves of *Eclipta prostrata* leaves (Chung et al. 2017); *Ginkgo biloba* leaves (Nasrollahzadeh and Mohammad 2015); *Plantago asiatica* leaves (Nasrollahzadeh et al. 2017); *Thymus vulgaris* (Issaabadi et al. 2017); Black tea leaves (Asghar et al. 2018); *Terminalia catappa* leaves (Muthulakshmi et al. 2017) and *Azadirachta indica* (Ahmed et al. 2015).

This research work synthesized CuNP from copper sulfate pentahydrate and aqueous extract from *Musa acuminata*. Advanced techniques characterized the copper nanoparticles, and an antimicrobial assay was performed.

MATERIALS AND METHODS

The fruits of *M. acuminata* (banana) were obtained from Sokoto central market for this study. The representative samples for the analysis were selected by random sampling. The samples of the banana fruits were peeled, and 20 g were accurately weighed. The sample was then crushed and finely macerated with mortar and pestle. The sample was then added to 100cm^3 of deionized water and boiled over a water bath at 60°C for 15 minutes. The extract was allowed to cool down and filtered with muslin cloth through Whatman filter paper and used immediately for the synthesis. A 0.01 M solution of $CuSO_4.5H_2O$ was prepared by dissolving 2.4955 g of the salt in 10 cm^3 of deionized water.

Green synthesis of copper nanoparticles

About 10 cm^3 of the *M. acuminata* extract was added to 100 cm^3 of 0.01 M $CuSO_4.5H_2O$ aqueous solution and mixed thoroughly. The mixture was heated to 80°C with constant stirring on a magnetic stirrer for six h. The suspension produced was centrifuged at 3,000 rpm for 10 min, and the supernatant liquid was decanted. The residue was repeatedly washed with 10 cm^3 of deionized water. Centrifugation-decantation-washing processes were repeated five times to remove impurities on the surface of the copper nanoparticles. The obtained precipitates were dried in an oven at 50°C for 24 h. The synthesized copper nanoparticles were then kept for characterization and antimicrobial studies.

Characterization

UV-Visible spectroscopy

UV-Visible spectroscopy technique was used in this research to confirm the formation of nanoparticles. For each analysis, 1.0 cm^3 of the aliquot suspension was diluted in 4.0 cm^3 deionized water, and its UV-Visible spectrum was measured at 80°C (Gnanasangeetha and Prathipa 2019).

Fourier transform infrared spectroscopy (FTIR)

FTIR analysis was carried out to identify the possible biomolecules responsible for reducing copper sulfate to CuNPs and the capping and stabilizing agents for CuNPs. After synthesizing the copper nanoparticles, the precipitate obtained using each extract was dried in an oven at 50°C for 24 h. The dried synthesized copper nanoparticles were grounded with KBr, cast into a pellet, and used for analysis on the FTIR spectrophotometer in the diffuse reflectance mode operating at a resolution of 4 cm^{-1} (Gnanasangeetha and Sarala 2015).

X-Ray Diffraction studies (XRD)

X-ray diffraction was used to determine the phase and crystallinity of the nanoparticles. The dried samples of the as-synthesized copper nanoparticles were grounded to fine powder and a thin film of each sample was made by dipping a cleaned glass plate into the powdered nanoparticles of the sample. The XRD analysis was performed passing monochromatic Cu radiation (θ = 1.5406 Å) operating at a voltage of 40kV and a current of 25mA on the sample film at room temperature (25°C). The XRD patterns were then collected at 2θ angles between 10° and 80°, 0.02 min$^{-1,}$ and at 1 second time constant. The peaks obtained were compared with the reference database in the Joint Committee on Powder Diffraction Standards (card no: 53-61386) library to ascertain the nature of the nanoparticles (Gnanasangeetha and Sarala 2015).

Scanning Electron Microscopy (SEM)

The SEM is a multipurpose instrument that provides qualitative information about the samples, such as topography, morphology, composition, and crystallographic arrangements, with high resolution (Gnanasangeetha and Suresh 2020). First, the surface morphology of the synthesized copper nanoparticles was studied using Scanning Electron Microscopy (SEM). Next, the sample was prepared to study size and crystallographic structure by placing a drop of colloidal solution of the synthesized copper nanoparticles in copper (II) sulfate on a carbon-coated copper slide and subsequently drying it in the air before transferring it to the microscope. The operation was carried out at an accelerated voltage of 130 kV, magnification of X^{10}, and resolution of 1 nm (Sarala 2015).

Energy Dispersive X-ray (EDX)

The use of EDX analysis investigated the presence of metallic copper. The copper nanoparticles' elemental composition, purity, and geometry were studied. Copper nanoparticles had a sorption peak at 1Kev (Ebrahimi et al. 2017), the point at which the index for metallic nanoparticles of copper was expected. The EDX observation was carried out by instrument coupled with SEM (Gnanasangeetha and Sarala 2015).

Test of antibacterial activity

The antibacterial activity of the synthesized copper nanoparticles was investigated according to the method outlined by Ebrahimi et al. (2017). The assays were conducted on agar well diffusion method with Gram-negative bacteria *Escherichia coli* and Gram-positive bacteria *Staphylococcus aureus.* The pathogenic bacteria were cultured using nutrient agar in petri dishes with an inner diameter of 9 cm to provide a thin agar plate of thickness 3.4-3.5 mm after solidification. The culture was regulated to 0.5 McFarland standards to get 1.5×10^6 CFU/mL (Ebrahimi et al. 2017).

The sample was prepared for an antibacterial test and labeled as A for the banana (*M. acuminata*) mediated Cu nanoparticles. The extracts were prepared at 1 mg/mL and dissolved in Dimethyl Sulfoxide (DMSO) in each case (Murthy et al. 2020). A Hollow of 6mm diameter was cut from each assay using a sterile cork-borer, and 50 µL of each of the three extracts was impregnated using a sterilized wire loop on the surface of Mueller-Hinton Agar (MHA) plates. The pathogens were incubated at 5-8°C for 2 hours to ensure good diffusion and then further incubated at 37°C for 24 hours (Ebrahimi et al. 2017). Ampicillin disc was used as a control. Next, using a micropipette, 10µL of antibiotic control (Ampicillin) was measured and used for both the *E. coli* and the *S. aureus* bacteria (Ebrahimi et al. 2017). The diameters of the inhibition zone in each case were measured millimeters using a ruler, and the results were recorded.

RESULTS AND DISCUSSIONS

UV-Visible absorption spectroscopic study

UV-Visible spectra of aqueous $CuSO_4.5H_2O$ mediated with the *M. acuminata* fruits extract are shown in Figure 1. The reduction of Cu^{2+} ions to Cu nanoparticles by the sample extract was indicated by the change of color from pale yellow to reddish brown.

The progress in reducing Cu^{2+} ions to Cu nanoparticles was indicated by the enhanced intensity of surface Plasmon absorption peak observed within 560nm to 600nm. The maximum absorption observed was 574nm. The appearance of the peak assigned to the Surface Plasmon Resonance band is within the range of 550nm-600nm for copper nanoparticles, as reported by Khodaie and Ghasemi (2018).

The metal nanoparticles show strong absorption of visible radiation due to their induced polarization in their conduction electrons concerning the immobile nucleus (Michael 2012). Furthermore, when a particular wave length is matched to the size of a nanoparticle, dipole oscillation is generated in the compensated form of the induced polarization, and the electrons in the nanoparticle resonate, introducing a strong absorption (Moskovits and Vlckova 2005).

Fourier Transform Infrared spectroscopic study (FTIR)

FTIR spectroscopic measurement was conducted to discover the possible biomolecules in the *M. acuminata* (banana) fruit extract and the synthesized Cu nanoparticles. Figure 2 showed active functional groups in the fruit extracts and the functional groups in the synthesized CuNPs. The figures showed a comparative Fourier Transform Infrared (FTIR) spectroscopic analysis of sample extracts and the synthesized CuNPs.

Peaks observed around 3,200 cm^{-1} and 2,900 cm^{-1} are characteristics of a carboxylic COOH (or N-H stretching mode) and alkynic ≡C-H stretching. Peaks at 1,700 cm^{-1}, 1,600 $cm^{-1}$and 1,618 cm^{-1} are corresponding to amide, arising due to carbonyl stretching in proteins, and the band at 1,604 cm^{-1} is a characteristic of N-H bending. The peaks at 1,474 and 1,039-1,381cm^{-1} correspond to methylene scissoring vibrations from the proteins in the solution and C-N stretching vibrations of amine (Michael 2012). The FTIR spectra showed that the flavonoids, alkaloids, protein molecules, and other metabolites in the fruit extract are responsible for reducing copper ions and stabilizing the Cu nanoparticles. The FTIR data were in agreement with the previous reports (e.g., Dadgostar 2008; Hailemariam 2011; Valodkar et al. 2011; Kalainila et al. 2014; Gnanasangeetha and Prathipa 2019). It is also clear from the relative FTIR data that the peaks of the functional groups of the spectra of the fruit extract and those of their corresponding synthesized copper nanoparticles are almost similar. That signifies the impact of the functional groups in the fruit extracts in reducing Cu^{2+} to CuO and further stabilization of CuNPs, as stated by Chandraker et al. (2020).

X-Ray Diffraction studies (XRD)

The XRD pattern of the synthesized Cu nanoparticles obtained by the green reduction of copper ions using banana (*M. acuminata*) fruit extracts is presented in Figure 3. The values of experimental diffraction peaks observed in the patterns at 2θ for CuNPs synthesized from banana fruit extract are 43.50, 50.40, and 74.10. These values of diffraction data conformed with Inorganic Structure Data Base (ICSD); file no. 04-0836 (Rajesh et al. 2018) as well as with International Centre for Diffraction Data (ICDD) standard for value for CuNPs; file number 04-0836 (Theivasanthi and Alagar 2011). The three distinct diffraction peaks for the copper nanoparticles synthesized from the banana correspond to (111), (200), and (220) lattice planes of the face-centered cubic structure (FCC) of copper nanoparticles.

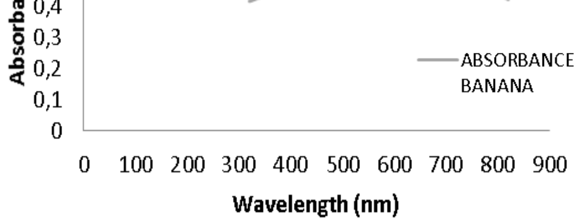


Figure 1. UV-Visible spectra of the synthesis of copper nanoparticles for the three fruits extracts

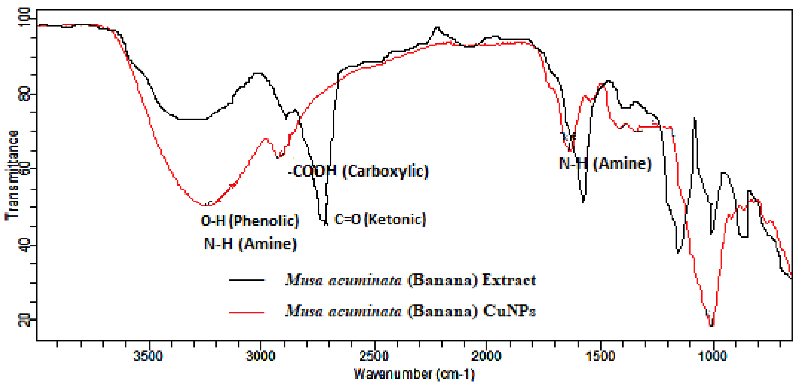


Figure 2. FTIR spectra of *Musa acuminata* (banana) extract and CuNPs synthesized using *M. acuminata* extract

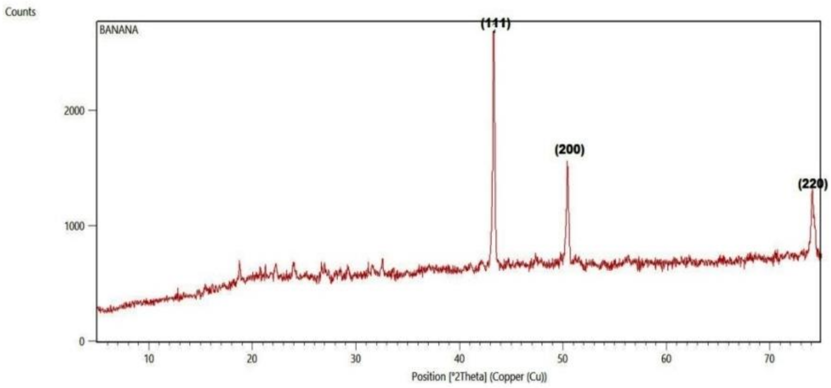


Figure 3. XRD Pattern of CuNPs synthesized using *Musa acuminata* (banana)

Scanning Electron Microscopy (SEM)

Figure 4 presents an image obtained from Scanning Electron Microscopy (SEM) of the copper nanoparticles synthesized from banana. Figure 4 presents SEM images of copper nanoparticles synthesized from bananas, which showed dispersed copper nanoparticles with irregular edges. The diameters of the copper nanoparticles were 18 nm for the banana, and the shapes of the CuNPs synthesized from banana synthesized nanoparticles appeared dispersed. The results were in agreement with Jaehoon et al. (2006) and Arya et al. (2018).

Energy Dispersive X-ray (EDX)

The use of EDX analysis confirmed the presence of metallic copper. The observed chart for Energy Dispersive X-ray (EDX) for as-synthesized copper nanoparticles is illustrated in Figure 5.

The EDX spectra of the copper nanoparticles synthesized are presented in Figure 5. The EDX spectra confirmed the presence of metallic copper and other elements, which corroborated the findings of Gnanasangeetha and Sarala (2015). The presence of elements such as C, S, and Cl may be attributed to contaminations originating from the biomolecules bound to the surface of the copper nanoparticles or physical absorption during sample preparation. In addition, oxygen and the Cu signal signify that phytoconstituents capped the CuNPs through oxygen atoms. In contrast, the presence of a trace amount of carbon demonstrated the involvement of plant phytochemical groups in the reduction and capping of the synthesized CuNPs.

Antibacterial activities of copper nanoparticles

The antibacterial activities of banana (*M. acuminata*) fruits extract mediated copper nanoparticles against two pathogenic bacteria, Gram-negative *E. coli* and Gram-positive *S. aureus* using agar well diffusion method shown in Table 1. Figure 6 illustrated the antibacterial activity of *M. acuminata* synthesized CuNps and Ampicillin, respectively, on *S. aureus* and *E. coli* bacteria.

The mean values of three replicates of the zone of inhibition in (mm) around each assay of the synthesized copper nanoparticles are presented in Table 1. The results of antibacterial activities of banana (*M. acuminata*) fruits extract mediated copper nanoparticles against two pathogenic bacteria, Gram-negative *E. coli* and Gram-positive *S. aureus* using agar well diffusion method and are presented in Table 1. The diameter of the assays' inhibition zones was measured in millimeters (mm) using a ruler. The results were recorded and compared to those obtained from the standard antibiotic drug, Ampicillin.

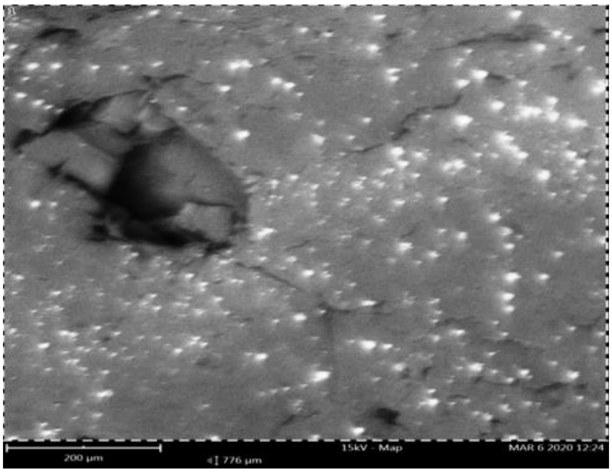


Figure 4. SEM image of CuNPs synthesized using *Musa acuminata* (banana)

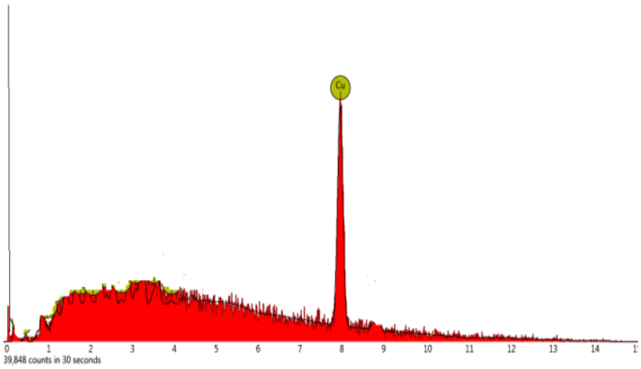
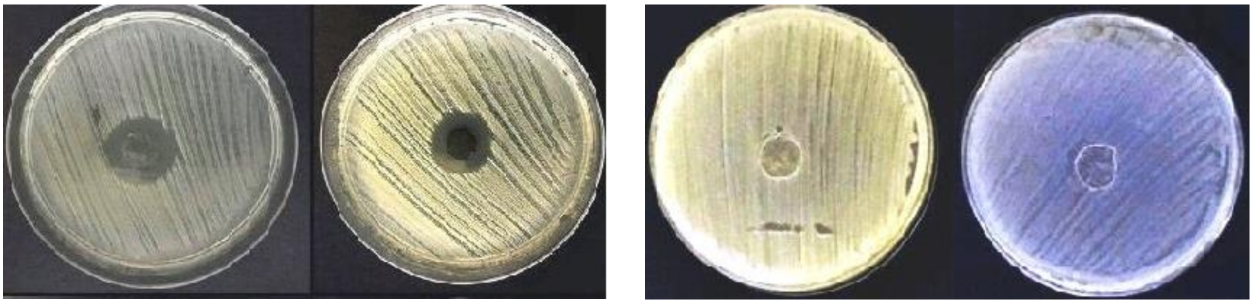


Figure 5. EDX figure of CuNPs synthesized using *Musa acuminata* (banana)



S. aureus *E. coli*
A

S. aureus *E. coli*
B

Figure 6. A. The antibacterial activity of *Musa acuminata* synthesized CuNps (A) and Ampicillin (B), respectively, on *S. aureus* and *E. coli* bacteria

Table 1. Mean values of three replicates of the zone of inhibition (mm) around the sample of the synthesized copper nanoparticles

Culture (bacteria)	Diameter of zone of inhibition (mm)	
	***Musa acuminata* (banana)**	**Ampicillin (Standard)**
Escherichia coli	16±2.2	17±0.50
Staphylococcus aureus	19±2.5	19±1.20

Note: The zone of inhibition was measured in mm. Values are expressed as Mean±SD, where n=3

From the obtained results, copper nanoparticles were found to have higher antibacterial activity against Gram-positive bacteria *S. aureus* than Gram-negative *E. coli*. The antibacterial activity was also more pronounceable against *S. aureus* compared to Ampicillin. The inhibition zones observed for *M. acuminata* synthesized CuNPs are 19±2.5 for *S. aureus* and 16±2.2 for *E. coli* and Ampicillin 19 mm and 17 mm are the values recorded for *S. aureus* and *E. coli*, respectively.

The higher activity against Gram-positive may be attributed to a greater abundance of amines and carboxyl groups on the cell surface of the Gram-Positive Bacteria and a greater affinity of copper towards these groups. According to Rupareli et al. (2008), copper ions released may interact with DNA molecules and insert between the nucleic acid strands. Moreover, copper ions inside bacterial cells also disrupt biochemical processes. The study showed that green synthesized copper nanoparticles from bananas have great potential in biomedical applications. That following with the previous observations of other researchers (Hailemariam 2011; Theivasanthi and Alagar 2011; Joseph et al. 2016).

In conclusion, this research confirmed the feasibility of green synthesis of copper nanoparticles using extracts of *M. acuminata* (banana) fruits. The results indicated a reduction of copper ions and stabilization of copper nanoparticles occurred due to the presence of proteins and other metabolites in the fruit extracts. The size of synthesized CuNPs calculated using powder XRD pattern is 18nm. FT-IR results confirmed that extracts of *M. acuminata* (banana) fruit are a suitable green pattern to prepare copper nanoparticles. It was also observed that *M. acuminata* (banana) copper nanoparticles were found to have a very good and fabulous antibacterial activity.

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