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Research paper

Effect of process variables on green synthesis of copper nanoparticles from solanum lycopersicum and Psidium guajava and its antibacterial activities

A.Muhammad[1*], Ashiru Umar[1], A.U. Birnin-Yauri[1], H. Abubakar Sanni[1], Ige A.R[1], C.M Elinge[1]

[1]Department of Pure and Applied Chemistry, Kebbi State University of Science and Technology, Aliero, Nigeria.
**Corresponding author E-mail:amnakowa@gmail.com*

Abstract

In this study copper nanoparticles were synthesized using extracts from Solanum lycopersicum (Tomato) and Psidium guajava (Guava) fruits. The synthesis process optimized experimental conditions such as temperature, concentration of precursor and amount of fruit extract. The synthesized nanoparticles were characterized using Uv-vis, FTIR, XRD, SEM and EDX techniques. Copper nanoparticles synthesized with tomato and guava extracts had diameters of 52nm and 25nm, respectively. The antibacterial activity of the synthesized nanoparticles was tested using pathogenic bacteria; Escherichia coli and Staphylococcus aureus and their results revealed that copper nanoparticles are promising antibacterial agents. The optimum conditions for the synthesis were 0.05 M, 80 ^{o}C and 10cm^3 for concentration, temperature and volume of fruit extract, respectively.

Keywords:*Copper Nanoparticles; Psidium Guajava; Contamination; Antibacterial Activity; Synthesis*

1. Introduction

In the last decades, synthesis of nanoparticles and research about them has attracted much attention from scientists from different areas of applied science. Nanotechnology is the research and development of materials at the atomic, molecular and macromolecular scales that manipulating structures to convert them at scales of 1–100 nm (Khodaie and Ghasemi, 2016).
The CuNPs have received considerable attention due to its optical, catalytic, mechanical and electrical properties. In addition, copper has advantages in the preparation of green-nanoparticles. CuNP has a wide range of uses as an ultra-strong material, sensor and antimicrobial activity against various bacterial and fungal strains.
Many methods have been developed for preparation of copper nanoparticles including electrochemical (Yang et al., 2003; Yu et al., 2008), electroreduction (Raja et al., 2008), chemical reduction (Qiuli et al., 2010; Granata et al., 2019), hydrothermal (Chen and Lee, 2010), thermal decomposition (Salavati-Niasari et al., 2008; Wei et al., 2010). Recently, researchers have focused on scientific research on the synthesis of CuNPs through the green pathway using leaf, fruit, bark and flowers extracts of environmentally friendly and non-toxic plants.
Copper nanoparticles have excellent applications in heat transfer systems, (Eastman et al, 2001), antimicrobial materials (Esteban-Cubillo et al., 2006), ultra-strong materials, (Guduru et al., 2007), sensors, and catalysts, (Kantam et al., 2007; Kang et al., 2007). Copper nanoparticles are easily oxidized to form copper oxide. When an application requires protecting copper nanoparticles from oxidation, the copper nanoparticles are usually encapsulated in an organic or inorganic material such as carbon or silica, (Moya et al., 2006). Copper monodisperse nanoparticles (2-5nm) embedded in a polysilicate called sepiolite showed strong antibacterial activity and were able to reduce the microbial concentration by 99.9% (Esteban-Cubillo et al., 2006).
Microbial contamination of water poses a great threat to public health. With the advent of microorganisms resistant to multiple antimicrobial agents, (Kolar et al., 2001) there is a growing demand for improved disinfection methods. Researchers have recommended the use of copper ions as an excellent disinfectant for public wastewater containing infectious microorganisms (Lin et al., 1998). However, residual copper and silver ions in the treated water can adversely affect human health (Blanc et al., 2005).
In this research work, copper nanoparticles (CuNPs) were synthesized from copper sulfate pentahydrate and aqueous extract from Solanum lycopersicum and Psidium guajava. The effects of concentration and volume of copper nitrate and temperature were investigated and characterized by advanced techniques.

2. Materials and methods

2.1. Materials

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Fruits of Solanumly copersicum (Tomatoes) and Psidium guajava (Guava) copper nitrate (from Merck Co., with a purity of 99.99%). Hydrochloric acid solution (0.1N) and Sodium hydroxide solution (0.1N) for pH adjusting and doubly distilled water was used in all the experiments. The devices used for characterization and sites are UV-Visible (SANYO SP65 GALANAKAMP, UK) and FTIR (SHIMADZU 1730, JAPAN). The spectroscopic data of the synthesized samples were analyzed at General Research Laboratory of Usmanu-Danfodiyo University, Sokoto. XRD (BRUKER D8 Advanced XRD, West Germany), SEM (JEOL, JSM Model 6390 LV, USA) and EDX (EDX-7000P/ EDX-8000P, SHIMADZU, AUSTRIA) measurements were carried out at General Research Laboratory Umaru Musa Yar'adua University, Katsina.

2.2. Samples collection

In this study, fruits of Solanum lycopersicum (Tomatoes) and Psidium guajava (Guava) were procured from Sokoto central market, washed with deionized water several times, dried and powdered for further experiments. The representative samples for the analysis were selected viarandom sampling.

2.3. Preparation of solanum lycopersicum (tomato) extract

Tomato were washed under tap water and then rinsed with distilled water. The skin was removed then quashed and juice was collected after filtration process. The extract was prepared by soaking 10g of tomato juice in 100 cm^3 deionized water. The mixture was boiled for approximately 15 minutes at 60 °C. The mixture was centrifuged for 15 minutes at 4000 rpm. The supernatant obtained was filtered through Whatman No.1 filter paper to obtain the extract, which was used in the synthesis of copper nanoparticles.

2.4. Preparation of Psidium guajava (guava) extract

10g of guava were accurately weighed and thoroughly washed under running tap water. The sample was washed with deionized water to remove surface impurities. The fruit was then crushed and finely macerated with mortar and pestle. The sample was added to 100 cm^3 of deionized water and boiled over water bath at 60 ^oC for 15 minutes. The extract was allowed to cool down. It was then filtered with muslin cloth through Whatmann No.1 filter paper and then used immediately for the synthesis.

2.5. Green synthesis of copper nanoparticles

10mL of Solanum lycopersicum extract was added to 100 cm^3 of 0.01M $CuSO_4.5H_2O$ aqueous solution and mixed thoroughly. The mixture was heated to 80^oC with constant stirring on a magnetic stirrer for 6h. The suspension produced was centrifuged at 3000 rpm for 10min and the supernatant liquid was decanted off. The residue was repeatedly washed with 10cm^3 of de-ionized water. Centrifugation-decantation and washing processes were repeatedly done five times to remove impurities on the surface of the copper nanoparticles. The procedure was repeated for Psidium guajava extract. The obtained precipitates were dried in an oven at 50^oC for 24h. The synthesized copper nanoparticles were then kept for characterization and antibacterial studies.

2.6. Effect of CuSO4.5H2O concentration, volume of sample extract and the temperature on the synthesis of CuNPs

Effects of three important experimental factors which are concentration, temperature and volume for the synthesis of CuNPs were investigated. The investigation was carried out for the volume of sample extract in cm^3 varied into 1 cm^3, 5 cm^3, 10 cm^3, 15 cm^3 and 20 cm^3; Concentration of $CuSO_4.5H_2O_{(aq)}$ and reaction temperature were maintained at 0.01M and 80 ^oC respectively. While for the concentration of Precursor ($CuSO_4.5H_2O_{(aq)}$); 0.01M, 0.02M, 0.03M, 0.04M and 0.05M were varied maintaining volume of the sample extracts and the reaction temperature at 10cm^3 and 80 ^oC respectively. In the case of reaction temperature in ^oC; temperature of reaction was varied into 60^oC, 70 ^oC, 80 ^oC, 90 ^oC and 100 ^oC, while concentration of $CuSO_4.5H_2O_{(aq)}$ and volume of sample extracts were maintained at 0.01M and 10 cm^3 respectively.

2.7. Characterization

2.7.1. 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 de-ionized water and its UV-Visible spectrum was measured at 80 ^oC.

2.7.2. Fourier transform infrared spectroscopy (FTIR)

FTIR analysis was carried out to identify the possible biomolecules responsible for the reduction of copper sulfate to CuNPs as well as the capping and stabilizing agents for CuNPs. Upon the synthesis of the copper nanoparticles the precipitate obtained using each of the extracts was dried in an oven at 50^oC for 24h. The dried synthesized copper nanoparticles were ground with KBr and casted into pellet and used for analysis on FTIR spectrophotometer in the diffuse reflectance mode operating at a resolution of 4cm^{-1}.

2.7.3. X-ray diffraction studies (XRD)

X-ray diffraction was used in determining the phase and crystallinity of the nanoparticles. The dried sample of the as-synthesized copper nanoparticles was ground to fine powder and a thin film of each of the 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^o and 80^o, 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 (JCPDS) library to ascertain the nature of the nanoparticles.

2.7.4. Scanning electron microscopy (SEM)

The surface morphology of the synthesized copper nanoparticles was studied using Scanning Electron Microscopy (SEM).The size and crystallographic structures were investigated. The sample was prepared 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 in air, before transferring it to the microscope. The operation was carried out at an accelerated voltage of 130kV, magnification of X^{10} and resolution of 1nm.

2.7.5. Energy dispersive x-ray (EDX)

The presence of metallic copper was investigated by the use of EDX analysis. Elemental composition, purity and geometry of the copper nanoparticles 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.

2.8. Test of antibacterial activity

Antibacterial activity of the synthesized copper nanoparticles was investigated according to the method outlined by Ebrahimi et al., (2017). The assays were carried by ager well diffusion method on Gram-negative bacteria Escherichia coli (E. coli) and Gram-positive bacteria Staphylococcus aureus (S. aureus). The pathogenic bacteria were cultured using nutrient ager in petri dishes with an inner diameter 9 cm to provide thin ager 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 two samples were prepared for antibacterial test and labeled as A and B for Tomatoes (Solanum lycopersicum) and Guava (Psidium guajava) synthesized Cu nanoparticles respectively. The extracts were prepared at 1mg/ml and dissolved in Dimethyl Sulfoxide (DMSO) in each case (Ananda Murthy et al., 2020). A Hollow of 6mm diameter was cut from each assay using a sterile cork-borer and50µl of each of the two extracts was impregnated using sterilized wire loop on the surface of Mueller-Hinton Agar (MHA) plates. The pathogens were incubated at 5 – 8 ºC for 2 hours to ensure good diffusion and then further incubated at 37 ºC for 24 hours (Ebrahimi et al., 2017). Ampicillin disc was used as a positive control white the extracts and precursor solution were used as negative control. Using a micropipette, 10µl of antibiotic control (Ampicillin) was measure and used for both the E. coli and the S. aureus bacteria (Ebrahimi et al., 2017).

The diameters of the zones of inhibition of each assay were measured in millimeter using a ruler and the results were recorded. The diameters of the zones of inhibition recorded for the two samples synthesized copper nanoparticles from Tomato and Guava were compared with those of standard drug ampicillin to evaluate the antibacterial activity of the synthesized copper nanoparticles.

3. Results and discussion

3.1. Uv-visible absorption spectroscopic study

The process of the reduction of copper ions to Cu nanoparticles using the two fruits extracts was indicated by the enhanced intensity of surface Plasmon absorption peak observed within the range of 560nm to 600nm as indicated in Fig. 3.1.

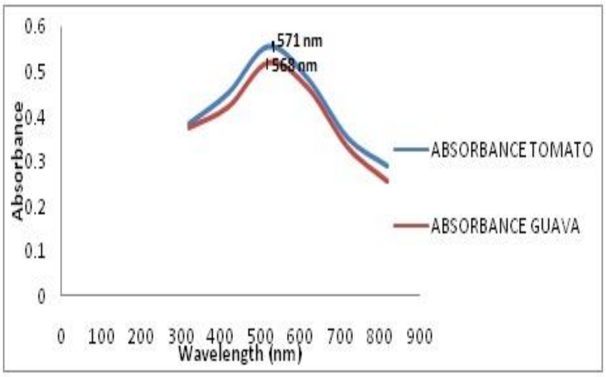


Fig. 3.1:UV-Visible Spectra of the Synthesis of Copper Nanoparticles for the Two Fruits Extracts.

Reduction of Cu^{2+} ions to Cu nanoparticles by the samples extracts were indicated by the change of color from pale yellow to reddish brown for Psidum gujava . While for solanum lycopasicum the colour change is from pale red to reddish brown. The progress in reduction of copper ions to Cu nanoparticles using the two fruits extracts was indicated by the enhanced intensity of surface Plasmon absorption peak observed within 560nm to 600nm. The maximum absorption observed for tomato (Solanum lycopasicum), and Guava (Psidum gujava) are 571nm and 568nm, respectively. The appearance of these peaks, assigned to the Surface Plasmon Resonance band is within the range of 550nm–600nm for copper nanoparticles as reported by (Khoidaie and Ghasemi, 2018).

Strong absorption of visible radiation is shown by the metal nanoparticles due to its induced polarization in its conduction electrons with respect to the immobile nucleus (Michael, 2012).

3.2. Effect of extract concentration, volume and temperature

Fig. 3.2a, 3.2b and 3.2c showed UV-Vis spectra observed for the effect of reaction variables; Concentration of Precursor, Volume of Sample Extract and Reaction Temperature respectively for the synthesis of copper nanoparticles.

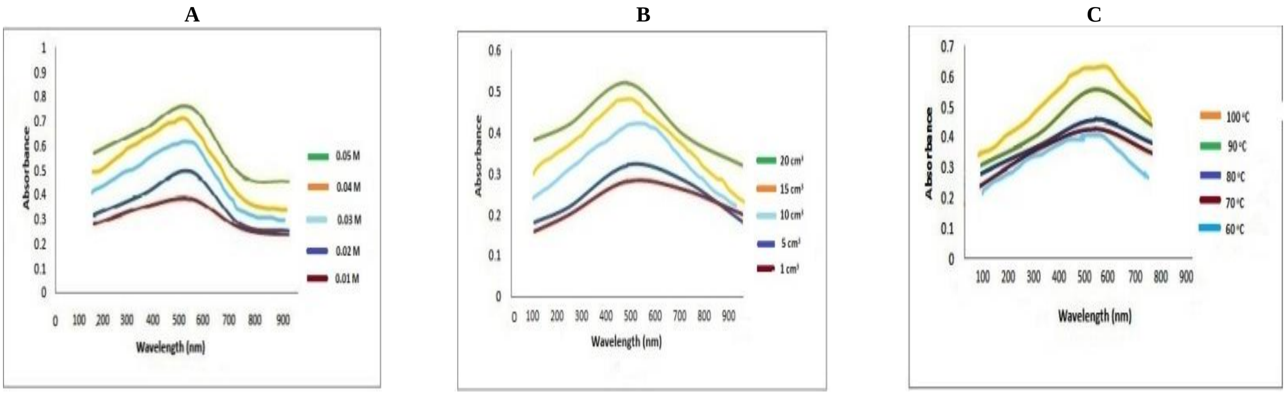


Fig. 3.2: A) Effect of Concentration of Precursor (Cuso$_4$.5H$_2$Oaq), B) Volume of Sample and, C) Extract and Reaction Temperature

It was observed that with increase in reaction temperature, absorption of surface Plasmon resonance increased significantly up to 80 °C. Above 80 °C absorbance decreased. It was observed that 80 °C was the optimum reaction temperature (Fig.3.2c).

Absorption increased when concentration was increased, but at higher concentration absorption reduced, which is related to the nanoparticles aggregation and reduction of the synthesized nanoparticles. The optimum concentration was found to be 0.05 M; this was in agreement with the finding by Khodaie and Ghasemi (2018). For change in volume of extract in cm^3, it was found that the absorbance increased slightly with increase in the volume of each sample extract. This is due to increased nucleation because of the amount of Cu^{2+} ions and generation of fewer nanoparticles in the solution as result of collision between smaller nanoparticles which leads to particle growth (Pavani et al., 2013). It was realized that the optimum amount of extract is 10cm^3. it was observed that absorption of surface Plasmon resonance increased significantly with increase in temperature. The process continued up to 80°C, the absorbance decreased at higher temperature. This is due to increase in size of the synthesized nanoparticles which results into agglomeration of particles. It was found that 80 °C was the optimum temperature for processes. The result is in agreement with the finding by Khodaie and Ghasemi (2018).

FTIR spectroscopic measurement was carried out to elucidate the possible biomolecules present in the fruit juice of Solanum lycopersicum (Tomato) and Psidium guajava (Guava) as well as their corresponding Cu nanoparticles.Fig. 3.3a and 3.3b showed active functional groups in the fruits extracts their respective synthesized CuNPs. The figures showed comparative Fourier Transform Infra-red (FTIR) spectroscopic analysis of sample extracts and their respective synthesized CuNPs.

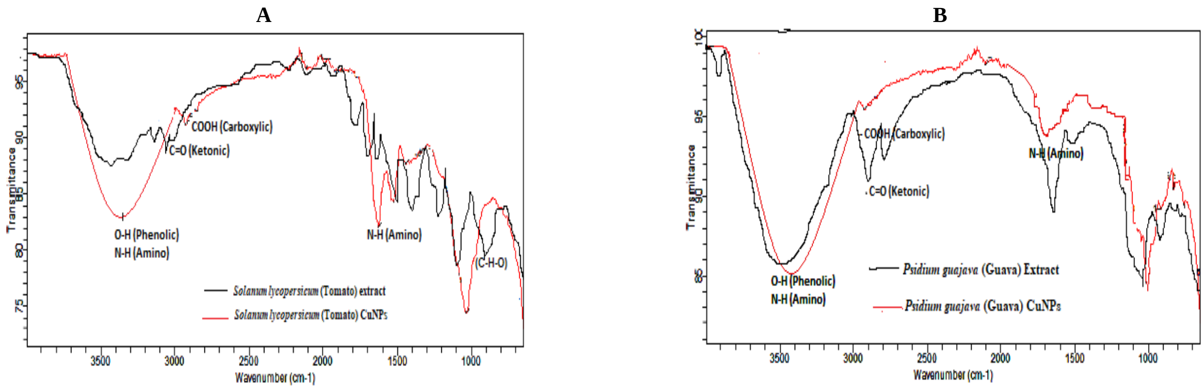


Fig. 3.3: A) FTIR Spectra of Solanum Lycopersicum (Tomato) Extract and Cunps Synthesized Using Solanum Lycopersicum Extract; B) FTIR Spectra of Psidium Guajava Extract and Cunps Synthesized Using Psidium Guajava (Guava).

The peaks around 3200cm^{-1}and 2900cm^{-1} are characteristics of a carboxylic COOH (or N-H stretching mode) and alkynic ≡C-H stretching. The bands at 1700cm^{-1}, 1600cm^{-1}and 1618cm^{-1}are corresponding to amide, arising due to carbonyl stretching in proteins and the band at 1604cm^{-1}is a characteristic of N-H bending. The peaks at 1474 and 1039 – 1381cm^{-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 fruits extract are responsible for the reduction of copper ions and stabilizing the Cu nanoparticles. The FTIR data concur with the previous reports (Dadgostar, 2008, Hailemariam, 2011, Kalainila et al, 2014 and Valodkar et al, 2011).

The similarity of the peaks of the fruits extract and those of the synthesized CuNPs is due to the impact of the functional groups in the fruits extracts in the reduction of Cu^{2+} to Cu^o and further stabilization of CuNPs as stated by Chandraker et al., 2020.

3.3. X-ray diffraction studies (XRD)

The XRD pattern of the synthesized Cu nanoparticles obtained by the green reduction of copper ions using Tomato (Solanumlycopersicum) and Guava (Psidium guajava) fruits extracts are presented in Fig. 3.4a and 3.4b.

A B

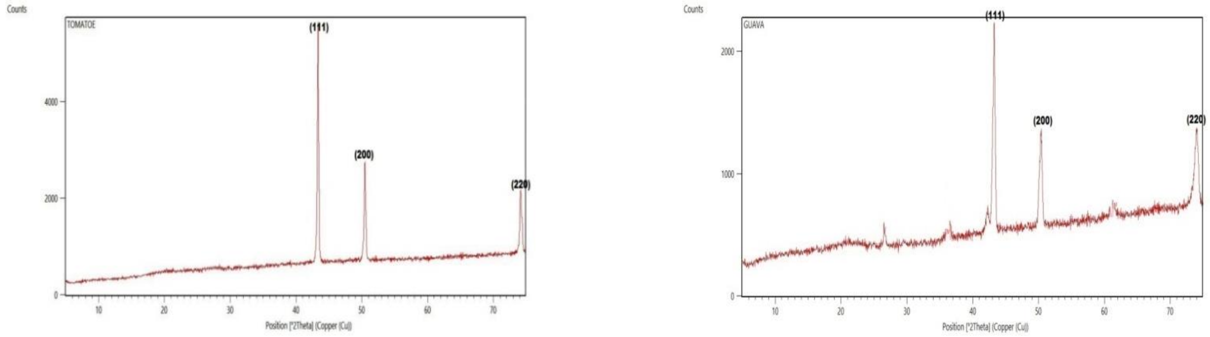


Fig. 3.4: A) XRD Pattern of Cunps Synthesized Using Solanum Lycopersicum (Tomato); B) XRD Pattern of Guava (Psidium Guajava) Synthesized Cunps.

The values of experimental diffraction peaks observed in the patterns at 2θ for tomato synthesized CuNPs are 43.50, 50.60 and 74.10; while for guava synthesized CuNPs, diffraction peaks found were 43.20, 50.20 and 73.90. These values of diffraction data were in agreement 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 Copper nanoparticles; file number 04-0836 (Theivasanthi and Alagar, 2011). The three distinct diffraction peaks for the synthesized copper nanoparticles from the Tomatoes and the Guava corresponds to (111), (200), and (220) lattice planes of face centered cubic structure (FCC) of copper nanaoparticles. The results were in good agreement with previous observations; Dash and Balto 2011; Theivasanthi and Alagar, 2011.

3.4 Scanning electron microscopy (SEM)

Fig. 3.5a and 3.5b below presented images obtained from scanning electron microscopy (SEM) of the copper nanoparticles synthesized from Tomato and Guava respectively.

A B

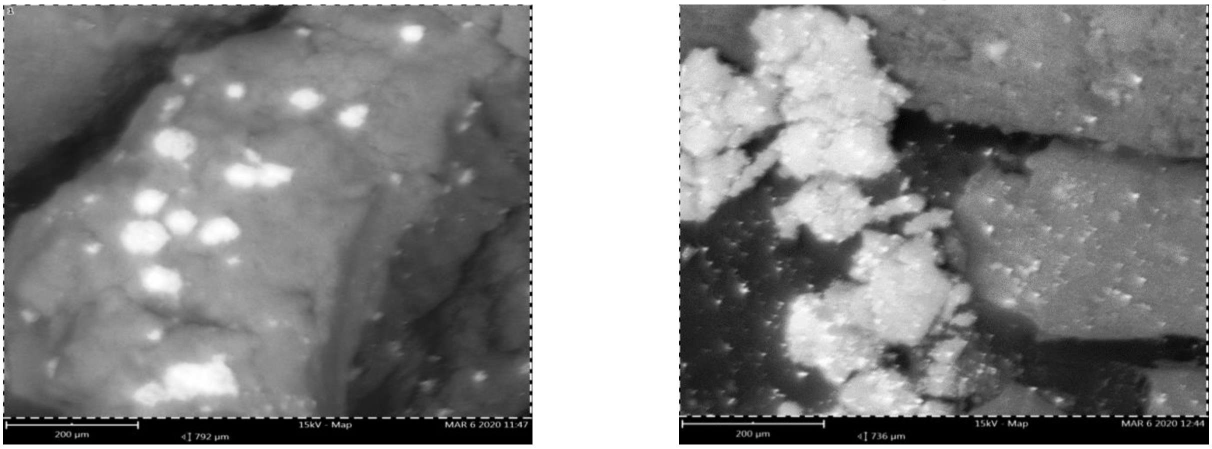


Fig. 3.5: A) SEM Image of Cunps Synthesized Using Solanum lycopasicum Extract; B) SEM Image of Cunps Synthesized Using Psidium Gujava (Guava) Extract

The images showed dispersed copper nanoparticles with irregular edges. The average diameters of the synthesized copper nanoparticles were found to be 52nm for tomato and 25nm for guava. The shapes are of the CuNPs synthesized from the guava extract and the tomato extract were found to look like flakes. The results were in agreement with Jaehoon et al., (2006); and Arya et al., (2018).

3.5. Energy dispersive x-ray (EDX)

The presence of metallic copper was confirmed by the use of EDX analysis. The observed charts for Energy Dispersive X-Ray (EDX) for as-synthesized copper nanoparticles are illustrated by Fig 3.6a and 3.6b below:

A B

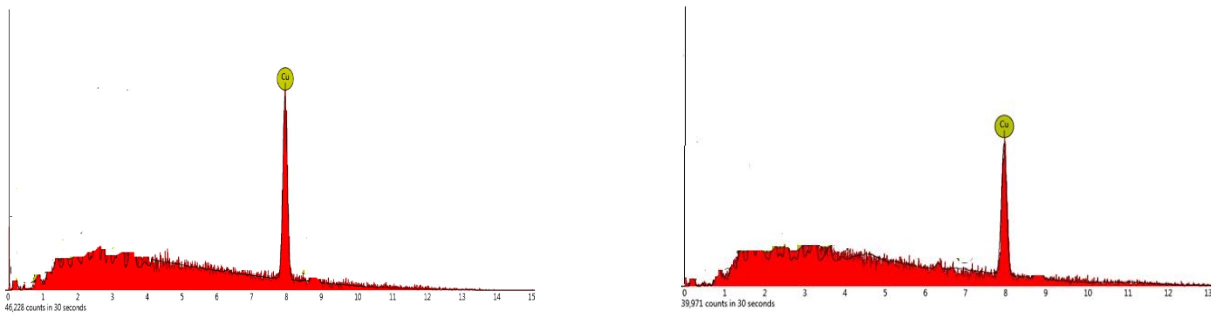
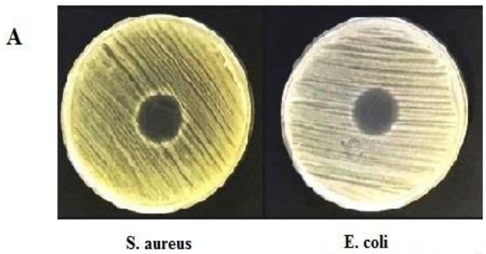


Fig. 3.6: A) EDX Figure ofSolanum Lycopasicum (Tomato) Synthesized Cunps; B) EDX Figure of Psidium Guajava (Guava) Synthesized Cunps

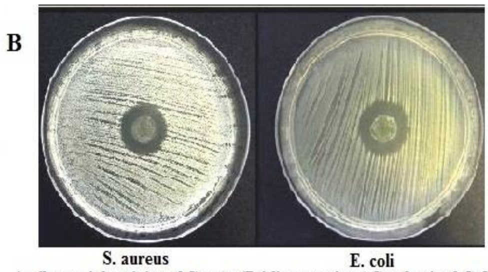
The EDX spectra confirmed the presence of metallic copper along with other elements. It was found from the EDX signals that weight percentages of elemental copper in the nanopaticles were 61.53%, and 63.85% for Tomato and Guava respectively.

3.6. Antibacterial activities of copper nanoparticles

The antibacterial activities of Tomatoes (Solanum lycopasicum) and Guava (Psjdium guajava) fruits extract mediated copper nanoparticles were carried out against two pathogenic bacteria, Gram-negative Escherichia coli and Gram-positive Staphylococcus aureus using agar well diffusion method.



Antibacterial activity of Tomato (*Solanum lycopersicum) Synthesized CuNPs*



Antibacterial activity of Guava *(Psidium guajava)* Synthesized CuNPs

Plates A and B above, illustrated the antibacterial activity of SolanumlycopersicumsynthesizedCuNps, Psidiumguajava synthesized CuNps and Ampicillin respectively S. aureus and E. coli bacteria. Extracts on the fruits were also assayed.

The mean values of three replicates of zone of inhibition in (mm) around each assay of the synthesized copper nanoparticles are presented in Table 3.1 below:

Table 3.1: Inhibition Zone (Mm) Around the Samples of the Synthesized Copper Nanoparticles and Fruits Extracts

	Diameter of zone of inhibition (mm)					
Culture (Bacteria)	Solanum lycopasicum (Tomato) CuNPs	Psydiumguajava (Guava) CuNps	Ampicillin (Standard)	Solanum lycopasicum (Tomato) extract	Psydium guajava (Guava) extract	$CuSO_{4(aq)}$
E. coli	16±2.5	15±1.5	17±1.8	13±2.0	9±2.5	12±1.7
S. aureus	20±3.5	18±2.8	19±1.5	16±2.8	13±3.0	13±1.2

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. aureusthan Gram negative E. coli. The anti bacterial activity was also more pronounced against S. aureus compared to ampicillin, the standard antibiotic. The zones of inhibition obtained in mm with Solanum lycopasicum synthesized CuNPs are 20±3.5 for S. aureus and 16±2.5 for E. coli. Zones of inhibition in mm recorded for Psidium guajava synthesized CuNps are 1.8±2.8 for S. aureus and 15±1.5 for E. coli. For the ampicillin 19±1.5 mm and 17±1.8 mm are the values recorded for S. aureusans and E. coli respectively.

The Higher activity against Gram positive maybe attributed to greater abundance of amines and Carbonyl groups on cell surface of the Gram Positive Bacteria and greater affinity of copper towards these groups Rupareli et al., (2008). The study showed that green synthesized copper nanoparticles from tomato and guava have a great potential in biomedical applications. This conform to previous observations of other researchers (Theivasanthi and Alagar, 2011a; Hailemariam, 2011, Joseph et al., 2016).

4. Conclusion and recommendation

The research confirmed the feasibility of green synthesis of copper nanoparticles using aqueous extracts of fruits juice Solanum lycopersicum (Tomato) and Psidium guajava (Guava).The results indicated reduction of copper ions and stabilization of copper nanoparticles occurred as a result of the presence of proteins and other metabolites present in the fruits extracts. The nanoparticles were of Face Centered Cubic (FCC) structure with high stability and without any impurity. The diameter of the prepared nanoparticle was 52nm for tomato and25nm for guava. The shapes of the nanoparticles were found looking like flakes. This green method of preparation of copper nanoparticle has the potential to be economical and cheap with no hazardous effect. Thus, the choice of green synthesis could be exploited in different fields where copper nanoparticle is desired.

Conflicts of interest

The authors declare no conflicts of interest

Authors contribution statement

Conceptualization and supervision; A. Muhammad; Experiment and Manuscript writing: Ashiru Umar; Project design and supervision: A. U. Birnin-Yauri; Manuscript review: H. Abubakar Sanni; Manuscript writing and editing: A. R Ige; Manuscript revision: C.MElinge.

References

[1] Ananda Murthy, H.C., Desalegn T, Kassa, AbebeB., and Assefa, T., 2020. Synthesis of Green Copper Nanoparticles Using Medicinal Plant Hageniaabyssinica (Brace) JF. Gmel. Leaf Extract:Antimicrobial Properties. *Hindawi*. Journal of Nanomaterials: https://doi.org/10.1155/2020/3924081.

[2] Blanc, D.S., Carrara, P., Zanetti, G., and Francioli, P., 2005. Water disinfection with ozone, copper and silver ions, and temperature increase to control Legionella.*J Hosp Infect*. (60): 69–72.https://doi.org/10.1016/j.jhin.2004.10.016.

[3] Chandraker S.K., Ghosh La M., Tiwari M. K., Ghorai V., and Shukla R., 2020. Green synthesis of copper nanoparticles using leaf extract of *Ageratum houstonianum Mill* and study of their photo catalytic and antibacterial activities.*Nano Express.*(1) 010033. https://doi.org/10.1088/2632-959X/ab8e99.

[4] Chen, H., Lee, J., 2010. Metallic copper nanostructures synthesized by a facile hydrothermal method. Journal of Nanoscience and Nanotechnology 10, 629-636. https://doi.org/10.1166/jnn.2010.1739.

[5] Dadgostar N. 2008. Investigations on Colloidal Synthesis of Copper Nanoparticles in a Two-phase Liquid-liquid System; A thesis presented to the University of Waterloo in fulfillment of the thesis requirement for the degree of Master of Applied Science in Chemical Engineering Waterloo, Ontario, Canada, URI: http://hdl.handle.net/10012/4005.

[6] Eastman, J. A., Cho, S. U., Yu, W and Thompson L. J. 2001. Anomalously increased effective thermal conductivity of eethylene glycol-based nanofluids containing copper nanoparticles. *Applied Physics Letters*. 78 (6): 718-720. https://doi.org/10.1063/1.1341218.

[7] Ebrahimi, K., Shiravand, H and Mahmoud H. 2017. Biosynthesis of copper nanoparticles using aqueous extract of *Capparisspinosa*fruit and investigation of its antibacterial activity. *Marmara Pharmaceutical Journal* 21(4): 866-871. https://doi.org/10.12991/mpj.2017.31.

[8] Esteban, A. C., Pecharroman, E. C.,Agilar, J. Santaren, and Moya, J. 2006. Antibacterial activity of copper monodispersed nanoparticles into sepiolite. *Journal of Material Science*.(41): 5208-5221. https://doi.org/10.1007/s10853-006-0432-x.

[9] Granata, G., Onoguchi, A., Chiharu Tokoro, C., 2019. Preparation of copper nanoparticles for metal-metal bonding by aqueous reduction with d-glucose and PVP. Chemical Engineering Science 209, 115210. https://doi.org/10.1016/j.ces.2019.115210.

[10] Guduru, R. K., Murty K. L., Youssef, K. M., Scattergood, R. O. and Koch, C. C.2007.Mechanical behavior of nanocrystalline copper.*Materials Science and Engineering A*. 463 (1- 2): 14-21. https://doi.org/10.1016/j.msea.2006.07.165.

[11] Hailemariam G., 2011. Kinetic Study of Silver ions Bioreduction for the Synthesis of Silver Nanoparticles and their Antibacterial Activity. A thesis Submitted to College of Natural and Computational Sciences, Department of Chemistry, School of Graduate Studies, Haramaya University in fulfillment of the Requirements for the Degree of Master of Science in Chemistry (Physical Chemistry).

[12] Jaehoon, L., Kim, D. K., and Kang, W. 2006. Preparation of Cu Nanoparticles from Cu Powder Dispersed in 2-Propanol by Laser Ablation, Bulletin of the *Korean Chemical Society.*, (27):2.1869-1872. https://doi.org/10.5012/bkcs.2006.27.11.1869.

[13] Jeong, S., Woo, K., Kim, D., Lim, S., Kim, J. S., Shin, H., Xia, Y. and Moon, J.2008. Controlling the thickness of the surface oxide layer on Cu nanoparticles for the fabrication of conductive structures by ink-jet printing. *Advanced Functional Materials.*18(5): 679-686. https://doi.org/10.1002/adfm.200700902.

[14] Joseph A. T., Prakash P., and Narvi S. S. 2016. Phytofabrication and Characterization of Copper Nanoparticles Using *Allium Sativum*and its Antibacterial Activity.International Journal of Science, Engineering and Technology (4): 2, 463-470, ISSN: 2348-4098.

[15] Kalainila P., Subha V., Ravindran , R. S. E. and Sahadevan, R. 2014. Synthesis and characterization of silver nanoparticle from *Erythrinaindic,* Asian J. Pharm. Clin. Res. ISSN: 2455-38917(2): 39-43.

[16] Kang, X., Mai, Z.,Zou, X., Cai, P. and Mo, J. 2007. A sensitive nonenzymatic glucose sensor in alkaline media with a copper nanocluster/multiwall carbon nanotubemodified glassy carbon electrode.*Analytical Biochemistry,* (363): 143-150. https://doi.org/10.1016/j.ab.2007.01.003.

[17] Kantam, M. L., Jaya, V. S., Lakshmi, M. J., Reddy,B. R.,Choudary B. M. and Bhargava, S. K., 2007. Alumina supported copper nanoparticles for aziridination and cyclopropanation reactions. *Catalysis Communications*. (8): 1963–1968. https://doi.org/10.1016/j.catcom.2007.03.012.

[18] Khodaie, M. and Ghasemi N. 2016. Green synthesis and characterization of copper nanoparticles using *Eryngiumcampestre*leaf extract. *Bulgarian Chemical Communication.* (50): 244 – 250

[19] Kolar, M., Urbanek K. and Latal, T. 2001. Antibiotic selective pressure and development of bacterial resistance. *Int J Antimicrob Ag*. (17): 357–363. https://doi.org/10.1016/S0924-8579(01)00317-X.

[20] Lin, Y. E., Vidic, R. D., Stout J. E., Mccartney, J., C. A. and Yu, V. L. 1998. Inactivation of Mycobacterium avium by copper and silver ions.*Water Res.* (32): 1997–2000. https://doi.org/10.1016/S0043-1354(97)00460-0.

[21] Michael, G. E. H. 2012. Biosynthesis of Copper Nanoparticles Using Some Plant Leaf Extracts, their Characterization and Antibacterial Activity. A M.Sc. Thesis Submitted to College of Natural and Computational Sciences, Department of Chemistry, School of Graduate Studies. Haramaya University.

[22] Moya, J. S., Pecharroman, C., Cubillo, A. and Montero, I. 2006. Monodisperse and corrosion resistant metallic nanoparticles embedded into sepiolite particles for optical and magnetic applications. *Journal of American Ceramic Society.*89 (10): 3043-3049. https://doi.org/10.1111/j.1551-2916.2006.01190.x..

[23] Qiu-Li, Z., Zhi-Mao, Y., Bing-Jun, D., Xin-Zhe, L., Ying-Juan, G., 2010. Preparation of copper nanoparticles by chemical reduction method using potassium borohydride. Transactions of Nonferrous Metals Society of China 20, s240-s244.https://doi.org/10.1016/S1003-6326(10)60047-7.

[24] Raja, M., Subha, J., Ali, F.B., S.H. Ryu, S.H., 2008. Synthesis of copper nanoparticles by electroreduction process. Materials and Manufacturing Processes 23, 782-785.https://doi.org/10.1080/10426910802382080.

[25] Rajesh, K. M., Ajitha, B.,Reddy, A. K., Suneetha, Y. and Reddy P. R. 2018. Assisted green synthesis of copper nanoparticles using Syzygiumaromaticum bud extract: Physical, optical and antimicrobial properties. *Optik,* (154) 593–600. https://doi.org/10.1016/j.ijleo.2017.10.074.

[26] Rupareli J.P., Chatterjee A.K., Duttagupta S.P. and Mukherji S. 2008. Strain specificity in antimicrobial activityof silver and copper nanoparticles.*ActaBiomaterialia,* (4)707-771. https://doi.org/10.1016/j.actbio.2007.11.006.

[27] Salavati-Niasari, M., Davar, F., Mir, N., 2008. Synthesis and characterization of metallic copper nanoparticles via thermal decomposition. Polyhedron 27, 3514-3518. https://doi.org/10.1016/j.poly.2008.08.020.

[28] Theivasanthi, T. and Alagar. M. 2011. Studies of Copper Nanoparticles Effects onmicroorganisms. *Annals of Biological Research*. 2 (3):82-87.

[29] Valodkar, M., Jadeja, R. N., Thounaojam, M. C., Devkar, R. V., and Thakore, S. 2011. Biocompatible synthesis of peptide capped copper nanoparticles and their biological effect on tumor cells. *Materials Chemistry and Physics*, *128*(1-2), 83-89. https://doi.org/10.1016/j.matchemphys.2011.02.039.

[30] Wei, Y., Chen, S., Kowalczyk, B., Huda, S., Gray, T.P., Grzybowski, B.A., 2010.. Synthesis of stable, low dispersity copper nanoparticles and nanorods and their antifungal and catalytic properties. Journal of Physical Chemistry C 114, 15612-15616. https://doi.org/10.1021/jp1055683.

[31] Yang, X., Chen, S., Zhao, S., Li, D., Ma, H., 2003. Synthesis of copper nanorods using electrochemical methods. Journal of the Serbian Chemical Society 68, 843-847.https://doi.org/10.2298/JSC0311843Y.

[32] Yu, Y., Shi, Y., Chen, C.H., Wang, C., 2008. Facile electrochemical synthesis of single-crystalline copper nanospheres, pyramids, and truncated pyramida nanoparticles from lithia/cuprous oxide composite. Thin Films. Journal of Physical Chemistry C 112, 4176-4179. https://doi.org/10.1021/jp800071h.