GSC Biological and Pharmaceutical Sciences

eISSN: 2581-3250 CODEN (USA): GBPSC2

Cross Ref DOI: 10.30574/gscbps

Journal homepage: https://gsconlinepress.com/journals/gscbps/

(RESEARCH ARTICLE)

Check for updates

Green synthesis of copper oxide nanoparticles using the outer layer of *Allium cepa L.* and evaluation of its antimicrobial properties

Ezealisiji, Kenneth M [1, 2, *] and Chiamaka Ruth Nwodo [2]

[1] *Department of Pharmaceutical and Medicinal Chemistry, Faculty of Pharmaceutical Sciences, University of Port Harcourt, East/West Road, PMB 5323 Choba Rivers State, 500001 Nigeria.*

[2] *Department of Pharmaceutical and Medicinal Chemistry, Faculty of Pharmaceutical Sciences, Madonna University Elele, Rivers State, Nigeria.*

GSC Biological and Pharmaceutical Sciences, 2023, 22(01), 302–311

Publication history: Received on 04 December 2022; revised on 20 January 2023; accepted on 23 January 2023

Article DOI: https://doi.org/10.30574/gscbps.2023.22.1.0032

Abstract

The recent antimicrobial resistant to the present antibacterial agents has been the biggest problem in the treatment of antimicrobial infection. Over the years, various strategies has been implemented to overcome the resistance the resistance to the available antibiotic agents. In regards to this, phytochemical found in plants like *Allium cepa*, which has exhibited potent antimicrobial activity has been used by researchers as natural products to fight against bacterial resistance. *Allium cepa* also known commonly as onion, is an herbaceous biennial plant in the amaryllis family (Amaryllidaceae) grown for its edible bulb. Copper oxide nanoparticles are one of the most vital nanomaterials used in biomedical application. The phytochemicals in the plant extract were used as reducing and capping agent. The aim of the study is to determine the antimicrobial activity of synthesized copper oxide nanoparticles using *Allium cepa* (onion) aqueous extracts. The dried outer layer of the *Allium cepa* was collected and boiled. 150 ml of the plant extract was mixed with 250 ml of the copper nitrate solution, for the purpose of green synthesis. After the reaction was completed, the synthesized nanoparticles was centrifuged, decanted and dried. The dried copper oxide nanoparticles were characterized and accessed for antimicrobial activity. The results of UV-Vis Spectroscopy, FTIR, DLS particles size analysis, TEM and XRD, were obtained and evaluated.

Keywords: Phytochemicals; Nanoparticles; Antimicrobial; *Allium cepa*

1. Introduction

Nanotechnology can simply be defined as a technology performed at a Nano scale, which is at a scale less than 100 nanometer. The word nano is an SI prefix and comes from the Greek word *nanos* meaning dwarf or something really small. Nanotechnology involves the use of various materials having dimensions of the order of a billionth of a meter. Although modern nanotechnology is relatively new, nanosize or nanoscale had been in existence, with their continuous use for centuries. Nanotechnology advancements and its application in various fields have revolutionized the world. The various fields that have potential application of nanotechnology includes; engineering, water purification, biomedical field, medicine, pharmacy etc. Copper is a semiconductor material considered to be an excellent candidate for the synthesis of metal-based nanoparticles. Besides being highly resistant to heat, it is also robust, stable, cheap and easily synthesized. [1, 2]. CuONPs can be synthesized by various processes. Among all, biocompatible processes emerged as the most investigated in the past few years. Independently of the selected method, during synthesis, $CuSO_4$, $CuCl_2 \cdot 2H_2O$, Cu $(NO_3)_2$ or Cu $(CH_3COO)_2$ are the most frequently used copper precursors. The two main approaches used in the synthesis of nanoparticles are the top down approach and the bottom up approach. In top-down approaches, bulk materials are broken down produce nano structured materials. Top-down methods include mechanical milling, laser ablation, etching, sputtering, and electro-explosion. Bottom-up, or self-assembly, approaches to nanofabrication

* Corresponding author: Ezealisiji, Kenneth M

use chemical or physical forces operating at the nanoscale to assemble basic units into larger structures. This approach includes supercritical fluid synthesis, spinning, sol-gel process, laser process, laser pyrolysis, molecular condensation, chemical reduction. Green synthesis using plant extracts such as *Lemon grass* [3], *Euphorbia tirualli* [4], *Gloriosa superba* [5] and *Cinnamomum camphora* [6] have been reported. *Allium cepa* also known as onion, is an herbaceous biennial plant in the amaryllis family (Amaryllidaceae) that is grown for its edible bulb. The onion is mostly native to southwestern Asia but is now grown throughout the world, chiefly in the temperate zones. Onions are low in nutrients but are valued for their flavour and are used widely in cooking. They add flavour to such dishes as stews, roasts, soups, and salads and are also served as a cooked vegetable. *Allium cepa* L. extracts were reported to inhibit the growth of both Gram-positive and Gram-negative bacteria, such as *Bacillus cereus, Staphylococcus aureus, Pseudomonas aeruginosa, Salmonella typhimurium*, and *Klebsiella pnuemoniae* [7, 8, 9, 10, 11]. Quercetin has been a most extensively studied flavonoid.

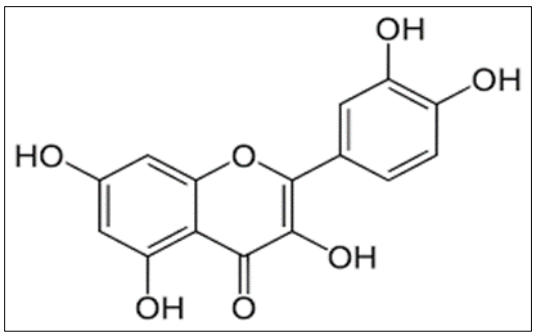


Figure 1 Chemical structure of quercetin

CuONPs have shown antimicrobial effects. The mechanism of antibacterial activity of CuONPs is not well elucidated yet, but it is believed that it involves bacterial cell wall adhesion triggered by electrostatic interactions. Dissociation of Cu^{2+} induces the generation of ROS that contact with cellular membranes. These ions also have the capacity to enter the cell, causing membrane damage which is associated with disruption of cells internal content and bacterial cell leakage [12, 13]. The study was conducted in order to evaluate the antimicrobial activities of cupper nanoparticles using the outer layer *Allium cepa* L., for its antimicrobial properties. Most researches on the antimicrobial effect of *Allium cepa* L. has been focused on the biological activity of *Allium cepa* L. alone against microorganisms. Very few researches has tried to explore and investigate into the antimicrobial (antibacterial and antifungal) properties of cupper nanoparticles, hence there are insufficient literature in that regard. Copper oxide nanoparticles combined with various antimicrobial agents has a better antimicrobial effect than cupper nanoparticles or the antimicrobial agent alone. Thus, this study might help discover more medicinal properties of cupper oxide nanoparticles synthesized the outer layer of *Allium cepa* L., especially its antimicrobial strength and properties, for further development in the field of medicine.

2. Material and methods

2.1. Materials

Copper Sulphate, and other analytical grade reagents were product of Merck, Germany, and Oxoid, Hampshire, UK. The bulb of *Allium cepa L*. (Onion) was collected from the southern part of Nigeria, Elele, Rivers state, and were further identified by Mr Boniface of the Department of pharmacognosy, Madonna University. The voucher specimen was deposited in the herbarium.

2.2. Extraction

The bulb of *Allium cepa* L. obtained from Elele, Rivers state, was used for the extraction process. The outer petals were separated from the inner portion of the allium cepa bulb, and placed in a 1000ml (1 Liter) beaker. 400ml distilled water was added to the beaker containing the outer petals of *Allium cepa* and the mixture was heated for 30 minutes at 100 ^{0}C . The mixture was filtered while hot, using a filter paper.

2.3. Green synthesis of cupper nanoparticles using *Allium cepa* L.

A 10 g quantity of copper sulphate were weighed using an electric weighing balance, and dissolved using a 150 ml volume of distilled water in a 50 ml beaker. It was then transferred into a 1 liter volumetric flask, where the volume was made up to the 1 liter mark of the volumetric flask. The copper sulphate solution afforded the copper ion required for

the reaction. 150 ml of the plant extract was mixed with 250 ml of the copper nitrate solution, while the plant extract was still hot. The reaction was incubated at a temperature of 27^{o}C, in the dark in order to avoid photochemical activation of the copper sulphate. A dark brown colour was observed after three hours, indicating the formation of copper oxide nanoparticles (CuONPs).

2.4. Isolation procedure

The synthesized copper oxide nanoparticles were centrifuged for 30minutes at 5000 rpm. The pallet containing copper oxide nanoparticles were rinsed using a small volume of distilled water. The nanoparticles was poured into a porcelain plate and placed in the oven at 115 ^{0}C to evaporate the solvent for 20minutes. The copper oxide nanoparticles were scrapped off the plate using a spatula and placed in sample tubes.

2.5. Instrumentation

The formation of CuONPs using the outer layer of *Allium cepa* L. was monitored by visual colour change The copper oxide nanoparticles (CuONPs) was scanned using the UV-VIS spectrophotometer (JENWAY, 6705) at a wavelength ranging from 180-700 nm.The presence of functional group was detected using the Fourier transform infrared spectrophotometer (FTIR). The Shimadzu FTIR Spectrophotometer (FTIR-8400S) was used to carry out the analysis, with a wave number ranging from 4000-5000 cm^{-}.The particle size distribution analysis was performed to determine the size of the copper nanoparticles. Dynamic light scattering (DLS) and Zeta potential of the synthesized nanoparticles was analyzed to know the average size and stability of particles using DLS-Nano 2s model, UK.The particle size and surface features of the CuONPs were determined by transmission electron microscopy (TEM) using a ZEISS LIBRA 120 KV-UK, at different magnification. The crystalline nature and the average size of the CuONPs was analyzed and calculated using Bruker: D8 Discover, Japan for the X-ray diffraction analysis.

2.6. Antimicrobial analysis of copper nanoparticles

2.6.1. Collection of bacterial isolate

The microorganisms were obtained from the diagnostic Labouratory of Madonna University Teaching hospital, Elele, Rivers State.

2.6.2. Preparation of Nutrient agar (Mueller Hinton)

All aseptic techniques were observed during the experiment. A 2.8 g quantity of the agar powder was weighed into a 500ml conical flask using an electric weighing balance. A 100 ml volume of water was added to the powder gradually while shaking to ensure the complete dissolution of the powder in water. The mixture was autoclaved at a temperature of 121 ^{0}C for 15 minutes. The valve of the autoclave was opened and the pressure was allowed to return to 0 before the autoclave was opened. The molten agar formed, was then poured into petri dishes, two-third full. The petri dish was covered and allowed to solidify.

2.6.3. Preparation of Sabouraud dextrose agar

All aseptic techniques were observed during the experiment. A 3.25 g quantity of sabouraud dextrose agar was weighed and transferred into a 500 ml conical flask. A 50 ml volume of water was added to the powder gradually while shaking to ensure a complete dissolution of the powder in water. The mixture was autoclaved at 121^{0}C for 15minutes. The valve of the autoclave was opened and the pressure was allowed to return to 0 before opening the autoclave. The molten agar formed was then poured into petri dishes, covered and allowed to solidify.

2.6.4. Preparation of nutrient broth

All aseptic techniques was observed during the experiment. A 0.65 g quantity of nutrient broth powder was weighed using an electric weighing balance and transferred into a 500 ml beaker. A 50 ml volume of distilled water was added gradually while shaking the mixture in order to ensure the complete dissolution of the agar powder in water. The mixture was then autoclaved at a temperature of 121^{0}C for 15 minutes. The valve of the autoclave was opened and the pressure was allowed to return to 0 before opening the autoclave.

2.7. Antimicrobial sensitivity screening

The solidified agar was dried in a hot air oven at a temperature of 45^{0}C for 30 minutes, after drying, a suspension of the organisms required for the experiment was swabbed on the agar plates using sterile swab sticks. Each agar plate contains a single organism. After the inoculation of the organisms, on the media, the filter paper were cut at a dimension

of 5 mm by 5mm. The filter papers were autoclaved at a temperature of 121^{0}C for 15minutes, and it was allowed to cool after removing it from the autoclave. The agar plates containing the different microorganisms were divided into four quadrants (first quadrant =40mg, second quadrant= 20mg, third quadrant= copper sulphate ($CuSO_4$) solution, forth quadrant= standard (antimicrobial agent)). The copper oxide nanoparticles were reconstituted and impregnated into the autoclaved filter paper, by picking up the sterile filer paper using a pair of sterile forceps and dipping them into the reconstituted nanoparticles that contains 20 and 40 mg of the copper nanoparticles. The filter papers were then placed on the inoculated agar plate. The agar plates were incubated for 24 hours and the minimum inhibitory concentration (MIC) was calculated.

2.8. Minimum inhibitory concentration (MIC) calculation

The agar well diffusion method was used in the determination of minimum inhibitory concentration. Serial dilutions of the reconstituted copper nanoparticles was prepared at different concentrations of 0.3125 – 20 mg/ml in separate test tubes. The Mueller Hinton agar for bacteria and sabouraud dextrose agar for the fungi were separately prepared, autoclaved and poured into the 7 petri dishes. The Mueller Hinton agar was added to the first 4 petri dishes, the sabouraud dextrose agar for the fungi was added to 1 petri dish and the 6th and 7th petri dishes contained only the Mueller Hinton agar and sabouraud dextrose agar respectively. The agar was allowed to solidify. The agar plates were divided into seven quadrants with a marker and labelled 1-7 which represents the different concentrations of copper nanoparticles, plant extracts and the antimicrobial agent used (quadrant 1= 20 mg/ml, quadrant 2= 10mg/ml, quadrant 3= 5 mg/ml, quadrant 4= 2.5 mg/ml, quadrant 5= 1.25 mg/ml, quadrant 6 = 0.625 mg/ml, quadrant 7 = 0.3125 mg/ml). The surface of the agar plate were streaked with the microorganism using a cotton swab and a cock borer was used to bore holes in the individual quadrants. The different concentrations of copper oxide nanoparticles, plant extracts and the antimicrobial agent used were added to their respective holes in the eight quadrants, after which, the agar plates were incubated at 37^{0}C for 24 hours. The agar plates were also observed for the one with the least observable growth, in order to determine the minimum concentration of the copper oxide nanoparticles that inhibit the growth of the microorganisms.

3. Results and discussion

The reaction between copper sulphate solution and aqueous extract of the outer layer of *Allium cepa* produced a colour change from bluish to dark brown, due to the reduction of the copper ions by the phytochemicals present in *Allium cepa* followed by the capping and stabilization of the nanoparticles formed, clearly indicates the presence of copper oxide nanoparticles (CuONPs). The mechanisms of the synthesis of copper nanoparticles follows a stepwise chemical reaction of copper (II) sulphate in water to dissociate into copper (II) ion (Cu^{2+}) and SO_4^{2-}. Copper (II) ion further reacts with Phyto-polyphenols of *Allium cepa* and reduced to copper (I) oxide. The subsequent aggregation and reduction of copper (I) oxide gave CuO nanoparticles as shown below.

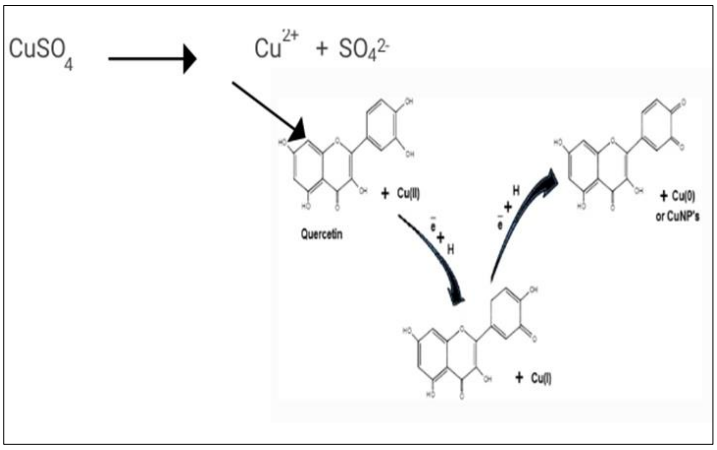


Figure 2 Reduction of Cu^{2+} to CuO

The CuO nanoparticles formed are stabilized and capped by phytochemicals, which imparts stability to the synthesized copper nanoparticles.

3.1. Ultraviolet-visible spectrophotometry (UV-Vis)

The sample analysis using UV- Vis spectrophotometer at a wavelength ranging between 180 – 700 nm gave a surface Plasmon resonance (SPR) spectra. The spectrum in figure 3 had a fairly broad peak at 280 nm, which corresponds to the copper oxide nanoparticles. This result was similar to the findings confirmed from copper oxide nanoparticles fabricated with the aid of *Syzygium alternifolium* stem bark aqueous extract [14]

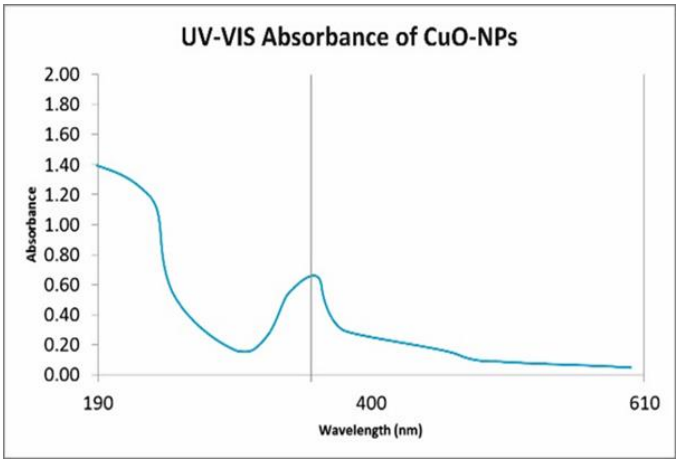
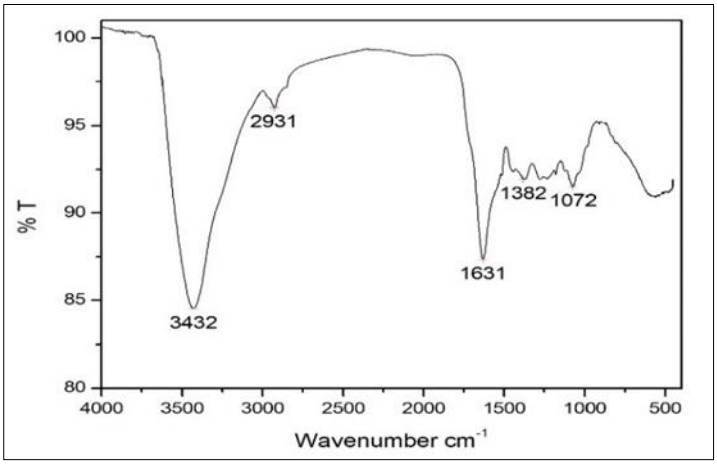


Figure 3 UV-Vis Absorbance of the synthesized CuONPs

3.2. Fourier-transform infrared spectroscopy (FTIR)

The FTIR spectrum of the synthesized copper nanoparticles (figure 4) shows a sharp narrow band at 3432 cm^{-1}, which corresponds to the N-H vibration mode. The peak at 1631cm^{-1} represents the N-H bending and the signal at 1072 cm^{-1} corresponds to the C-N bond stretching.



Keyword: T = Transmittance

Figure 4 FT-IR of the synthesize CuONPs

3.3. Dynamic light scattering (DLS) Particle size distribution

The DLS study (Fig: 5) of the synthesized Copper oxide nanoparticle (CuONPs) gave an average size distribution of 396±1.64 nm. It is expected that the size of CuONPs obtained from the DLS study could be larger than those obtained from XRD and TEM. The increased size could be due to the fact that the DLS measures not only the apparent size (hydrodynamic radius or diameter) of a particle but also the hydrodynamic layers that could be formed around hydrophobic particles. The result could also be attributed to the influence of salvation layer effect of electrolyte dispersion and the restrictions in bond and rotation angles, which leads to larger sizes in colloidal water media than the `geometric` sizes obtained from TEM and XRD. The synthesized copper nanoparticles are positively charged and evenly dispersed in the medium and the Zeta potential was estimated to be 48.3 mV.

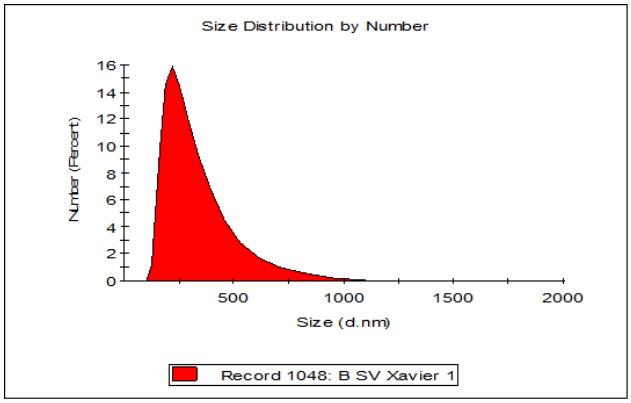


Figure 5 Dynamic light scattering (DLS) Particle size distribution of synthesized CuONPs

3.4. Transmission electron microscope (TEM)

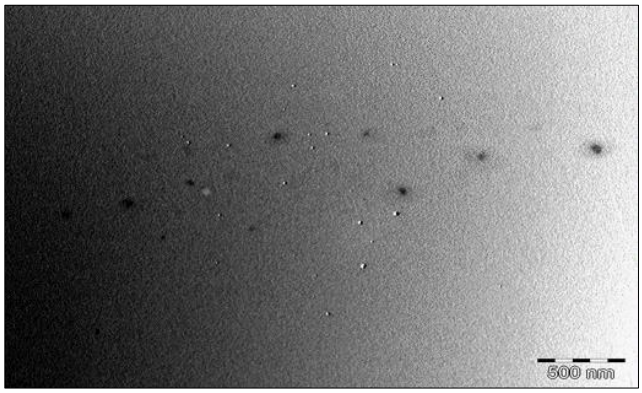


Figure 6 TEM image of synthesized CuNPs

Transmission electron microscopy (TEM) were used to reveal the particle size and morphology of the synthesized CuONPs. The S\synthesized CuONPs were spherical in shape, non-clustering, well dispersed with a size ranging between 28 and 45 nm with a mean particle size of 32±0.25 nm (Figure 6).

3.5. X-ray diffraction analysis (XRD)

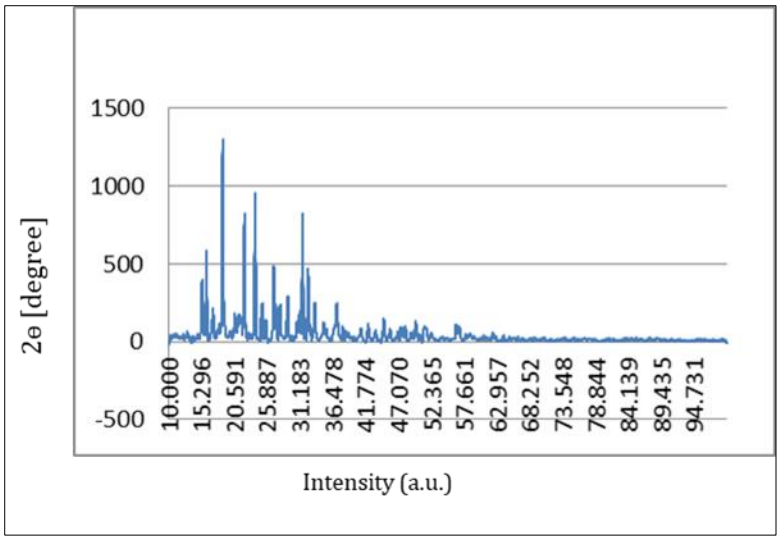


Figure 7 XRD image of synthesized CuONPs

The X-ray diffraction (XRD) pattern (figure 7) of the biosynthesized CuONPs was analyzed using the Bruker d8 Advanced X-ray diffractometer, using CuKα radiation (λ=1.5406 Ä) 40 Kv, 2 Θ/Θ scanning mode. Data was obtained for the 2 Θ range of 10 – 120 degree in step proceeding of 0.0206 degree. Four prominent peaks at 2 Θ values of 18.406°, 22.102°, 23.672°, and 30.902° corresponding to, (020), (022), (022), and (111) integer on the `hkl` planes respectively. This may be recorded as bands for face-centred and crystalline particulate matter which is spherical in nature. Present result showed an excellent correlation with data obtained from International Centre for Diffraction Data (ICDD). Using Debby-Scherrer equation

$$D = \frac{K\lambda}{\beta \, Cos\, \Theta}$$

Where D = the crystallite size of CuNPs
λ = the wavelength of x-ray source (1.54059 nm) used in XRD
β = the full width at half maximum of the diffraction peak
K = the Scherrer's constant with value from 0.9 to 1
θ = the Bragg angle

The average particle size of the CuONPs was calculated to be 22.76 nm at an operational Optimum Bragg reflection obtained at 2Θ of 18.204°. This was confirmed by the TEM study as well.

3.6. Antimicrobial screening results

Table 1 Inhibition zone diameter (mm) result

S/N	Concentration	*Staphylococcus aureus*	*Escherichia coli*	*Bacillus subtilis*	*Salmonella typhi*	*Candida albicans*
1	CuNPs (40mg)	7mm	4 mm	9 mm	6 mm	4 mm
2	CuNPs (20mg)	5 mm	4 mm	7mm	5 mm	2 mm
3	Copper sulphate solution	NZ	NZ	NZ	NZ	NZ
4	Gentamicin (10µg)	24 mm	22 mm	23 mm	19 mm	-
5	Fluconazole	-	-	-	-	19

Well size= 8mm; Keyword: NZ = No zone

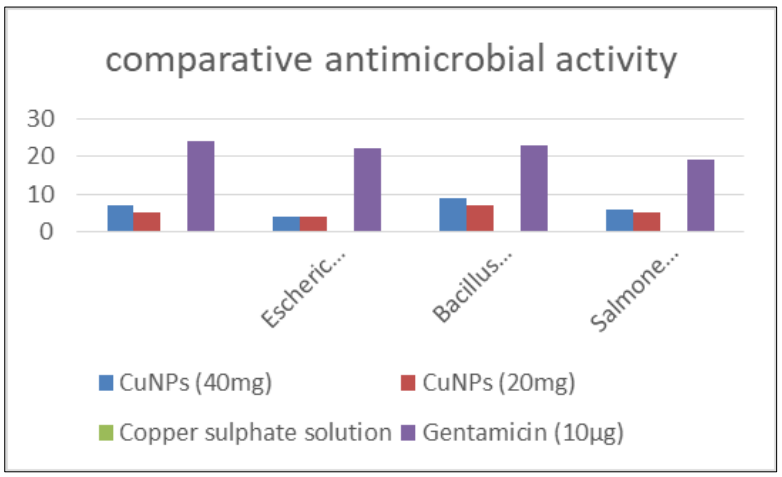


Figure 7a Comparative chart of antimicrobial activity of CuONPs against *Staphylococcus aureus, Escherichia col*i, *Bacillus subtilis* and *Salmonella typhi*

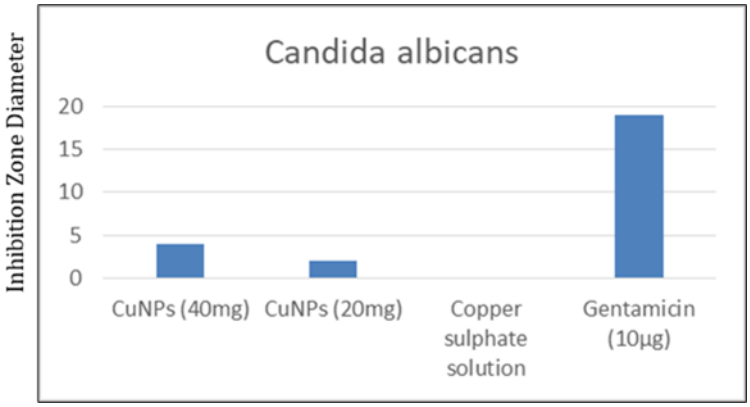


Figure 7b Chart of antifungal activity of CuONPs against *Candida albicans*

The zones of inhibition of the synthesized copper oxide nanoparticles against the selected microorganism (*Staphylococcus aureus, Escherichia coli, Bacillus subtilis, Salmonella typhi* and *Candida albicans*) at concentrations of 40mg/ml, 20mg/ml, copper sulphate and control which includes gentamycin and fluconazole were shown in table 3.1. The highest zone of inhibition against the test organisms was observed at the control (gentamycin and fluconazole). In copper sulphate solution, there was no zone of inhibition at any of the test organisms. It has been reported that nanoparticles combined with various antibiotics have better antimicrobial properties, than the effects of the nanoparticles alone (Li *et al*, 2005). Even though there would be no zone of inhibition by the copper nanoparticles at the concentration similar to that of the control, there was zone of inhibition in concentrations of 40mg/ml and 20mg/ml, as shown in table 1.

Table 2 Minimum inhibitory concentration result

S/N	Microorganism	20 mg/ml	10 mg/ml	5 mg/ml	2.5 mg/ml	1.25 mg/ml	0.625 mg/ml	0.3125 mg/ml
1	*Staphylococcus aureus*	+	_	_	_	_	_	_
2	*Escherichia coli*	+	_	_	_	_	_	_
3	*Bacillus subtilis*	+	_	_	_	_	_	_
4	*Salmonella typhi*	+	_	_	_	_	_	_
5	*Candida albicans*	_	_	_	_	_	_	_

Keyword: + = Activity; _ = No activity

The minimum inhibitory concentration of the synthesized copper oxide nanoparticles against selected microorganisms (*Staphylococcus aureus, Escherichia coli, Bacillus subtilis, Salmonella typhi* and *Candida albicans*) at concentrations ranging between 20mg/ml-0.3125mg/ml (diluted using the two fold serial dilution) were shown at table 3.2. From table 3.2 the minimum concentration of the synthesized copper oxide nanoparticles that would inhibit the growth of the test microorganism was 20mg/ml, although the concentration had no effect against candida albicans.

4. Conclusion

This study provided scientific proof that copper oxide nanoparticles synthesized using the outer layer of *Allium cepa* has antimicrobial properties against both gram positive and gram negative organisms. It also has activity against fungi, although not as effective as the standard antimicrobial agents. The synthesized CuONPs showed remarkable stability in the characterization using different techniques such as UV-Visible spectroscopy which was used in noting the rate reaction at a given time, FTIR which was used to determine the different functional groups, DLS particle size which was used to determine the size distribution of the particles, Transmission electron microscopy (TEM) which was used to reveal the particle size and morphology of the synthesized CuONPs and finally the X-ray diffraction analysis which was used to analyze The crystalline nature and the average size of the CuONPs.

Compliance with ethical standards

Acknowledgments

The authors wish to acknowledge the Chemistry Department, Rhodes University, PO Box 94, Grahamstown, 6140, South Africa for their unprecedented permission to access their state of the art Nanotechnology Laboratories.

Disclosure of conflict of interest

The authors declare no conflict of interest.

References

[1] Losasso C., Belluco S., Cibin V., Zavagnin P., Mičetić I., Gallocchio F., Zanella M., Bregoli L., Biancotto G., and Ricci A. (2014). Antibacterial activity of silver nanoparticles: Sensitivity of different Salmonella serovars. Frontiers in Microbiology, 5, 227.

[2] Qing Y., Cheng L., Li R., Liu G., Zhang Y., Tang X., Wang J., Liu H., and Qin Y. (2018). Potential antibacterial mechanism of silver nanoparticles and the optimization of orthopedic implants by advanced modification technologies. International Journal of Nanomedicine, 13(1), 3311–3327.

[3] Iravani, S. (2011) Green Synthesis of metal nanoparticles using plants. Green Chemistry, 13(10); 2638-2650.

[4] Lee, H.J., Lee, G., Jang, N.R., Yum, J.H., Song, J.Y., and Kim, B.S. (2011). Biological Synthesis of Copper nanoparticles using plant extract. Nanotechnology, 128(1-2); 83-89.

[5] Naika, R.H., Lingaraju. K., Manjunath, K., Kumar, D., Nagaraju, G., Suresh, D., and Nagabhushana, H. (2015). Green Synthesis of Copper Oxide (CuO) Nanoparticles using Gloriosa superb L. extract and their antimicrobial activity; Journal of Taibah University for Science, 9(1); 7-12.

[6] Huang, J., Li, Q., and Sun, D. (2007). Biosynthesis of silver and gold nanoparticles by Novel sundried Cinnamomum camphora leaf. Nanotecnology, 18(10); 105104.

[7] Eltaweel, M. (2013). Assessment of antimicrobial activity of onion extract (Allium cepa) on Staphylococcus aureus; in vitro study. International Conference on Chemical, Agricultural and Medical Sciences, 12, 29– 30.

[8] Induja, M. P., and Geetha, R. V. (2018). Antimicrobial activity of Allium cepa against bacteria causing enteric infection. Drug Invention Today, 10(12), 35– 38

[9] Loredana, L., Giuseppina, A., Filomena, N., Florinda, F., & Donatella, A. (2019). Biochemical, antioxidant properties and antimicrobial activity of different onion varieties in the Mediterranean area. Journal of Food Measurement and Characterization, 13(2), 1232– 1241.

[10] Sharma, K., Ko, E. Y., Assefa, A. D., Ha, S., Nile, S. H., Lee, E. T., and Park, S. W. (2015). Temperature-dependent studies on the total phenolics, flavonoids, antioxidant activities, and sugar content in six onion varieties. Journal of Food and Drug Analysis, 23(2), 243– 252.

[11] Sharma, K., Mahato, N., and Lee, Y. R. (2018). Systematic study on active compounds as antibacterial and antibiofilm agent in aging onions. Journal of Food and Drug Analysis, 26(2), 518–528.

[12] Slavin, Y. N., Asnis, J., Häfeli, U. O., and Bach, H. (2017). Metal nanoparticles: understanding the mechanisms behind antibacterial activity. Journal of nanobiotechnology, 15(1), 1-20.

[13] Lara, H.H., Ayala-Nuñez, N.V., Ixtepan-Turrent, L., and Rodriguez-Padilla, C. (2010). Mode of antiviral action of silver nanoparticles against HIV-1. Journal of Nanobiotechnology, 8(1), 1–10.

[14] Pulicheria, Y., Thirumalandhuni, V., Yagani, J.R., Palempalli, U. M. D., Golla, N., and Nataru, S. (2018). Cost Effective, Green Synthesis of Copper Oxide Nanoparticles Using Fruit Extract of Syzygium alterniflium (Wt.) Walp., Characterization and Evaluation of Antiviral Activity. Journal of Cluster Science, 28(4), 743-755.

[15] Khanna, P., Kaur, A., and Goyal, D. (2019). Algae-based metallic nanoparticles: Synthesis, characterization and applications. Journal of microbiological methods, 163, 105656.

[16] Bahram-Parvar, M., and Lim, L. T. (2018). Fresh-cut onion: A review on processing, health benefits, and shelf-life. Comprehensive Reviews in Food Science and Food Safety, 17(2), 290– 308.

[17] Chakraborty, A.J., Uddin, T.M., Zidan, M., Redwan, B.M., Mitra, S., Das, R., Nainu, F., Dhama, K., Roy, A., Hossain, M. and Khusro, A. (2022). Allium cepa: A Treasure of Bioactive Phytochemicals with Prospective Health Benefits. Evidence-Based Complementary and Alternative Medicine, 2022.

[18] Charles P. Poole, Jr. and Frank J. Owens. (2003). Introduction to nanotechnology, John Wiley and sons Inc., United States, pp: 1-7.

[19] Gao, W., Thamphiwatana, S., Angsantikul, P., and Zhang, L. (2014). Nanoparticle approaches against bacterial infections. Wiley Interdisciplinary Reviews-Nanomedicine and Nanobiotechnology 6(6), 532–547.

[20] Kumar, K. S., Bhowmik, D., Chiranjib, B., and Tiwari, P. (2010). Allium cepa: A traditional medicinal herb and its health benefits. Journal of Chemical and Pharmaceutical Research, 2(1), 283-291.

[21] Mirhosseini, M. (2015). Synergistic antibacterial effect of metal oxide nanoparticles and ultrasound stimulation. Journal of Biology and Today's World, 4(6), 138–144.

[22] Ramos, F. A., Takaishi, Y., Shirotori, M., Kawaguchi, Y., Tsuchiya, K., Shibata, H., and Takeuchi, M. (2006). Antibacterial and antioxidant activities of quercetin oxidation products from yellow onion (Allium cepa) skin. Journal of Agricultural and Food Chemistry, 54(10), 3551– 3557.

[23] Sánchez-López, E., Gomes, D., Esteruelas, G., Bonilla, L., Lopez-Machado, A.L., Galindo, R., Cano, A., Espina, M., Ettcheto, M., Camins, A. and Silva, A.M. (2020). Metal-based nanoparticles as antimicrobial agents: an overview. Nanomaterials, 10(2), 292.