https://chemclassjournal.com/
ChemClass Journal Vol. 8 Issue 1 (2024); 189-200
e-ISSN:0198-2734 p-ISSN:0198-5680
doi.org/10.33003/chemclas_2024_0801/016

Green Synthesis, Characterization, and Antimicrobial Activities of Copper Oxide Nanoparticles (CuONPS) Using Bitter Leaf (*Vernonia amygdalina)* Aqueous Extract

[1]Ahmed Wasiu Ayinde, [1]Afolabi Nafisa Ajibola, [2,4]Adebisi Toheeb Olohungbebe, [1]Adepoju Ridwan Adetunji, [3]Yekeen Bayo Hammed and [1]Idris Abdul Akeem

1. Department of Science Laboratory Technology, The Oke-Ogun Polytechnic, Saki
2. Department of Chemistry, Federal University of Technology, Minna
3. Department of Environmental Management and Toxicology, Federal University of Agriculture, Abeokuta
4. Nigeria Natural Medicine Development Agency

Corresponding Author: ahmedayinde80@gmail.com Phone no: 09057943650

Abstract

Nanotechnology, a rapidly evolving field, focuses on designing, synthesizing, and utilizing materials at the nanoscale, offering revolutionary applications across industries. This study investigates the synthesis, characterization, and application of nanoparticles for specific functional applications, employing ultraviolet-visible (UV-Vis) spectroscopy, Fourier-transform infrared spectroscopy (FTIR), and scanning electron microscopy (SEM) for detailed analysis. Nanoparticles were synthesized through the green method and subjected to comprehensive characterization. UV-Vis spectroscopy was used to evaluate the optical properties, confirming the surface plasmon resonance (SPR) indicative of nanoparticle formation. FTIR analysis identified functional groups and chemical interactions, while SEM provided detailed morphological insights and subjected to antimicrobial screening. UV-Vis spectra confirmed a characteristic SPR peak at 250 nm, correlating with the desired particle size and uniformity. FTIR analysis revealed FTIR analysis identified functional groups, including hydroxyl (-OH), carboxyl (C=O), and amine (C-N), which reflect the presence of flavonoids, phenols, and proteins from the *Vernonia amygdalina* extract. These functional groups likely play a vital role in reducing and stabilizing Cu ions, enhancing nanoparticle bioactivity, and highlighting successful chemical bonding and stability of the nanoparticles. SEM images depicted uniform morphology and size distribution with an average particle size of 20nm, supporting consistent synthesis. CuONPs showed minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) against several organisms: Staphylococcus aureus (S.a) (MIC 6.25 µg/ml, MBC 12.5 µg/ml), Bacillus subtilis (B.s) (MIC 3.125 µg/ml, MBC 50 µg/ml), Escherichia coli (E.c) (MIC 12.5 µg/ml, MBC 50 µg/ml), and others. Compared to controls like ciprofloxacin, CuONPs demonstrated broader efficacy, especially against resistant strains like Pseudomonas aeruginosa (PS.a) (MIC 6.25 µg/ml).

Keywords: Green synthesis, characterization, antimicrobial, copper nanoparticles, Vernonia amygdalina

Introduction

Green synthesis of nanoparticles using living cells through biological pathways is a more efficient technique and yields a higher mass when compared to other related methods. Plants are the sources of several components and biochemicals that can play a role as stabilizing and reducing agents to synthesize green nanoparticles. The green synthesized methods are eco-friendly, non-toxic, cost-effective, and also more stable when compared to other biological, physical, and chemical methods [1].

Green synthesis of nanoparticles is categorized into three groups, viz. extracellular, intracellular, and phytochemicals. The nanoparticle synthesis from plant extract is an inexpensive process and it results in higher yield due to the huge quantity of phytochemical components in the plant extract that can also act as reducing and stabilizing agents converting metal ions into metal nanoparticles [2]. Green synthesized metal and metal oxide nanoparticles have emerging applications in the biomedical sector like diagnostics, wound healing, tissue treatment, immunotherapy, regenerative medicine, dentistry, and biosensing platforms. Bio-toxicology and its antimicrobial, antifungal, and antiviral characteristics were passionately contested [3].

Plant-mediated copper oxide nanoparticles synthesized from various plant extracts play various roles like diverse biological activities, environmental remediation, photocatalysis, catalytic reduction, sensing, energy storage, and several organic transformations such as coupling, reduction, and multicomponent reactions [4]. Green synthesized selenium nanoparticles help improve the activity in antioxidants, catalysis, anticancer, photocopiers, xerography, rectifiers, and solar cells [5]. Green synthesized cerium oxide nanoparticles have potential photocatalytic dye degradation, antioxidant activity, antidiabetic, anticancer antibacterial, and antifungal activity properties [6].

Green synthesized stannic oxide nanoparticles have potential photocatalytic, antioxidant, and antibacterial activity, and these nanoparticles help enhance the environment and human health applications [7]. Green synthesized silver chloride nanoparticles are used to develop environmental and biomedical applications [8].

Green synthesized metal nanoparticles are produced from different parts of the plants and also these methods are eco-friendly, non-toxic, and cost-effective method. Green synthesized nanoparticles have more active performance in removing dyes, antibiotics, and metal ions compared to other physical and chemical methods [9]. The green synthesis method is the best method for the preparation of nanoparticles, and these methods help reduce the toxicity, increase stability, and are eco-friendly, and cost-effective methods. Green synthesis methods have more beneficial responses in environmental and biomedical applications [10]. Plants contain many types of

phytochemical compounds like phenolics, terpenoids, polysaccharides, and flavonoids that possess oxidation–reduction capabilities. Thus, they are preferably utilized for the green synthesis of nanoparticles [11]. Phytochemical compound synthesis of nanoparticles is not a general procedure as essential knowledge of the exact phytochemical components is needed for the synthesis of stabilized nanoparticles [12]. It is a general point of view that plants' secondary metabolites (polyphenols) are the main elements playing a very important function in the progression of the green synthesis of nanoparticles.

The green synthesis practice is more advanced, safe cost-effective, easily reproduced, and stable [13]. There are some positive impacts on the plant-based green synthesis of nanoparticles when compared to the other related biological methods using bacteria, fungi, actinomycetes, and algae [14]. Diverse plant parts (roots, stem, leaf, seed, and fruit) are concerned with such synthesized green nanoparticles because of the presence of notable phytochemicals [15].

Vernonia amygdalina is a plant reach in phytochemicals and limited work has been reported on the use of the plant for green synthesis of CuONPs [16].

Very insignificant work has been found on literature review on the green synthesis of CuO nanoparticles on the use of *V. amygdalina* for biomedical and antimicrobial applications. Thus, we present eco-friendly green synthesis of CuONPs The nanoparticles have been characterized by SEM, UV, FTIR and screened for antibacterial activity.

Methods

Reagent and Apparatus

$Cu(NO_3)_2.3H_2O$ purchased from Pascal Scientific Ltd was utilized as the starting material. Fresh *V. amygdalina* leaves were collected from the Irshad Area in Oyo State, Saki of Latitude $8^0 41' 26"$ N and Longitude $3^0 25' 36"$ E. The identification of the plant was done at Biological Department, the Oke-Ogun Polytechnic, Saki. Distilled water was used to prepare reagent solutions. All chemicals were of analytical grade and they were used without further purification**.**

Preparation of the aqueous *V. amygdalina* leaf extract

V. amygdalina leaves were surface cleaned and repeatedly washed with distilled water to completely remove all dust particles. The thoroughly washed leaves were air-dried for five days and after that ground using a mechanical grinder. This was subsequently sieved and stored in an airtight container. The extraction was carried out by measuring 10 g of powdered leaves were weighed and boiled in 250 ml conical flask of 100 mL distilled water at 60 ℃ for 10 min. The extract was then filtered through Whatman 185 mm filter paper, yielding a light brown filtrate. The extract was kept in the refrigerator between 4°C and utilized to synthesize CuONPs.

Preparation of $Cu(NO_3)_2.3H_2O$ Solution

0.2M of $Cu(NO_3)_2.3H_2O$ was prepared by accurately weighing 4.63g of the salt and dissolved in 50 ml of distilled water which was then transferred to a 100 ml standard flask and top to the marked with distilled water.

Phytochemicals Screening (qualitative analysis)

Test for alkaloids

To 2 ml of the plant extract, 1 ml of Dragendoff's reagent was added in 100 ml volumetric flask. Formation of an orange or reddish-brown precipitate indicates the presence of alkaloids.

Test for flavonoids

Alkaline reagent: 2-3 drops of NaOH + 2 ml of plant extract were added together. Initially a deep yellow but gradually turns colorless by adding a few drops of HCl.

Test for glycosides

Keller Killani test: 2 ml of the plant extract, a solution of 0.5ml containing glacial acetic acid and 2-3 drops of ferric chloride were mixed together and 1ml of concentrated H_2SO_4 was added along the wall of the test tube. A reddish-brown color appears at the junction of the liquid layers and the upper layers appear bluish-green.

Test for saponins

Approximately 2 g of powdered material was boiled with 20 mL of distilled water in a water bath and filtered. Next, 1 mL of the filtrate was mixed with 5 mL of distilled water shaken vigorously, and observed for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously again and then observed for the formation of emulsion as an indication of saponin.

Test for steroids

Approximately 3ml of chloroform with concentrated H_2SO_4 and 5ml of plant extract. In the lower layer chloroform layer appears color that indicates the presence of steroids.

Test for tannin

Approximately 1.5 g of dried powdered sample was boiled in 20 mL of water in a test tube and filtered. A few drops of ferric chloride were added and observed for brownish green or a blue-black coloration as an indication of tannins.

Test for terpenoids

Approximately 5 mL of extract was mixed with 2 mL chloroform and 3 mL H_2SO_4 was carefully added to form a layer. A reddish-brown coloration of the interface was an indication of terpenoids.

Synthesis of Copper Nanoparticles from Plant Extracts

The synthesis of Copper (Cu) nanoparticles was carried out with slight modifications. To 30 mL of the aqueous leaf extract was added 10 mL of 0.2M $Cu(NO_3)_2.3H_2O$ in a clean 250 mL beaker. The mixture was stirred for 6 hours on a magnetic stirrer/hot plate at 60 ^{0}C (Fig. 3). The mixture was allowed to stand for 24 h and was centrifuged at 4000 rpm for 30 min. The synthesized mixture was allowed to stand for 24 h before being separated by centrifugation at 4000 rpm for 30 min. The

transparent liquid was decanted, and the settling layer (i.e., nanoparticles) was dried in the oven at 30 °C and stored in a 5 mL plastic sample vial with the appropriate labeling [17].

Antimicrobial Activities

Materials

Liquid cultures bacterial at suitable growth phase (diluted to 10-2), Sterile 96 well Microliter Plates, extract, Sterile diluents (methanol 50%) and Test-tubes.

Minimum inhibitory concentration

The test organisms were sub cultured into nutrient broth medium to make overnight broth culture. Dissolve the antibacterial to the concentration desired in the test medium to double fold the top wells. The dissolved antibacterial (oil) was then dilute in the test tube medium to 2x the concentration desired in the test, that is the desired concentration at the first column was 50% but 100% of the oil was dispensed.

Using the micropipette, 100ml of triptone soya broth (TSB) was dispensed into the wells of each of the microtitre plate respectively the lids were labeled with the names of organism.

100 uL appropriate 2x extract and positive control was dispensed into wells in row one

Using the multipipe set at 100uL, the extracts were mixed in row one by sucking up and down without splashing.

100uL was withdrawn from the first well and diluted serially up to well 8. The same procedure was repeated for the other rows respectively.

10uL of 1:100 bacterial and fungi culture were dispensed into wells in row 7 to 1 in the order. The organism was not added to column 8 which serves as broth sterility control.

The plates were incubated at 370C and 250C for 24hrs and 48hrs

The lowest concentration of the extract and which showed no turbidity were taken as MIC.

Minimum bactericidal concentration

The concentration which showed no color change (Red Coloration) after the addition of 0.2mg/ml of p-INT dye were streaked on solidified nutrient agar plates and incubated at 370C for 24hrs.

The lowest concentration which showed no growth on nutrient agar medium was taken as MBC.

Results and Discussion

Results

The result for the *V. amygdalina* aqueous extract is presented in table 1. The test for alkaloids, glycoside, saponin, steroid and terpenoids were positive while flavonoid and tannin were absent.

Table 1: Phytochemical Screening of *V. amygdalina* aqueous extract

Test	Sample
Alkanoid (Dragendorff's test)	+
Flavonoid (Alkaline)	-
Glycoside(Keller Kelani test)	+
Saponin	+
Steroid	+
Tannin(Ferric chloride)	-
Terpenoid	+

Key: + = positive, - = negative

The phytochemical screening of *Vernonia amygdalina* aqueous extract highlights its rich content of alkaloids, terpenoids, saponins, steroids, and glycosides—key compounds that play a significant role in the synthesis of CuO nanoparticles. Alkaloids and terpenoids act as natural reducing agents, helping convert copper ions into stable nanoparticles.

Saponins, with their surface-active properties, aid in stabilizing and capping the nanoparticles, preventing agglomeration and ensuring a uniform size. Steroids and glycosides may further enhance the stabilization process and contribute to the nanoparticles' biocompatibility. However, the absence of tannins and flavonoids may slightly reduce the antioxidant activity during synthesis, potentially influencing the final properties of the CuO nanoparticles [18]. Overall, *V. amygdalina* extract provides a natural, eco-friendly medium for synthesizing effective and stable CuO nanoparticles.

Characterization of Cuo Nanoparticles

UV-Visible Characterization of CuONPs

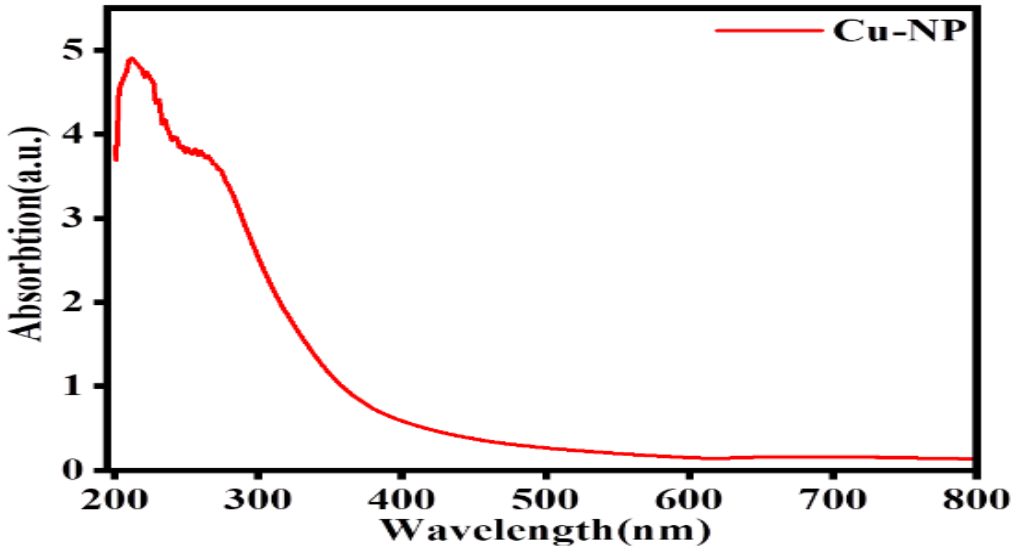

Figure 1: UV-Visible Spectra of CuONPs

The UV-Vis spectrum of CuO nanoparticles typically shows a prominent absorption peak in the 200–300 nm range due to the electronic transitions in CuO. In this spectrum, a strong absorption around 250 nm is observed, which is characteristic of CuO nanoparticles, indicating successful synthesis [9]. This peak can be attributed to the charge transfer transitions of CuO and confirms the nanoparticle's formation. The gradual decrease in absorption at higher wavelengths suggests stability and uniformity of the nanoparticles, as no additional peaks imply minimal impurities or aggregation.

FTIR Characterization of CuONPs

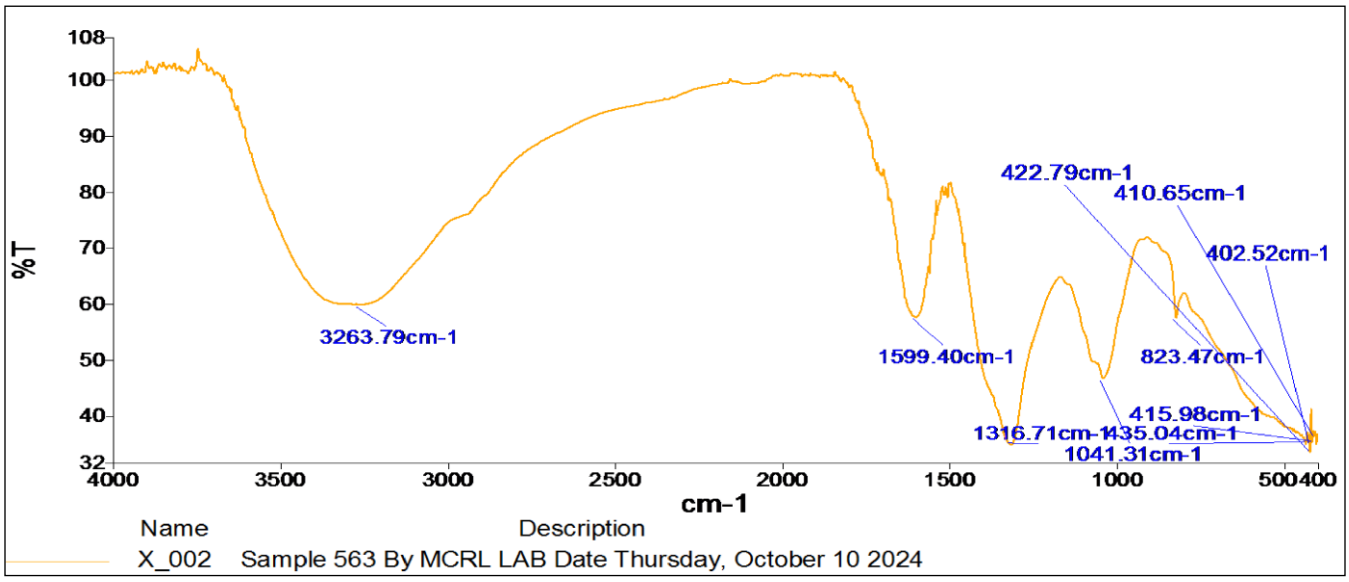

Figure 2: The FTIR Spectrum of CuONPS

The FTIR spectrum of the synthesized CuO nanoparticles provides crucial insights into the functional groups involved in their formation and stabilization. The broad absorption band at **3263.79 cm^{-1}** corresponds to O–H stretching vibrations, which could indicate the presence of hydroxyl groups, likely contributed by water molecules or phytochemicals such as flavonoids, alkaloids, and saponins during synthesis. The peak at **1599.40 cm^{-1}** suggests the presence of C=C stretching vibrations from aromatic compounds, possibly from terpenoids or glycosides in the *V. amygdalina* plant extract [5].

The absorption at **823.47 cm^{-1}** and **410.65 cm^{-1}** is characteristic of Cu–O vibrations, confirming the successful synthesis of CuO nanoparticles. Peaks at **1041.31 cm^{-1}** and **1316.71 cm^{-1}** may indicate C–O and C–N stretching, respectively, highlighting the potential involvement of alkaloids or glycosides as capping and stabilizing agents.

The plant-based phytochemicals likely played a dual role: reducing copper (ii) ions to CuO nanoparticles by electron transfer and stabilizing them to prevent agglomeration. Terpenoids and saponins, known for their reducing and stabilizing abilities, likely contributed significantly to this process [18]. The absence of tannins and flavonoids, as shown in earlier screening, may influence the nanoparticles' antioxidant properties but does not hinder their synthesis or stabilization.

SEM Characterization of CuO nanoparticles

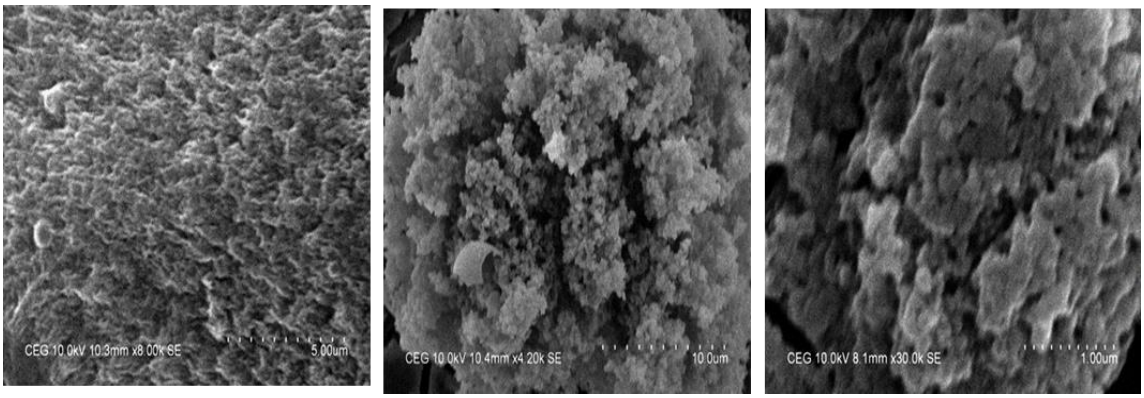

Plate I: SEM micrograph of CuO nanoparticles

The SEM image of the synthesized CuO nanoparticles reveals a highly aggregated structure with a roughly spherical morphology. The particles appear to form clusters, which could be due to natural aggregation during synthesis or drying with different particle sizes as indicated by the magnification with average diameter of 25nm [17], the nanoscale size suggests the particles are well within the nanometer range [9]. This small size and high surface area contribute to the nanoparticles' potential for enhanced reactivity, stability, and antimicrobial efficiency. The observed uniformity in shape and distribution also highlights the effectiveness of using plant-based phytochemicals as reducing and stabilizing agents during synthesis.

Antimicrobial Activity

Table 2: Antimicrobial Screening of CuONPs

	Sample x		BROTH ONLY	BROTH AND EXTRACT	BROTH AND ISOLATES	CONTROL			
						Cipro[ng/ml]	Ket 1%		
Organisms	MIC	MBC				MIC. MBC	MIC.MBC		
S.a	6.25	12.5	-	-	+	5	5	NA	NA
B.s	3.125	50	-	-	+	5	10	NA	NA
E.c	12.5	50	-	-	+	10	10	NA	NA
PS.a	6.25	>50	-	-	+	>10	>10	NA	NA

T.r	3.125	50	-	-	+	NA	NA	0.25	5
C.a	25	50	-	-	+	NA	NA	1	1

Copper oxide nanoparticles (CuONPs) synthesized using bitter leaf extract demonstrated notable antimicrobial activity. In broth-only tests, CuONPs showed minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) against several organisms: Staphylococcus aureus (S.a) (MIC 6.25 µg/ml, MBC 12.5 µg/ml), Bacillus subtilis (B.s) (MIC 3.125 µg/ml, MBC 50 µg/ml), Escherichia coli (E.c) (MIC 12.5 µg/ml, MBC 50 µg/ml), and others. Compared to controls like ciprofloxacin, CuONPs demonstrated broader efficacy, especially against resistant strains like Pseudomonas aeruginosa (PS.a) (MIC 6.25 µg/ml).

This effectiveness correlates with phytochemical screening of the bitter leaf extract, revealing the presence of alkaloids, terpenoids, saponins, and steroids. Alkaloids and terpenoids likely enhanced nanoparticle stabilization, while saponins, known for membrane-disruptive properties, may synergize with CuONPs to disrupt bacterial integrity. The absence of tannins and flavonoids suggests these compounds were not contributing to the formation of CuONPs [15]. Thus, the synergy between CuONPs and bioactive phytochemicals underpins their antimicrobial potency.

Conclusion

In conclusion, the study highlights the successful synthesis of copper oxide nanoparticles (CuONPs) using *Vernonia amygdalina* (bitter leaf) extract as a natural, eco-friendly medium. Phytochemical analysis of the extract revealed alkaloids, terpenoids, saponins, and steroids, which played crucial roles in reducing and stabilizing the nanoparticles. The synthesized CuONPs were characterized by UV-Vis, FTIR, and SEM analyses, confirming their nanoscale size, stability, and effective functionalization with plant-based compounds.

Antimicrobial testing demonstrated the significant efficacy of CuONPs against a variety of pathogenic organisms, including *Staphylococcus aureus*, *Bacillus subtilis*, and *Escherichia coli*, with MIC and MBC values comparable to or exceeding conventional antibiotics like ciprofloxacin. Notably, CuONPs were effective against resistant strains such as *Pseudomonas aeruginosa.* The absence of tannins and flavonoids in the extract did not impair nanoparticle synthesis or antimicrobial performance, underscoring the pivotal role of other phytochemicals. Overall, the study underscores the potential of plant-mediated CuONPs as sustainable and potent antimicrobial agents.

Acknowledgment

The authors acknowledge the contribution of Science Laboratory Technology Department, The Oke-Ogun Polytechnic Saki.

References

[1]. Manimaran, M.; Kannabiran, K. Actinomycetes-mediated biogenic synthesis of metal and metal oxide nanoparticles: Progress and challenges. *Lett. Appl. Microbiol.* **2017**, *64*, 401–408.

[2]. Kumar, H.; Bhardwaj, K.; Kuc̆a, K.; Kalia, A.; Nepovimova, E.; Verma, R.; Kumar, D. Flower-Based Green Synthesis of Metallic Nanoparticles: Applications beyond Fragrance. *Nanomaterials* **2020**, *10*, 766.

[3]. Mao, X.; Wu, L.-F.; Guo, H.-L.; Chen, W.-J.; Cui, Y.-P.; Qi, Q.; Li, S.; Liang, W.-Y.; Yang, G.-H.; Shao, Y.-Y.; Guo, Y.n. The Genus *Phyllanthus*: An Ethnopharmacological, Phytochemical, and Pharmacological Review. *Evid.-Based Complement. Altern. Med.* **2016**, *2016*, 7584952.

[4]. Sepeur, S. *Nanotechnology: Technical Basics and Applications*; Vincentz Network GmbH & Co KG: Hannover Deutschland, Germany, 2008.

[5]. Xu, L.; Wang, Y.Y.; Huang, J.; Chen, C.Y.; Wang, Z.X.; Xie, H. Silver nanoparticles: Synthesis, medical applications and biosafety. *Theranostics* **2020**, *10*, 8996–9031.

[6]. Rezvani Amin, Z.; Khashyarmanesh, Z.; Fazly Bazzaz, B.S.; Sabeti Noghabi, Z. Does Biosynthetic Silver Nanoparticles Are More Stable with Lower Toxicity than Their Synthetic Counterparts? *Iran. J. Pharm. Res.* **2019**, *18*, 210–221.

[7]. Soni, V.; Raizada, P.; Singh, P.; Cuong, H.N.; S, R.; Saini, A.; Saini, R.V.; Le, Q.V.; Nadda, A.K.; Le, T.T.; et al. Sustainable and green trends in using plant extracts for the synthesis of biogenic metal nanoparticles toward environmental and pharmaceutical advances: A review. *Environ. Res.* **2021**, *202*, 111622.

[8]. Rajan, P.I.; Vijaya, J.J.; Jesudoss, S.K.; Kaviyarasu, K.; Kennedy, L.J.; Jothiramalingam, R.; Al-Lohedan, H.A.; Vaali-Mohammed, M.-A. Green-fuel-mediated synthesis of self-assembled NiO nano-sticks for dual applications—Photocatalytic activity on Rose Bengal dye and antimicrobial action on bacterial strains. *Mater. Res. Express* **2017**, *4*, 085030.

[9]. Tangthong, T.; Piroonpan, T.; Thipe, V.C.; Khoobchandani, M.; Katti, K.; Katti, K.V.; Pasanphan, W. Water-Soluble Chitosan Conjugated DOTA-Bombesin Peptide Capped Gold Nanoparticles as a Targeted Therapeutic Agent for Prostate Cancer. *Nanotechnol. Sci. Appl.* **2021**, *14*, 69–89.

[10]. Khoobchandani, M.; Khan, A.; Katti, K.K.; Thipe, V.C.; Al-Yasiri, A.Y.; MohanDoss, D.K.D.; Nicholl, M.B.; Lugão, A.B.; Hans, C.P.; Katti, K.V. Green nanotechnology of MGF-AuNPs for immunomodulatory intervention in prostate cancer therapy. *Sci. Rep.* **2021**, *11*, 16797.

[11]. Albanese, A.; Tang, P.S.; Chan, W.C. The effect of nanoparticle size, shape, and surface chemistry on biological systems. *Annu. Rev. Biomed. Eng.* **2012**, *14*, 1–16.

[12]. Arasu, M.V.; Arokiyaraj, S.; Viayaraghavan, P.; Kumar, T.S.J.; Duraipandiyan, V.; Al-Dhabi, N.A.; Kaviyarasu, K. One step green synthesis of larvicidal, and azo dye degrading antibacterial nanoparticles by response

surface methodology. *J. Photochem. Photobiol. B* **2019**, *190*, 154–162.

[13]. Arya, A.; Gupta, K.; Chundawat, T.S.; Vaya, D. Biogenic Synthesis of Copper and Silver Nanoparticles Using Green Alga Botryococcus braunii and Its Antimicrobial Activity. *Bioinorg. Chem. Appl.* **2018**, 7879403.

[14]. Barabadi, H. Nanobiotechnology: A promising scope of gold biotechnology. *Cell. Mol. Biol.* **2017**, *63*, 3–4.

[15]. Eckelman, M.J.; Zimmerman, J.B.; Anastas, P.T. Toward Green Nano. *J. Ind. Ecol.* **2008**, *12*, 316–328.

[16]. Ananda, M.; Tegene, D.; Kar, B.T.; & Suresh, K.G. Enhanced Multifunctionality of CuO nanoparticles Synthesized Using Aquoeus Leaf Extract of *Vernonia amygdalina* Plant. *Elsevier B.V.* 2021, 2211-7156

[17]. Fuku, X.; Modibeji, M.; Mathe, M. Green Synthesis of Cu_2/CuO Nanosturctures and the Analysis of their Electrochemical Properties. *SN Applied Sciences*. 2020, 2, 902.

[18]. Sanchez-Sanhieza, G.; Fuentes-Rodriguez, D.; Bello-Toledo. Copper Nanoparticles as Potential Antimicrobial Agent in Disinfecting Root Canals. A systematic Review, *Int. J. Odontosmatology*. 2016, 10., 547-554