Biosynthesis, Characterization and Antifungal Investigation of Ag-Cu Nanoparticles from Bark Extracts of *Garcina kola*

Akintelu Sunday Adewale [1] and Folorunso Aderonke Similoluwa [2*]

[1]Department of Pure and Applied Chemistry, Ladoke Akintola University of Technology, Ogbomoso, Nigeria.
[2]Department *of* Chemistry, Louisiana State University, Louisiana, USA
aderonkesimi@gmail.com
+2348136872649

Abstract: The scarcity of effective antifungal agents capable of eradicating the increasing antifungal resistance has been acknowledged as one of the most challenge battling with many orthodox health care centres. This study aimed at the investigation of the antifungal competence of Ag-Cu nanoparticles synthesized from the bark of *Garcina kola* against *Candida tropicalis, Fusarium oxysperium, Candida albican* and *Aspergillus flaws*. Fresh matured barks of *Garcina kola* were collected from Gebeleju Farm land in Irele area of Ondo State. The bark was washed, air dried, chopped, pulverized, extracted and properly stored. The Ag-Cu nanoparticles formed where characterized and the Agar disk diffusion method was followed to determine its antifungal activity at 150, 100 and 50mg/ml using Amphotericin-B as controls. The characterization of the Ag-Cu nanoparticles revealed a spherical shape nanoparticles of size 21-32 nm with an average diameter of 16nm which are of disperse distribution. The inhibitory zones of the Ag-Cu nanoparticles at 150, 100, 50mg/ml and Amphotericin-B against the test fungi ranges from 20 to 13mm, 13 to 7mm, from 10 to 4 mm and from 29 to 20mm respectively. The order of the antifungal competence of the Ag-Cu nanoparticles against test fungi was *Aspergillus flaws* > *Candida albican* > *Fusarium oxysperium* > *Candida tropicalis*. The broad inhibitory spectrum obtained from the antifungal screening of Ag-Cu nanoparticles confirmed its application as remedy against fungal infections and its usage as highly relevant ingredient that could be useful in the production of effective novel antifungal agents.
[Akintelu Sunday Adewale and Folorunso Aderonke Similoluwa. **Biosynthesis, Characterization and Antifungal Investigation of Ag-Cu Nanoparticles from Bark Extracts of *Garcina kola*.** *Stem Cell* 2019;10(4):30-37]. ISSN: 1945-4570 (print); ISSN: 1945-4732 (online). http://www.sciencepub.net/stem. 5. doi:10.7537/marsscj100419.05.

Key words: *Garcinia kola* bark, nanoparticles, characterization, fungal strains, Amphotericin-B, antifungal agents

Introduction

The scarcity of novel antifungal agents and increasing antifungal resistance has been acknowledged as a main defy experienced by the world health sector for decades [1, 2]. The need for effective, inexpensive and novel antifungal agents to prevent and treat fungal infections must be met as one-half of deaths that occur yearly are due to infectious diseases, especially in remote parts of the globe [3, 4].

The pervasive, inappropriate and extensive usage of antibiotics has been reported as factors contributing to the increase in the development and growth of antimicrobial resistant fungal strains [5]. Antibiotic resistance in humans, plants and animals will forever be a challenge except it is urgently attended to [6].

The chemical and medicine application of nanotechnology has spawned great enthusiasm over the decades [7]. Nanotechnology comprises of various approaches that involves the formulation of nanoparticles with fascinating chemical and physical properties [8-10]. The wide application of nanoparticle synthesis in the field of biomedicine, phytochemistry and chemistry has led to new area of research for many researches [11]. Silver and copper nanoparticles have shown many application in the medicinal field among which are antimicrobial activity against pathogens [12,13]. However, diverse manufacturing techniques involve in the synthesis of nanoparticles are very luxurious and are of great environmental concerns, [8].

Therefore, the need to develop inexpensive and eco- friendly environmentally procedures for the synthesis of nanoparticle is of great importance. Hence, rendering the biological approach for the synthesis of nanoparticles crucial [9]. The biological procedures for the synthesis of nanoparticles offers a relatively non-toxic and eco-friendly procedure which are sometime referred to as Green chemistry [10]. In contrast to the chemical methods adopted for the synthesis nanoparticles are environmentally offensive [7].

Numerous organisms and plants have been used for the green synthesis of many nanoparticles among which are copper, gold, silver, nickel e.t.c [14].

Plants are made up of diverse bioactive compounds and phytochemicals that constitutes plant derived antimicrobial substances which are highly competent in treating various microbial infections [15]. Many of these plants are grown in tropical Africa among which we have *Garcina kola* that is commonly

use in the whole of Nigeria as therapeutic agent in healing several ailments as a result of the medicinal virtues it possess [16-18].

The ethnobotanical studies and pharmacological investigations of *Garcina kola* plant's parts, had revealed its competence as pharmaceutical agent in healing several diseases and infections due to the bioactive compounds found in them [19]. Previous research conducted by [20-21] showed that *Garcina kola* plant contains chromanols, Kolaviron, prenylate benzophenones, kolanone and oleoresin. However, the antifungal efficacy of the bark of *Garcina kola* has not be well reported. Thus, this research centered on the investigation of the antifungal efficiency of Ag-Cu nanoparticles synthesized from *Garcina kola.*

Materials and methods

All the solvents and chemicals used were of analytical grade and were purchased Sigma Aldrich, Germany.

***Garcina kola* bark Collection**

The bark of *Garcina kola* was selected for this study based on available review on its pharmacological activities and relative abundance. Matured fresh barks of *Garcina kola* were collected from 'Gebeleju' Farm land in Irele Ondo State.

***Garcina kola* bark extraction**

The bark was washed to remove impurities, it was air dried, chopped into small pieces, pulverized and properly stored in an air tight sample bottle to prevent microbial contamination and environmental defect before usage. The extraction of was carried out by quantitatively transfer of 20g of the pulverized sample into an extracting vessel containing 50 mL of distilled methanol for 48 hours. The extracting vessel was continuously agitated at an interval of 6hours to aid absolute extraction. The filtration of the mixture was done using filter paper to obtain *Garcina kola* bark extract.

The Synthesis of Ag-Cu nanoparticles

About 5 mL of *Garcina kola* bark extract was added to an Erlenmeyer flask containing 25 ml of 1mM silver nitrate solution and 25 ml of 1mM aqueous copper sulphate solution. The yellowish brown colour of the content of the Erlenmeyer flask changes to reddish brown when *Garcina kola* extract was added. The Erlenmeyer flask and its content was placed on a shaker with to aid proper agitation for 7 hours after which a brownish red colouration was observed affirming a completion reaction and formation of Ag-Cu nanoparticles. Similar colour changes have been reported in previous studies [23, 24].

Characterization of synthesized Ag-Cu Nanoparticles

The surface plasmon peak of the Ag-Cu nanoparticle synthesized was monitored with UV-vis spectrometer ((UV-245 Shimadzu)). Few drops of the neat solution of the biosynthesized Ag-Cu nanoparticle was placed in a quartz cell of 1cm path length and was scanned in the range of 200-700 nm.

The FT-IR analysis were carried out with FTIR spectrophotometer (Scimitar Series FTS 2000 Digilab) The *Garcina kola* extract and the Ag-Cu nanoparticles were scanned as neat in the region of 500 to 400cm^{-1} at a resolution of 4 cm^{-1}.

The morphological study of the Ag-Cu nanoparticle was determined with Scanning Electron Microscopy SEM (Zeiss GeminiSEM 5000). The dispersion of the nanoparticles in acetone was followed by placing the suspension on the sample holder (carbon specimen). TEM (JEOL 200CX) was equally used in determining the size of the nanoparticle. A drop of the sample was dropped into holey carbon copper grid of 200-mesh. The excess liquid on the grids was gotten rid of and the grids were air-dried before examination.

The investigation of the elemental composition of the biosynthesized Ag-Cu nanoparticles were monitored with an energy dispersive x-ray analyzer (Oxford-Horiba Inca X-Max 50 instrument). The phase and crystal structure of Ag-Cu nanoparticles were characterized using X-Ray diffraction. The scan of theta 2 was taken in the range of 30-80^0. Williamson-Hall procedure was adopted in the estimation of the average crystalline size by considering the most projecting peak (111). The morphology of the surface of Ag-Cu nanoparticles was evaluated using a Gemini Supra (DSM 982, 40 VP FESEM).

Antifungal screening of Ag-Cu nanoparticles against test fungal strain

Four clinical isolate fungal strains namely; *Candida tropicalis, Fusarium oxysperium, Candida albican* and *Aspergillus flaws* were used for the investigation of the growth inhibitory effects of Ag-Cu nanoparticles. The synthesized Ag-Cu nanoparticles was tested against the test fungi. Agar disk diffusion test was used for the antifungal screening. 0.3g of previously obtained synthesized Ag-Cu nanoparticles was dissolved 2ml of methanol and to a stock solution of 150 mg/ml which was further diluted to obtain concentrations of 100 and 50mg/ml while 0.2g of Amphotericin-B acting as the controls was dissolved in 2ml of methanol to obtain concentration of 10 mg/ml.

Sterile petri dishes were used for the screening. 25 mL of sterile base agar was transferred to sterile petri dishes using a sterile which was the lower layer

and was left to coagulate. Subsequently 25 mL of Saboraud agar was added as the upper layer. The petri dishes were left to set and were kept in refrigerator at a temperature of 4^0C.

The fungal strains were inoculated onto the solid Saboraud agar slants and were incubated at 37°C for 48 hours. The Fungi from the agar slants were transferred into 2 mL of sodium chloride solution in a sterile glass tube via sterile inoculation loop. The absorbance of the suspensions were adjusted to 0.1 by diluting 1 mL of the each of the suspension with sodium chloride solution. 200 µL of each of the diluted fungal suspensions were evenly distributed on each petri dish and left to dry by opening of the lid for several seconds.

Several equidistance holes were made on the agar surface of the petri dishes by a cork borer of 11mm diameter. 200 µL of 150, 100 and 50 mg/mL of synthesized Ag-Cu nanoparticles and 10 mg/mL of Amphotericin-B were carefully transferred into the properly labelled holes using a dropping pipetted. 200 µL of methanol was used as solvent controls which was unable to inhibit the growth of the fungi. The petri dishes were firstly incubated for 1hour at 4^0C and were later incubated for at 37 °C for 48 hours.

The diameters of the inhibitory zones around the dishes were measured with a transparent ruler and were recorded as zones of inhibition. This experimental process was repeated twice.

Results and Discussion

UV-vis analysis of Ag-cu nanoparticles

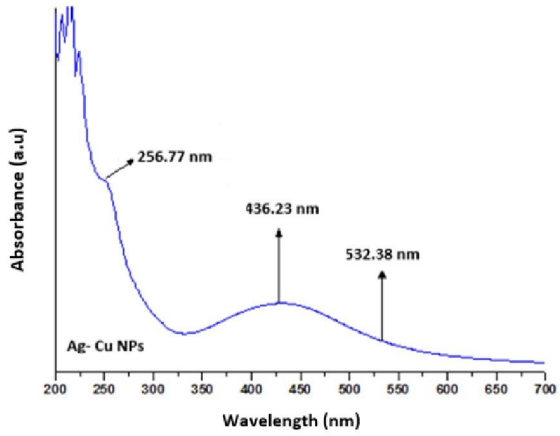

Figure 1: UV-vis spectrum of synthesized Ag-Cu nanoparticles

The UV-vis spectrum of the Ag-Cu nanoparticles showed in Figure 1 indicated an absorption at wavelength 256.77 nm, 436.23 nm, and 532.38nm which correspond to the wavelength of a metabolite in the plant extract, silver and copper respectively which occur as a result of the surface plasmon resonance of Ag-cu nanoparticles. These absorption bands confirmed the rapid bioreduction of the metal ions using *Garcina kola* extract. Similar report has been documented from previous study [25,26].

FTIR analysis of Ag-cu nanoparticles

The FTIR spectra of the extract and Ag-Cu nanoparticles showed in Figure 2a and 2b revealed prominent peaks of absorption wavelength 3331.09 cm^{-1} which suggested the availability of O–H functional group sample. Other observed peaks at 1648.11 to 1633.26 cm^{-1} correspond to the absorption wavelength of denote –C = O, absorption band at wavelength 2090.23- 2091.13 cm^{-1} -O– C- and 2918.23 cm^{-1} alkane (C–H), respectively. The absence of the band at 2940.99 cm^{-1} of in the FTIR spectrum of the extract and the shift in the absorption band at 1648.11 to 1633.26 cm^{-1} of the spectrum of the extract and Ag-Cu is an indication of the bioreduction and stability of the Ag-Cu nanoparticles [25]. However, these bands occurred as a result of some secondary metabolite present in *Garcina kola* extract. This result is similar to previous study [26]

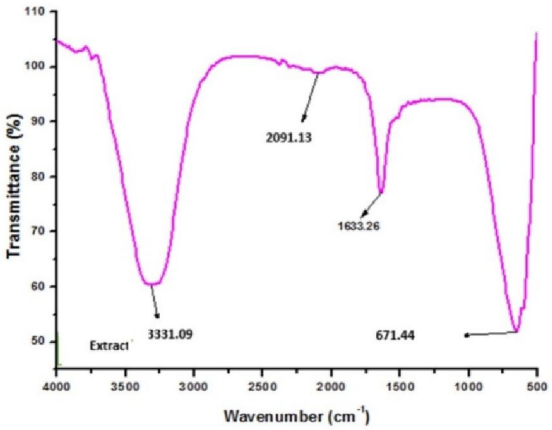

Figure 2a: FTIR spectrum of *Garcina kola* extract.

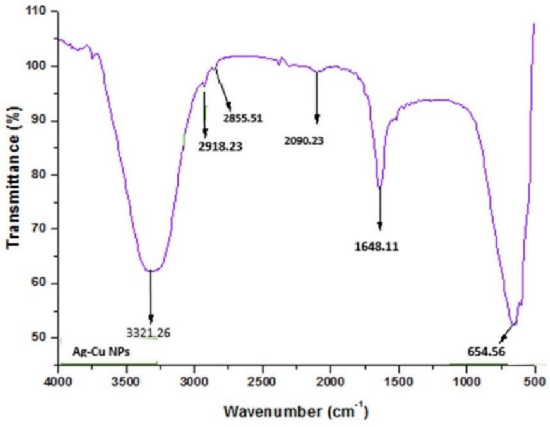

Figure 2b: FTIR spectrum of Ag-cu nanoparticles

SEM Analysis of Ag-Cu nanoparticles

The SEM picture showed that the Ag-Cu nanoparticles are mostly spherical in shape with agglomerates formation as showed in Figure 3. This is possible because of the nature of the capping agents present in the extracts. The shifts and variation in the peaks obtained from FTIR analysis also supported this finding. In addition, the SEM analysis also shown that the Ag-Cu nanoparticles had an average size in the range of 21- 35 nm diameter. These findings agreed with the findings of Pirtarighat et al [28].

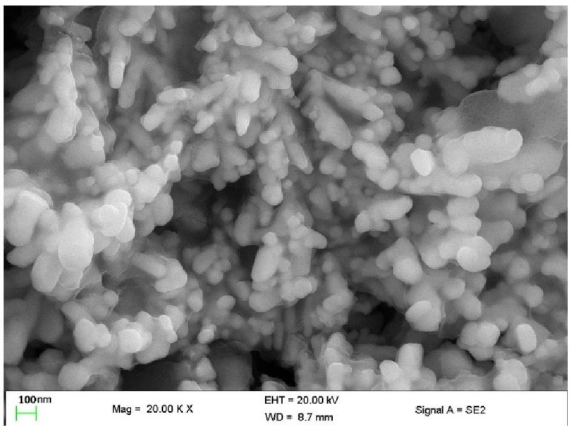

Figure 3: SEM picture of synthesized Ag-Cu nanoparticles

TEM Analysis of Ag-Cu nanoparticles

Micograph obtained from TEM analysis has shown in Figure 4 revealed a dispersed distribution of spherical shapes Ag-Cu nanoparticles with particle size ranging from 21 to 31nm and an average particle size of 16nm. This result is similar to the report of Akintelu and Folorunso [29].

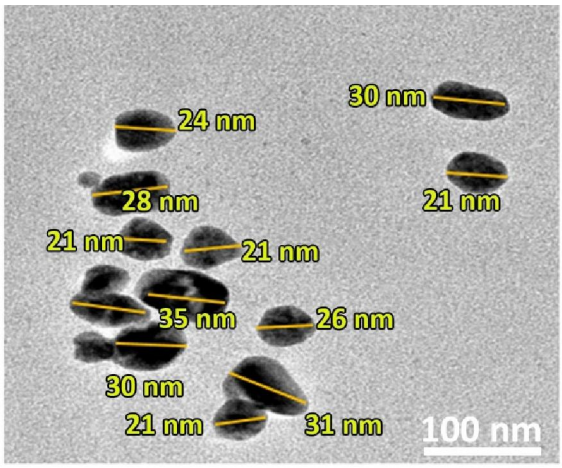

Figure 4: TEM image of Ag-Cu nanoparticles

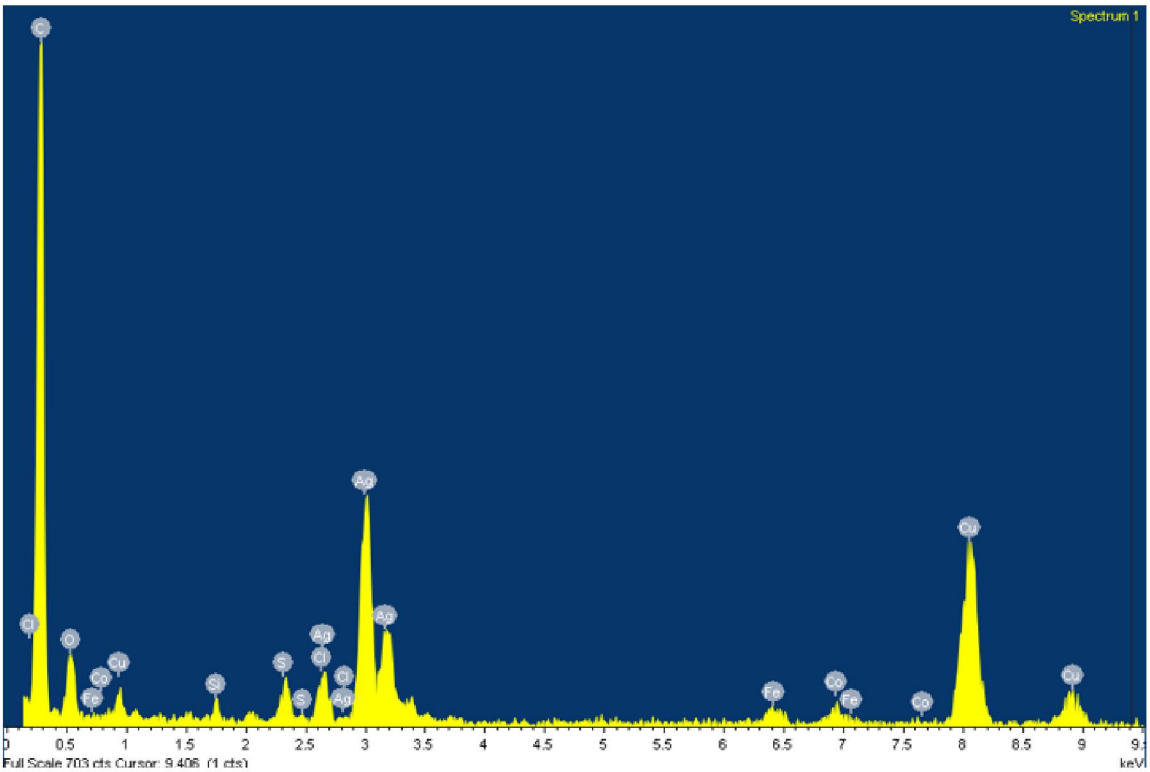

Figure 5: EDX pattern of Ag-Cu nanoparticles

Table. 1.0: Elemental composition of the Ag-Cu nanoparticles

Element	Weight %	Weight % sigma	Atomic %
O	6.70	0.94	32.98
Si	3.56	0.33	3.23
S	4.77	0.48	7.66
Cu	34.18	0.53	8.28
Fe	23.73	0.47	3.05
Co	1.84	0.50	2.80
Ag	45.22	1.30	42.00
Total	100		

EDX Analysis of Ag-Cu nanoparticles

The elemental composition of the Ag-Cu nanoparticles obtained from the EDX spectrum of the Ag-Cu nanoparticles showed in Figure 5 revealed the presence of silver, copper, iron, oxygen, silicon, sulphur, cobalt. Also Table 1.0 showed the percentage weight of the elements.

The signal of silver and copper from the EDX spectrum confirmed the formation of Ag-Cu nanoparticles. Among these elements silver and copper had higher weight percentage. These signals might have originated from the biomolecules bound to the surface of the Ag-C u nanoparticles.

Nanoparticles synthesized using plant extracts has been reported to contain organic material found in the plant which has the tendency of been stable for a month after synthesis [30]. This suggested that other elements found in the EDX spectrum aside silver and gold might be from the *Garcina kola* extract. This findings agrees with the findings of previous researchers [31]

X-Ray diffraction Analysis of Ag-Cu nanoparticles

The crystal structure of the synthesized Ag-Cu nanoparticles at 138^0C obtained from the XRD pattern showed in figure 6 indicated the diffraction peaks for silver and copper at 2θ as 38^0 (111), 44^0 (200), 64^0 (220) and 43^0 (111), 50^0 (200), 74^0 (220) respectively as showed in Figure. These diffraction peaks correspond to the peaks obtained from the study of Hikmah et al. [32]. It can be clearly stated that Ag and Cu phases are the only phases identified on the detected on the XRD pattern of the Ag-Cu nanoparticle synthesized. This therefore suggested that Cu-Ag nanoparticles can be safely synthesized using *Garcinia kola* bark.

The XRD pattern also revealed Ag-Cu nanoparticle synthesized are crystalline in nature and they are mixtures of spherical and hexagonal structures. This findings correspond to the findings of Antonio et al. [33].

The result obtained from the Scherrer's calculation using the prominent peaks of the synthesized Ag-Cu nanoparticles gotten from the XRD pattern shows that the crystallite size for silver ranges from 22 to 29 nm and that of for copper was in the range of 29 to 32 nm, as shown in Table 2.

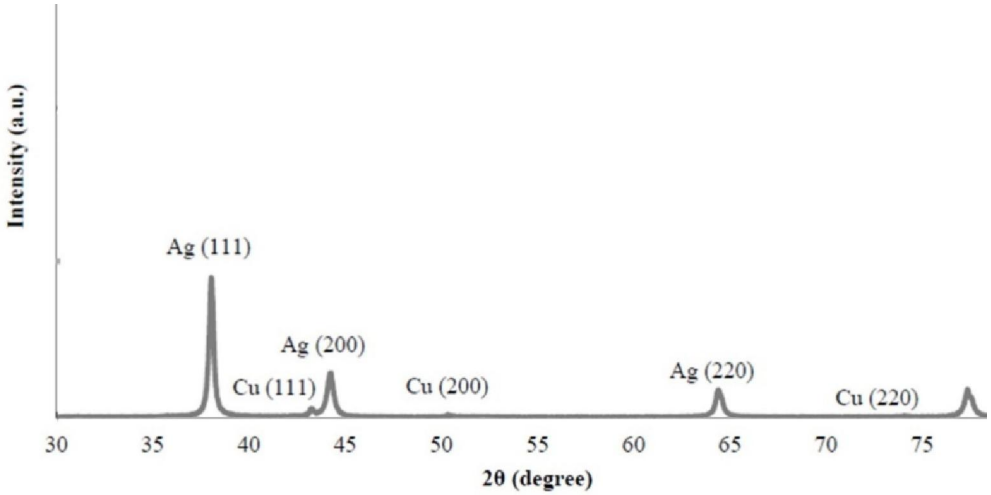

Figure 6: X-Ray diffraction pattern of Ag-Cu nanoparticles

Table 2. XRD parameters for synthesized Ag-Cu nanoparticles

Element	2Θ	Intensity	FWHM	Crystal size	h k l
Ag	38	100	0.29	29	1 1 1
Ag	44	38.2	0.37	22	2 0 0
Ag	64	26	0.4	24	2 2 0
Cu	50	1.7	0.30	29	2 0 0
Cu	74	1.1	0.35	30	2 2 0
Cu	43	5.4	0.29	32	1 1 1

Where FWHM = full width at half maximum

Antifungal screening of synthesized Ag-Cu nanoparticle from *Garcinia kola*

The zones of inhibition demonstrated by the Ag-Cu nanoparticle against *Candida tropicalis, Fusarium oxysperium, Candida albican* and *Aspergillus flaws* were illustrated in Table 1. The zones of inhibition of the Ag-Cu nanoparticle at 150, 100 and 50mg/ml ranges from 20 to 13mm, 13 to 7mm and from 10 to 4 mm respectively. The inhibitory zones displayed by Amphotericin-B against the test fungi ranges from 29 to 20mm. The inhibitory zones displayed by this Ag-Cu nanoparticle correspond to the study of Oyebamiji et al [34].

It was observed that at high concentration the antifungal activity of the Ag-Cu nanoparticle against test fungi was more effective due to higher inhibitory zones. Among the test fungal strains *Candida tropicalis* displayed the highest resistance against the activity of Ag-Cu nanoparticle while *Aspergillus flaws* exhibited most susceptibility toward the activity of the Ag-Cu nanoparticle.

The order of the antifungal activity of the Ag-Cu nanoparticle against test fungi was *Aspergillus flaws* > *Candida albican* > *Fusarium oxysperium* > *Candida tropicalis*. This is an indication that Ag-Cu nanoparticle can be beneficial in the development of novel antifungal agents that will be effective for the treatment of fungal infection. The antifungal property of Ag-Cu nanoparticle was in line with previous study of (Folorunso et al 2019).

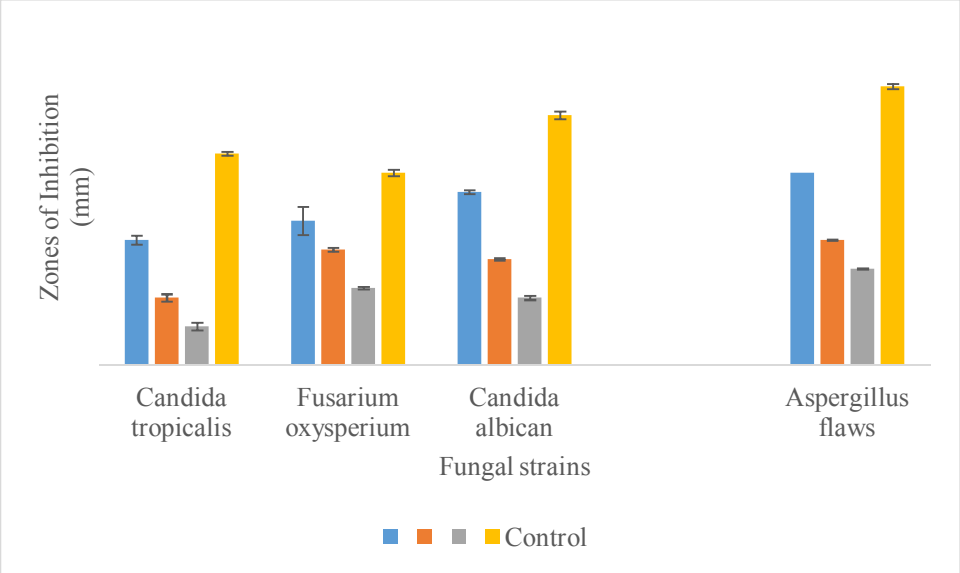

Figure 7: Antifungal screening of the of Ag-Cu nanoparticle.

Conclusion

The analytical characterization of the synthesized nanoparticle confirmed the formation and stabilization of non-toxic and eco-friendly Ag-Cu nanoparticles which signified the efficiency of the approach adopted for the synthesis of the nanoparticle.

Also, the promising result obtained from the antifungal investigation of Ag-Cu nanoparticles confirmed its application as ingredient of high relevance needed in the production of effective novel antifungal agent. At high concentration, the Ag-Cu nanoparticles showed activity comparable to that of the control.

The analytical procedure of this study can be modified by scientist or pharmaceutical professionals and included in the pharmacopeia as procedure for the production of antifungal agents from natural products.

Further research on the isolation, characterization, identification and structural elucidation of the compounds responsible for such antifungal activity is recommended as it could be vital for the design and production of effective antifungal agents that can be used alone or in combination with other orthodox antibiotics to reduce or prevent challenges associated with fungal infection.

Declaration

This manuscript has no competing interests, all the authors have consent for publication of this manuscript and all the authors contributed equally.

References

1 Theuretzbacher U, Mouton JW. Update on antibacterial and antifungal drugs - Can we master the resistance crisis? Curr Opin Pharmacol. 2011;11: 429–32.

2 Walsh T, Toleman M. The emergence of pan-resistant gram-negative pathogens merits a rapid global political response. J Antimicrob Chemother. 2012;67:1–3.

3 Awouafack MD, McGaw LJ, Gottfried S, Mbouangouere R, Tane P, Spiteller M, Eloff JN. Antimicrobial activity and cytotoxicity of the ethanol extract, fractions and eight compounds isolated from Eriosema robustum (Fabaceae). BMC Complement Altern Med. 2013; 13:1.

4 Srivastava J, Chandra H, Nautiyal AR, Kalra SJS: Antimicrobial resistance (AMR) and plant-derived antimicrobials (PDAms) as an alternative drug line to control infections. Biotech. 2013;4:451–60.

5 Lowy F. Antimicrobial resistance: the example of Staphylococcus aureus. J Clin Invest. 2003; 111:1265–73.

6 Andersson DI, Hughes D. Persistence of antibiotic resistance in bacterial populations. FEMS Microbiol Rev. 2011;35:901–11.

7 Ahmad A, Senapati S, Islam Khan M, Kumar R, Ramani R, Srinivas V, Sastry M 2003 Intracellular synthesis of gold nanoparticles by a novel alkalotolerant actinomycete, *Rhodococcus* species *Nanotechnology* 14 824-828.

8 Husseiny M I, Abd El-Aziz M, Badr Y, Mahmoud M A 2006 Biosynthesis of gold nanoparticles using *Pseudomonas aeruginosa Spectrochimica Acta Part A* 67 1003–1006.

9 Mandal D, Bolander M E, Mukhopadhyay D, Sarkar G, Mukherjee P 2006 The use of microorganisms for the formation of metal nanoparticles and their application *Appl Microbiol. Biotechnol.* 69 485-492.

10 Du L, Jiang H, Liu X, Wang E 2007 Biosynthesis of gold nanoparticles assisted by *Escherichia coli DH5α* and its application on direct electrochemistry of haemoglobin *Electrochem. Commun*. 9 1165-1170.

11 Khan A K, Rashid R, Murtaza G, Zahra A 2014 Gold nanoparticles: synthesis and application in drug delivery *Trop. J. Pharm. Res*. 13 7 1169-1177.

12 Anju_A, Khushbu_G, Tejpal S_C and Dipti_V (2018) Biogenic Synthesis of Copper and Silver Nanoparticles Using Green Alga Botryococcus braunii and Its Antimicrobial Activity. Bioinorganic Chemistry and Applications 1-9 doi.org/10.1155/2018/7879403.

13 Thakur S, Sharma S, Thakur S, and Rai R, (2018.)"Green synthesis of copper nano-particles using Asparagus adscendens roxb. Root and leaf extract and their antimicrobial activities," International Journal of Current Microbiology and Applied Sciences, (7) 4, 683–694.

14 Mohanpuria P, Rana N K 2008 Biosynthesis of nanoparticles: technological concepts and future applications *J. Nanopart Res* 10 507-517.

15 Fernebro J. Fighting bacterial infections - Future treatment options. Drug Resist Updat. 2011;14:125–39.

16 Fennell CW, Lindsey KL, McGaw LJ, Sparg SG, Stafford GI, Elgorashi EE, Grace OM, Van Staden J. Assessing African medicinal plants for efficacy and safety: pharmacological screening and toxicology. J Ethnopharmacol. 2004;94:205–17.

17 McGaw LJ, Lall N, Hlokwe TM, Michel AL, Meyer JJM, Eloff JN. Purified compounds and extracts from Euclea species with antimycobacterial activity against Mycobacterium.

18 Terashima K, Takaya, Niwa M (2002). Powerful antioxidative agents based on garcinoic acid from Garcinia kola. Bioorganic Medicinal Chemistry 10(5):1619-1625.

19 Onayade OA, Looman AMG, Scheffer JJC, Gbile ZO (1998). Lavender lactone and other volatile constituents of the oleoresin from seeds of Garcinia kola Hechel. Flavour fragrance Journal, 13(6):409-12.

20 Seanego CT, Ndip RN (2012). Identification and antibacterial evaluation of bioactive compounds from Garcinia kola (Heckel) seeds. Molecules 17(6):6569-6584.

21 Lacmata ST, Kuete V, Dzoyem JP Tankeo SB, Teke GN, Kuiate JR, Pages JM (2012). Antibacterial activities of selected cameroonian plants and their synergistic effects with antibiotics against bacteria expressing MDR phenotypes. Evidence Based Complementary and Alternative Medicine. doi: 10.1155/2012/623723

22 Krishnaraj C, Jagan EG, Rajasekar S, Selvakumar P, Kalaichelvan PT, Mohan N (2010) Synthesis of silver nanoparticles using Acalypha indica leaf extracts and its antibacterial activity against water borne pathogens. Colloids Surf B: Biointerfaces 76:50–56.
23 Lalitha A, Subbaiya R, Ponmurugan P (2013) Green synthesis of silver nanoparticles from leaf extract Azhadirachta indica and to study its anti-bacterial and antioxidant property. Int J Curr Microbiol App Sci 2:228–235.
24 Singhal G, Bhavesh R, Kasariya K, Sharma AR, Singh RP (2011) Biosynthesis of silver nanoparticles using Ocimum sanctum (Tulsi) leaf extract and screening its antimicrobial activity. J Nanoparticle Res 13:2981–2988.
25 Banerjee P, Satapathy M, Mukhopahayay A and Das P Leaf extract mediated green synthesis of silver nanoparticles from widely available Indian plants: synthesis, characterization, antimicrobial property and toxicity analysis. Bioresources and Bioprocessing 2014 1:3.
26 Sre, P.R., Reka, M., Poovazhagi, R., Kumar, M.A., Murugesan, K.: Antibacterial and cytotoxic effect of biologically synthesized silver nanoparticles using aqueous root extract of Erythrina indica lam. Spectrochim. Acta, Part A 135, 1137–1144 (2015)
27 Song JY, Kim BS (2009) Rapid biological synthesis of silver nanoparticles using plant leaf extract. Bioprocess Biosyst Eng 32:79–84.
28 Folorunso A, Akintelu S, Oyebamiji AK, Ajayi S, Abiola B, Abdusalam I, Morakinyo A, (2019) Biosynthesis, characterization and antimicrobial Activity of gold nanoparticles from leaf extracts of Annona muricata Journal of Nanostructure in Chemistry. 1-11.
29 Dada, A.O., Inyinbor, A.A., Idu, I.E., Bello, O.M., Oluyori, A.P., Adelani- Akande, T.A., Okunola, A.A., Dada, O., 2018b. Effect of operational parameters, characterization and antibacterial studies of green synthesis of Silver Nanoparticles, using Tithonia diversifolia. Peer J 6, e5865.
30 Pirtarighat. S, Maryam G, Saeid B (2019) Green synthesis of silver nanoparticles using the plant extract of Salvia spinosa grown in vitro and their antibacterial activity assessment Journal of Nanostructure in Chemistry 9:1–9.
31 Hikmah N, Idrus N F, Jai J and Hadi A 2016 Synthesis and characterization of silver-coppercore-shell nanoparticles using polyol method for antimicrobial agent IOP Conf. Ser.: Earth Environ. Sci. 36 012050.
32 Akintelu SA and Folorunso AS, Characterization and Antimicrobial Investigation of Synthesized Silver Nanoparticles from Annona muricata Leaf Extracts J Nanotechnol Nanomed Nanobiotechnol 2019, 6: 022.
33 Antonio Z, Annalaura I, Stefano N and Roberto L (2019) Green Synthesis of Silver Nanoparticles Using Bilberry and Red Currant Waste Extracts Processes 2019, 7, 193 1-11.

12/23/2019