RESEARCH ARTICLE

Green Synthesis and Characterization of Bimetallic (Ag-Cu) Nanoparticles from Leaf extract of Celtis integrifolia (Hackberry) and its Antimicrobial Activity

Nasiru Pindiga Yahaya*[*, 1], Wilson Danbature Lamayi [1], Suleiman Mohammed Bawaji [1]

[1] *Department of Chemistry Gombe State University, Gombe Nigeria*

ARTICLE INFO

Article History:

Received 2022-03-25

Accepted 2022-08-06

Published 2023-02-15

Keywords:

Celtis integrifolia,

Bimetallic Nanoparticles,

Antimicrobial Activity.

ABSTRACT

The (Ag-Cu) bimetallic nanoparticles were synthesized from the leaf extract of Celtis integrifolia using the silver nitrate ($AgNO_3$) copper chloride ($CuCl_2$) as metal precursors. The synthesized bimetallic nanoparticles were characterized using UV-visible spectrophotometry, the Celtis extract shows highest peak at 300nm due to plasma vibration and excitation of bio reduction capping agent Ag-CuNPs that shows peak at 300nm which is due to surface Plasmon vibration, indicating the formation of bimetallic nanoparticles due to the excitation of the surface Plasmon vibration and phytochemical constituent present in the extract. FT-IR was used for the identification of the functional groups present; SEM showed the morphology of the bimetallic Ag-CuNPs is partially merely crystalline and spherical in nature. XRD analysis of Ag-CuNPs showed face centered cubic structure (FCC) with the average particle size of 54.42. The (Ag-CuNPs) bimetallic nanoparticles showed significant antibacterial and anti-fungal activity when tested against Escherichia-coli, pseudomonas aureginosa (Gram-negative), staphylococcus aures, Klebsella Pneumonia (Gram-positive) bacteria's and two fungi Aspagillus Niger and Candida using Augomentine and Fulcin as control drugs.

How to cite this article

Yahaya N.P., Lamayi W.D. Bawaji S.M., Green Synthesis and Characterization of Bimetallic (Ag-Cu) Nanoparticles from Leaf extract of Celtis integrifolia (Hackberry) and its Antimicrobial Activity. J. Nanoanalysis., 10(1): 419-428, Winter 2023. 10.22034/jna.2022.1955547.1298

INTRODUCTION

Hackberry (Celtis) is a group of medium-sized, deciduous trees with long ovately-shaped leaves, clusters of small fuzzy spring flowers, and small purple fruits locally available. Hackberry trees are hardy trees that withstand many conditions, including drought, wet soil, strong winds, and air pollution [1]. Nano-Chemistry has been the topic of concern from past few years and recent advances have made it more applicable in both industries pharmaceuticals and academia [1-3]. Usages of nanoparticles in various applications make them impotent, otherwise impossible. Nanoparticles are formed in deferent ways

such as bi-metallic nanoparticles which we are interested in this study [2]. Metal nanoparticles can be classified based on their origin, dimension as well as structure and their synthesis process can be physical, chemical as well as greener route i.e. biological [4]. Bimetallic nanoparticles are of more interest than metal nanoparticles as they show better optical, electrical and medical applications due to their peculiar mixing patterns and synergistic effects of two metal nanoparticles that form bimetallic nanoparticles [5]. Their synthesis from plants and microbes is of great importance, because it is environmentally benign and friendly [2]. Application of bimetallic nanoparticles as catalysts is most promising one due to large surface area and small size, but bimetallic nanoparticles as biosensors, antimicrobials, in ground water remediation and for drug delivery has promising values [6]. Nanoparticles envelop frameworks, whose size is above sub-atomic measurements and under naturally visible ones (i.e. > 1 nm and < 100 nm). Nanoparticles are of several types and can be classified on the basis of: Origin, Dimension, and Structural organization [**Figure 1**].

Nano-chemistry speaks to the plan, creation and use of materials at atomic, molecular and macromolecular level, keeping in mind that the end goal is to deliver new nano-sized materials [7-8]. As of late, the meeting of nano sized scale advancements and biological techniques has paved way for the new field of nano biotechnology. It is generally centered on the creation, control, and utilization of materials at the nanometer scale for cutting edge biotechnology [8]. Green syntheses of nanoparticles are more eco-friendly and nontoxic and more stable than chemical synthesis. Biosynthetic processes for nanoparticles would be more useful if nanoparticles were produced extracellular using plants or their extracts and in a controlled manner according to their size, dispersity and shape. Plants have been used to support mankind to sustain its well-being since the dawn of medicine. They are the most cherished bio resources of both traditional and modern drugs and have been the basis of health preservation and care [1]. Anarado *et al,* [9] suggested majority of the species of the genus Celtis have been found to possess medicinal values. C. Africana, C. australis and C. occidentals have been reported to possess phyto-compounds that exhibit numerous important pharmacological activities such as antioxidant and cytotoxic properties, [10]. C. Integrifolia is implicated in the management of epilepsy, mental disorder, weakness, as pain killer, treatment of Chicken pox, Measles, Gout, Ecbolic, treatment of Diarrhea, Sore throat [11]. Cancer wound healing, bleeding, Spices and Aphrodisiac in Northern Nigeria. With new discovery in science of the adverse effects of synthetic drugs, people are clamoring for the days when medication was comprised solely of natural products, which have low toxicity and no known side effects. Hence there is upsurge in the use of herbal remedies. There is therefore the need for a thorough scientific evaluation to validate the supposedly therapeutic effects of some of these medicinal plants [12]. Pindiga *et al,* [4] Suggested nano technology is gaining tremendous attention in the present century due to its expected impact on many important areas such as medicine, energy, electronics, and space industries. In this context, biosynthesis of nanoparticles is a reliable, eco-friendly and important aspect of green chemistry approach that intersects biotechnology and nanotechnology [13]. Research has shown that microbes and plants are supportive in the biosynthesis of nanoparticles with good surface and size characteristics [12].

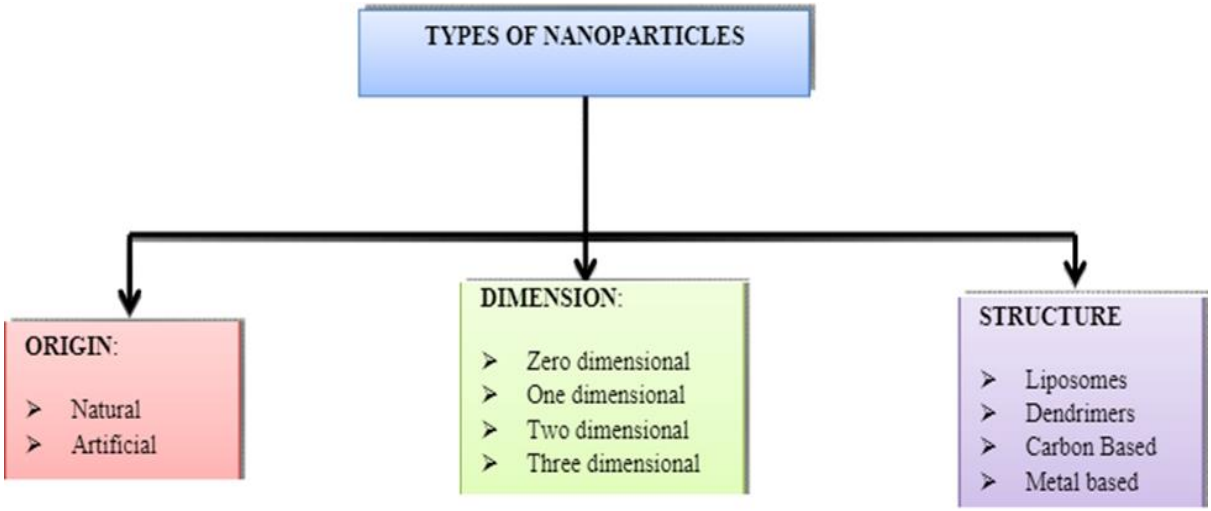

Figure 1. Types of Nanoparticles Flowchart

EXPERIMENTAL

MATERIALS

Reagent and chemicals

All the chemicals were of analytical grade and were used as purchased without further purification. They are as follows Silver nitrate ($AgNO_3$) Cadmium Chloride ($CdCl_2$) Cupper (ii) chloride dehydrate ($CuCl_2$) Mercury (ii) oxide ($HgCl_2$) Mangnase dioxide (MnO_2) Chromium (iii) chloride hexahydrate ($CrCl_36H_2O$) Ehrlich reagent (paradimethyl amino Benzaldehyde absolute alcohol hydrochloric acid). Hydrogen Peroxide, Miller Hilton broth, Nutrient Agar, Potatodestros Agar, Sterile cotton, dimethyl sulphuroxide, dimethyl hydrochloride, crystal violet, lugols iodine, acetone, ethanol oil immersion, human plasma, Fulcin, Augomentine, Distilled water and *Celtis Integrifolia* leaf extract.

Apparatus\ Equipment

The apparatus used are magnetic stirrer, Heating mantle, FT-IR Machine (PerkinElmer), spectrum version 10 03 09, UV-visible spectrophotometry machine (Jenway 6705), SEM machine (Hitachi 4160), XRD machine, Incubator, staining rags, autoclave, microscope, wooden mortar and pestle, Petridish.

Sample Collection and Authentication

The leaves of *Celtis integrifolia* plant were collected from Ndala-diyo (Jalam district) Dambam L.G.A of Bauchi State, The plant material was identified and authenticated by a specialist, given voucher number GUH 192 and deposited at the herbarium of Department of Botany, Gombe State University Gombe, Nigeria. The Fresh matured leaves of the plant were collected.

Sample preparation

Fresh *celtis integrifolia* (Hackberry) leaves were washed for three times with tap water and then rinsed with distilled water to remove adhered dirty and then ground using a wooden mortar and pestle with addition of distilled water thereby obtaining a fine homogeneous paste sample, [14].

METHODS

SYNTHESIS OF Ag-Cu BIMETALLIC NANOPARTICLES (Ag-CuNPs) [FIGURE 2]

By using slightly modified method of [15]. For the synthesis of Ag-Cu bimetallic nanoparticles, the prepared Ag-Cu solution were added drop wise collectively into the plant extract by the ratio of 1:9 (that is 10ml extract and 90ml bimetallic precursor) with constant stirring at 60^0C for 60 minutes using magnetic stirrer. Within the first 15 minutes the color change was observed which indicated the formation of

nanoparticles and the mixture was allowed to settle for 24h after that it was decanted and the collected nanoparticles were dried at 100°C for 2h and ground into powder for further analysis.

CHARACTERIZATION

UV-Visible spectroscopy analysis was done to confirm the formation of nanoparticles and observe the extract Plasmon vibration and excitation. The wavelength varied at regular wavelengths of 200nm, 300nm, 400nm, 500nm, 600nm, 700nm and 800nm respectively.

FT-IR analysis was done on leaf extract and the synthesized Ag-Cu, bimetallic nanoparticles for determination of the functional group present in the samples.

The SEM analysis was done in order to determine the morphology, size and the composition of the element present in the synthesized Ag-Cu, bimetallic nanoparticles. It is done using powdered sample.

XRD analysis was done to find out the average crystalline size. The Debye Scherer equation was used to calculate the average crystalline size, [10]. The Debye Scherer equation as follows$D = K\lambda/\beta COS\theta$

Where D= Particles size = 0.94

K= Constant volume

λ= X-ray wavelength (0.154nm)

β=Line broadening at half the maximum intensity

θ= Braggs angle (in degree).

ANTIMICROBIAL ACTIVITY

The antimicrobial activity of the plant extract and synthesized bimetallic nanoparticles were carried out using the agar well method to test the antimicrobial activity of the plant extract and the Green synthesized Ag-Cu, on two gram negative bacteria's *Escherichia-coli, pseudomonas aureginosa*, Two gram positive bacteria *Staphylococcus aureus, klebsella pneumonia* and two fungus *Aspagillus Niger and Candida albicans* as well. This is conducted by creating 6mm hole in the prepared agar (media) inside the petri dish. The organism was inoculated all over the surface of the petri dish and the synthesized drugs (bimetallic nanoparticles) were also inoculated in to each hole with a control drug at the center. It was then be incubated overnight at 37°C after which the zone of bacterial and fungi growth inhibition was measured in millimeters (mm), [16].

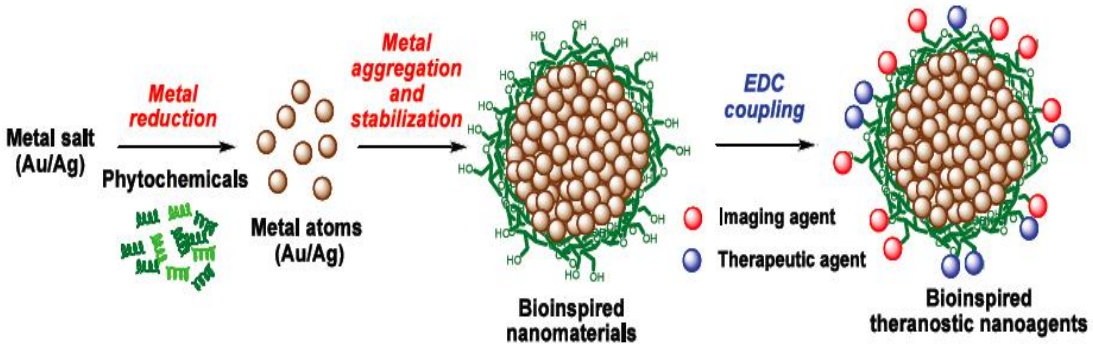

Figure 2. General synthetic pathway of nanoparticles

RESULTS AND DISCUSSION

UV-visible spectroscopy

The UV spectrophotometer was used to monitor the synthesis of nanoparticles as it gives us information about the path of synthesis. When plant extract was mixed with silver salt solution, the reduction of Ag^+ ion was reduced due to the presence of active molecules present in the plant extract which gives light green color to the solution with absorption in UV-Vis spectroscopy near 435 nm but when copper salt solution was mixed the spectrum became different due to the formation of composite structure which incorporates Cu^+ as second center in the nanostructures. Ag-Cu resulted during the green synthesis of bimetallic silver and copper bimetallic nanoparticles, the color change was observed as the first indication of the formation of bimetallic nanoparticles Table 1. The Ag color is white and Cu color is blue and the Ag-Cu bimetallic nanoparticles changes to dark green, [13].

The *Celtis extract* Figure 3 showed the highest absorbance at 300nm which is due to the surface Plasmon vibration and bio-reduction and the Ag-CuNPs nanoparticles (Ag-CuNPs). Figure 4 showed the highest absorbance at the peaks of 300nm and 700nm which is also due to the surface Plasmon vibration and excitation of bio-reduction and capping or stabilizing agent present in the leaf extract. This corresponded with the literature of [17] which showed the highest absorbance peaks at 300nm and 650nm and also suggested that, this variation depends on the reducing agent and *the* type of metal salt used as a precursor.

FT-IR Spectroscopy

IR spectroscopy was done using plant material as well as synthesized bimetallic nanoparticles. Table 2 exhibits that the functional groups present in the plant material have been transferred to nanoparticles [Figure 5 and 6]. Many functional groups such as hydroxyl, Alkene group and multiple bonds are present in the nanoparticles [Figure 5 and 6], see similar to [18-19].

SEM Analysis

The SEM result [Figure 7] of bimetallic nanoparticles shows that the Ag-Cu nanoparticles are merely crystalline and partially spherical in nature which is similar to [20].

XRD Analysis

Table 1. Uv-visible Spectrophotometry Analysis Results

SAMPLE	ABSORBANCE
Celtis extract	300nm
Ag-CuNPs	300nm, 700nm

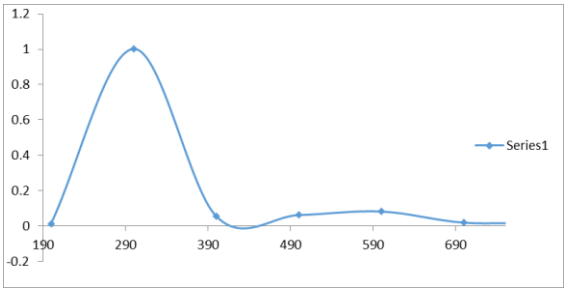

Figure 3. UV-Visible spectrum for Celtic Integrifolia extract

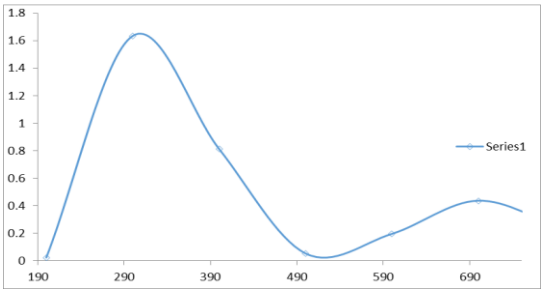

Figure 4. UV-Visible spectrum for Cd-MnNPs

The XRD result [**Fig 8]** of the bimetallic Nanoparticles was done to determine the average crystalline size using Derby Scherer equation. Ag-Cu nanoparticles shows the average crystalline size of 54.42nm, with peaks 2 Θ = 25.22^0, 27.95^0, 29.17^0 and 35.42^0, with respect to the plane of (111), (210), (211) and (311), [21].

Table 2. FT-IR Spectroscopy Analysis Results Figure 3 and 4.

Samples	***Celtis extract***			**Ag-CuNPs**		
Absorption bands	3546cm^{-1}*	1037 cm^{-1}	1033 cm^{-1}	3444 cm^{-1}	3444 cm^{-1}	1622 cm^{-1}
Functionalgroups		C-C	C-O	O-H	O-H	C=C

Stretching due to: O-H = Alcohols, C-C = Aromatics, C-O = Carboxylic acid, *Alcohols and phenols

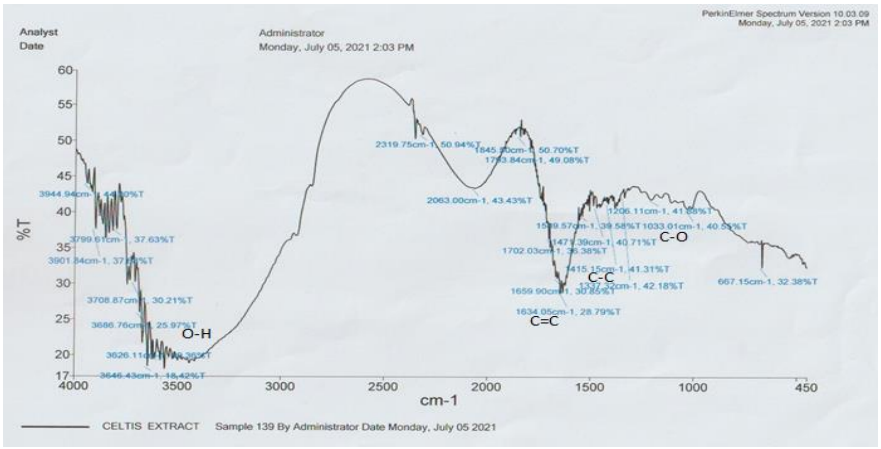

Figure 5. Showing the FT-IR spectrum of the Celtis leaf extract

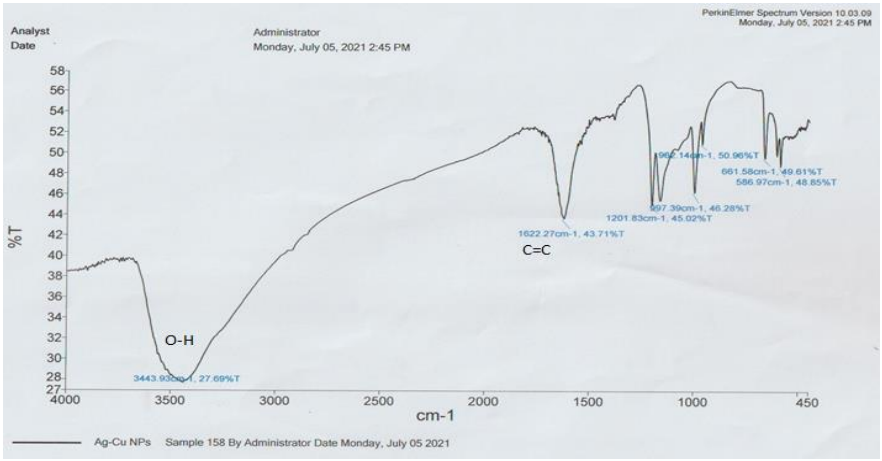

Figure 6. Showing the FT-IR spectrum of the Ag-CuNPs

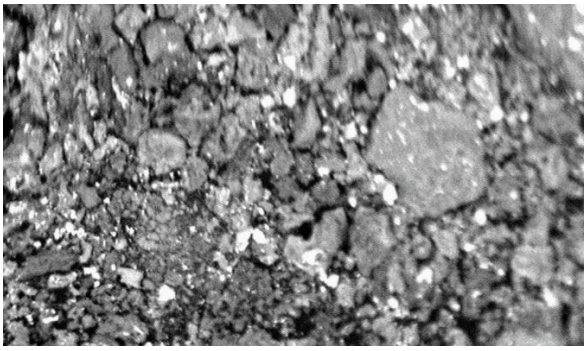

Fig 7. SEM result of Ag-CuNPs

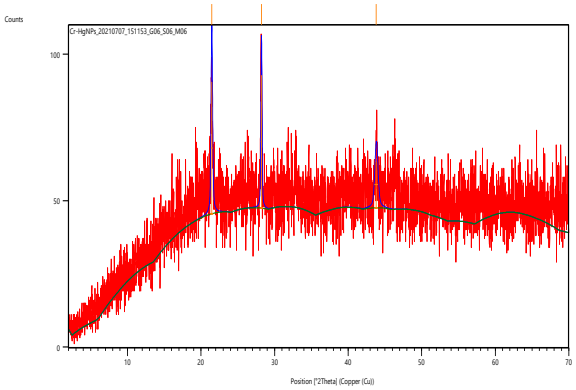

Figure 8. XRD result of Ag-CuNPs

ANTIMICROBIAL ACTIVITY OF LEAF EXTRACT AND BIMETALLIC NANOPARTICLES OF Ag-CuNPs

The plant extract showed that, the *Escherichia –coli* showed an increase in the bacterial growth inhibition with an increase in concentration of the plant extracts. *Pseudomonas aureginosa* showed an increase in inhibition of the bacterial growth with an increase in the concentration of the plant extract which is almost closed to that of the control drug. Staphylococcus aureus showed an increase in the inhibition with an increase in the concentration of plant extract. Klebsella pneumonia exhibited an increase in bacterial growth with an increase in the concentration of the plant extract which was also almost closed to that of the control drug.

The antibacterial activity test result for silver-copper nanoparticles (Ag-CuNPS) showed that, the *Escherichia-coli* revealed an increase in the bacteria's growth inhibition with increase in concentration of the nanoparticles. It showed the highest inhibition at 300ug/ml and started to decrease with increase in concentration. *Pseudomonas aureginosa* showed an increase in the bacterial growth inhibition with increase in concentration of the synthesized drug (NPS). *Staphylococcus aureus* shows an increase in bacterial growth inhibition with increase in concentration of the synthesized drug. *Klebsella pneumonia* also shows an increase in bacterial growth inhibition with increase in concentration of the nanoparticles which is closed to the controlled drug (Augmentin) inhibition value (Table 3) [16].

The plant extract *Aspagillus Niger* shows an increase in fungal inhibition with an increase in the concentration of the plant extract, which shows the high rate of inhibition at 500ug/ml and the *Candida.* Showing an increase in the fungal growth as the concentration increases and the highest inhibition was observed at 500ug/ml which shows that as the concentration increases the zone of inhibition increases, [22]. The silver-copper bimetallic nanoparticles (Ag-CuNPs), *Aspagillus Niger* showed an increase in fungal growth inhibition with increase in concentration of the synthesized drug (nanoparticles). It showed the highest fungi inhibition rate at concentration 300µg/ml but it decreased in concentration 400µg/ml and 500µg/ml respectively. This decrease depends on the type of fungi in question. Each fungi has its own activity and resistance to the drug used. The same applies to *candida;* it also showed an increase in fungal growth inhibition with increase in concentration of the nanoparticles (Table 4).

Table 3. Antimicrobial activities, zone of Inhibition in Millimeters (mm)

Test Organism	Concentration in µg/ml					Control
	100	200	300	400	500	300
			Leaf Extract			
E. Coli	7	9	12	15	18	21
P. Aureg	7	9	14	17	21	26
S.Aureu	9	11	15	17	19	26
K.Pneu	12	19	20	22	24	27
			Ag-CuNPs			
E. Coli	6	7	7	8	10	18
P. Aureg	7	8	10	13	18	20
S.Aureu	8	6	8	11	12	22
K.Pneu	7	11	13	14	19	21

Control = Augmentin *E. Coli* -= Escherichia Coli, *P. Aureg* = Pseudomonas Aureginosa, *S.Aureu* = Staphyloccocus Aureus, *K.Pneu* = Klebsella Pneumonia, µg/ml = Microgram per mil

Table 4. Antifungal Activity of The Plant Extract And Bimetallic Nanoparticles Zone of Inhibition (mm)

Test organism	Concentrations in µg/ml					Control (µg/ml)
	100	200	300	400	500	500
			Plant extract			
A.Niger	7	6	7	8	9	17
Candida	6	8	7	9	10	18
			Ag-CuNPs			
A.Niger	8	10	13	11	14	21
Candida	10	13	16	19	22	25

CONCLUSION

This research work entailed the Green synthesis and antimicrobial studies of bimetallic Ag-CuNPs. It can be concluded that the bimetallic nanoparticles were green synthesized from the leaf extract of *Celtis integrifolia* and characterized using UV-visible spectrophotometry, FT-IR, SEM and XRD analysis. The bimetallic nanoparticles showed more antibacterial activity than the plant extracts. It also showed good antibacterial and antifungal activity when tested against gram-positive and negative bacterial *Escherichia-coli, pseudomonas aureginosa, staphylococcus aureus* and *Klebsella pneumonia*, and also two fungi *Aspagillus Niger* and *candida.*

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