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Antimicrobial and Dye Degradation Activities of Eco-Friendly Synthesised Silver-Copper Bimetallic Nanoparticles

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Abstract

Indiscriminate discharge of industrial wastewater without proper treatment and bacterial resistance due to an increase in antibiotic use pose a great threat to the environment and living things. There has been an increase in epidemics associated to various microorganisms and these poses a great threat to global economic development and environmental safety. Although various methods are presently being used to mitigate these effects however, most of these methods have some disadvantages such as high cost, low efficiency and risk of reintroducing more pollutants to the environment. Literature has revealed that nanoparticles, due to their large surface to volume ratio and excellent adsorbent properties, have been applied as antimicrobial agents and in wastewater treatment. In this study, ecofriendly silver-copper nanoparticles (Ag-CuNPs) was successfully synthesized using aqueous leaves extract of *Hierochloe odorata*. The ecofriendly synthesized Ag-CuNPs was first confirmed by the observation of a distinct colour change from deep blue to murky green after addition of the aqueous leaves extract to 0.1 M silver nitrate and copper chloride salt solution. The Ag-CuNPs was examined using UV-vis spectroscopy which revealed a characteristics peak maxima at 305 nm. FTIR analysis revealed the possible biomolecules present in the leaves extract responsible for capping and stabilization of Ag-CuNPs. XRD analysis revealed the crystalline nature of Ag-CuNPs. SEM-EDS revealed the surface morphology and elemental composition. Further dye degradation properties and antimicrobial activity of Ag-CuNPs were also investigated. It was observed that Ag-CuNPs showed antimicrobial activity against *Staphylococcus aureus* and *Salmonela typhi* and dye degradation activity against Congo red dye.

Keywords: Silver-Copper nanoparticles, wastewater, Ecofriendly synthesis, *Hierochloe odorata* antimicrobial, dye degradation

1. Introduction

There has been a surge in bacterial resistance due to an increase in antibiotic use. Globally, there has been an increase in epidemics associated to various microorganisms and these poses a great threat to global economic development and environmental safety [1, 2]. Literature studies has also shown that bacteria resistance has constituted a major drawback in the fight against disease causing microorganisms. Data obtained in 2019 revealed that 1.27 million people died directly from antibiotic-resistant infections, while 4.95 million deaths were indirectly linked to this issue, surpassing the number of deaths caused by AIDS or malaria [3]. Thus, the quest for novel and efficient antibacterial agents cannot be overemphasized.

Nanotechnology has shown significant applications in different fields including agriculture, medicine, energy, climate, chemistry, and consumer goods [4–6]. The most significant properties of nanoparticles is its high surface to volume ratio and these properties accounts for why nanomaterials are widely used in diverse fields of science [7, 8]. Various methods, including physical and chemical methods, have been adopted in the synthesis of nanoparticles but, these physical and chemical methods possess some drawbacks such as high energy consumption and toxic chemical utilization hence the need for more for eco-friendly methods of synthesis [9]. Eco-friendly method of synthesis which also includes the use of plants are straightforward, cost-effective, and environmentally friendly. Plant extract contains bioactive compounds such as polyphenols, alkaloids, steroids, flavonoids, and terpenoids which act as both reducing and stabilizing agents during synthesis of nanoparticles [10].

Hierochloe odorata or Anthoxanthum nitens (commonly known as sweet grass, Manna grass or Vanilla grass) is an aromatic herb native to northern Eurasia and North America. It is considered sacred by many Indigenous peoples in Canada and the United States. It is used as a smudge, in herbal medicine and in the production of distilled beverages. It owes its distinctive sweet scent to the presence of coumarin and possess high antioxidant properties. The name *Hierochloe odorata* is from the Greek and Latin. *Hierochloe* means "holy grass" and *odorata* means "fragrant". Some North American indigenous people burn Sweet Grass in ceremonies to attract good spirits. [39]

Nanoparticles have proven to be very effective against bacterial resistance microbes via direct killing, membrane inhibition, and targeted mechanisms [11]. Inorganic metal nanoparticles offers facile, simple low toxicity and reduced resistance compared to organic based nanoparticles. Their small size, charge, and surface area enhances the control of microorganisms [12]. Silver nanoparticles (Ag NPs) have proven to possess excellent antimicrobial efficacy and minimal resistance tendencies. This is achieved by disrupting the bacterial membranes and interfering with the DNA and protein process by releasing silver ions and taking advantage of reactive oxygen species for effective microbial control [13-15]

Several researchers have inferred that the antibacterial mechanism of Copper nanoparticles (Cu NPs) involves basically the initiation of oxidative stress through the creation of reactive oxygen species, the introduction of metal ions, and the internalization of nanoparticles [16-18]. The efficacy of Cu NPs is equivalent to that of Ag NPs though the difficulty of oxidation during their production and storage processes still persists [19]. Ag-Cu bimetallic nanoparticles have been shown to exhibit a strong synergistic antibacterial potency due to increased cell permeability. Therefore, it was suggested that employing silver-copper alloy nanoparticles would be a cost-effective alternative to traditional antibiotics [20, 21].

Bimetallic nanoparticles (BNPs) have attracted large attention due to their exceptional physical properties, which has aided the integration of different metals to enhance optoelectronic, catalytic, and antimicrobial activities [22, 23]. Experimental findings have also revealed that synthesized Ag-Cu NPs exhibits minimal genotoxicity and sustained antimicrobial potency. Hence, the incorporation of silver and copper nanoparticles into the BNPs system as a promising route for achieving more effective and safer antimicrobial applications have been proposed [24].

Researchers have shown that Ag-Cu NPs address the restrictions of mono-metallic copper and silver nanoparticles, enhancing antimicrobial effects, improving stability, and mitigating nanotoxicity concerns [25, 26]. Bimetallic copper–silver nanoparticles showed catalytic activity for the reduction of nitrophenol into aminophenol [27].

Organic dyes commonly found in wastewater, are harmful to human health and are hazardous to the environment. Congo red is a toxic organic azo dye found in untreated effluent streams discharged from different industries. Currently, nanocatalysis is an area of research in which nanobiocides such as metal nanoparticles is used in the treatment of water and wastewater to remove dye pollutant [28]. The objective of this research work is to eco-friendly synthesize copper–silver bimetallic nanoparticles using aqueous leaves extract of *Hierochloe odorata* and investigate its antimicrobial and dye degradation activities.

The aqueous leaves extract of *Hierochloe odorata* used as capping and stabilizing agent was effective (Due the the wide range of biomolecules present in the extract) in minimizing the average particle size and reduced the tendency of agglomeration of the eco-friendly synthesized nanoparticles. The eco-friendly synthesized bimetallic nanoparticles were characterized using UV-Vis spectroscopy, FTIR, XRD and SEM-EDS analysis. The eco-friendly synthesized bimetallic nanoparticles were verified for their catalytic activity on Congo red dye degradation from aqueous solution and the antimicrobial ability for both gram-positive and gram-negative bacterial strains.

2. Materials and Methods

Materials

Hierochloe odorata leaves were collected and identified from the premises of Federal College of Forestry Jos, Plateau State. Analytical grade Copper (II) chloride ($CuCl_2$ 99.5% pure), silver nitrate ($AgNO_3$), Congo red ($C_{32}H_{22}N_6Na_2O_6S_2$, purity > 97%) were purchased from British Drug House Chemicals Ltd. Poole England, nutrient agar was obtained from Fisher Scientific. The bacterial strains of *Staphylococcus aureus* and *Salmonella typhi* were maintained at the department of Microbiology laboratory of the University of Jos, Jos. All the chemicals and reagents were used as received without further purification.

Methods

Preparation of Aqueous leaves extract of *Hierochloe odorata*

Exactly 10 grams of fresh uninfected leaves of *Hierochloe odorata* was weighed and then washed several times with running tap water and rinsed with deionized water for removal of dust particles. Afterwards, the leaves was chopped into smaller pieces to obtain better yield of the extract. The chopped and weighed material was then transferred into a 500 ml beaker containing 100 ml of deionized water and then heat on a hotplate at 30^{o}C. The mixture was brought to stand for 15 minutes and allowed to cool after which it was filtered using Whatman filter paper No. 1. The filtrate was then kept for onward use.

Preparation of 0.1 M $AgNO_3$ and $CuCl_2$ Salt Solutions

Exactly 1.70 and 1.34 grams of silver nitrate, copper (II) chloride and salts respectively was weighed and transferred into beakers containing 100 ml of deionized water. The mixtures were stirred to ensure that the salt dissolves properly after which both salt solutions were mixed together to obtain a homogenous mixture. The prepared salt solutions was then stored for onward use

Synthesis of Ag-Cu bimetallic Nanoparticles

The bimetallic nanoparticles was synthesized by reducing the precursor salts of silver and copper concurrently using aqueous leaves extract of *Hierochloe odorata*. 20 ml of the freshly prepared leaf extract was added drop wise to 100 ml of 0.1 M $AgNO_3$-$CuCl_2$ solution (50 ml of each salt solution) with constant stirring on a magnetic stirrer. On addition of the extract and after a period of time, a colour change was observed indicating the formation of the bimetallic nanoparticles. The bimetallic nanoparticles formed was centrifuged at 3000 rpm for 15 minutes, washed with distilled water, allowed to dry at room temperature and kept for further analysis.

Characterization

The eco-friendly synthesized bimetallic nanoparticles was subjected to various characterization techniques to determine the size, morphology and composition of the ecofriendly synthesized nanoparticles. UV-Visible measurements of the nanoparticles was recorded using the T70 PG Instruments' UV- Spectrophotometer at different wavelength and absorption and also for the quantification of the aqueous Congo red solution. FTIR (Cary 630 Agilent Technologies) was used to identify the possible biomolecules present in the plant extract responsible for capping and stabilization of the nanoparticles. SEM–EDS analysis was carried out using Quanta 250 FEG instrument to investigate the surface morphology and elemental composition of the nanoparticles. The crystalline structure and particle size of the eco-friendly synthesized nanoparticles was analyzed using X-ray diffraction (XRD Empyrean Malvern Panalytical diffractometer). XRD spectra were obtained in the 2θ angle range of 20°–100°.

Applications

Antimicrobial studies

Antimicrobial susceptibility test of the eco-friendly synthesized Ag-Cu bimetallic nanoparticles was carried out in the microbiology laboratory of the department of Microbiology, University of Jos Plateau state. The agar diffusion test (well diffusion method) was adopted [29]. The analysis was carried out against two strains of bacteria;

Staphylococcus aureus and *Salmonela typhi* respectively. A sterilize molten Muller Hinton Agar (HiMedia) was prepared according to manufacturers' instruction and dispensed into sterile Petri plates previously adjusted at 45 °C in a water bath. The agar plates were then left to solidify under Bunsen burner flame for 60 min. Subsequently, a sterile cotton swab was dipped into 18 hours old bacterial suspensions adjusted to turbidity of 0.5 McFarland Standard. The swab was then used to inoculate the solidified agar by evenly streaking cotton swab over the medium. After 30 min, equidistant wells were cut in the medium using a 6mm diameter flame sterilized cork borer and labeled. A 1ml of the test nano particles of the different concentrations (400, 200, 100 and 50 mg/ml) and controls were then dispensed into the wells and labeled. The plates were incubated at 37 °C for 24 h and diameters of growth inhibition zones around the wells formed were measured in millimeters. Antibiotic gentamicin (10 μg ml^{-1}) was used as positive and 5% DMSO as negative control agents.

Dye Degradation Activity

The photocatalytic dye degradation potential of Ag-Cu NPs was evaluated for congo red. Before the dye degradation experiment, the dye solution (1 mM Congo red solution) at various exposure time intervals was scanned between 200 nm and 800 nm to obtain absorbance maxima to serve as control for the analysis. 1 mg Ag-Cu NPs was added to 5 ml of the dye solution to prepare a 200 ppm solution and exposed to sunlight. The dye degradation/photocatalytic study was performed with the prepared sample, it was scanned regularly between 300 nm and 800 nm at different time intervals of 2 hours, 4 hours, 6 hours and 8 hours to measure the change in intensity via the λmax. The λmax value was used to determine the percentage as well as rate of degradation. This procedure was repeated for 2 mg, 3 mg, 4 mg and 5 mg of Ag-Cu NPs corresponding to 400, 400, 800 and 1000 ppm respectively. The degradation of dyes in term of percentage was calculated as per the following equation (1) [30]:

$$\boldsymbol{Dye\ degradation}\ \% = \frac{\mathbf{Ao - At}}{\mathbf{Ao}}\ \boldsymbol{X\ 100} \quad (1)$$

Where Ao denotes absorbance at zero time; At denotes absorbance at t time.

3. Results and Discussion

The novelty of this study was to utilize the aqueous extract (at room temperature) of the natural biomolecules present in the *Hierochloe odorata* leaves as an efficient reducing, capping and stabilizing agent for the eco-friendly synthesis of Ag-Cu bimetallic nanoparticles which is a facile and green technology means. Furthermore, the efficacy of the eco-friendly synthesized bimetallic nanoparticles was also examined for their potential catalytic activity for dye degradation and antibacterial assay which in turn highlights the potency of the eco-friendly synthesized bimetallic nanoparticles.

Optical Property

Plate 1: (a) Hierochloe odorata aqueous leaves extract (b) $AgNO_3$ & $CuCl_2$ salt solution (c) solution of Ag-Cu nanoparticle

One of the distinct characteristic properties of nanoparticles is their optical properties. The optical property of the bimetallic salt solution of Ag-Cu, *Hierochloe odorata* leaf extracts and the bimetallic nanoparticles of Ag-Cu are shown on plate 1. After addition of 20 ml of aqueous leaf extract of *Hierochloe odorata,* the colour of the 100 ml 0.1 M bimetallic salt solution of Ag-Cu changed from a deep blue to murky green (plate 1) indicating the creation of Ag-Cu bimetallic nanoparticles. The excitation of the surface plasmon resonance action in the nanoparticles is responsible for the colour variations. The size and shape of metal surface plasmon oscillation, as well as their optical properties are well understood.

UV-Vis spectrophotometry Analysis

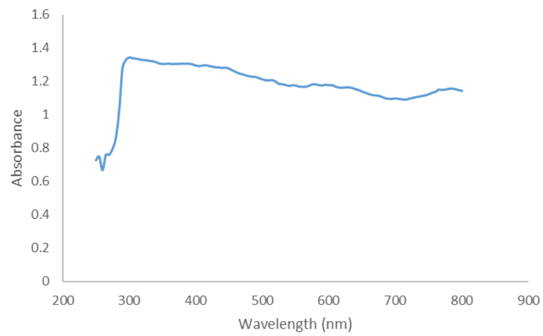


Figure 1: UV-visible spectrum of Ag-Cu nanoparticles

UV-visible spectroscopy was used to monitor the reduction of aqueous silver and copper metal ions after treatment with *Hierochloe odorata* aqueous leaves extract which acted as the reducing agent. The UV-visible spectrum of the reaction mixture indicated the presence of surface plasmon resonance (SPR) absorption band of approximately 295-315 nm with maximum absorbance at 305 nm which can be attributed to the presence of silver-copper nanoparticles. The absorption bands in the range 295 to 315 nm observed indicates that sufficient amount of reductive biomolecules are present in the extract. Also, the nearly symmetrical structure of the plasmon band can be attributed to the fact that the nanoparticles are not homogenous and also not uniformly dispersed. This non- uniformity of the nanoparticles might be responsible for the slightly broad absorption peak. The particle size, shapes, homogeneity and capping agent determines the position of the plasmon resonance.

FTIR spectroscopy Analysis

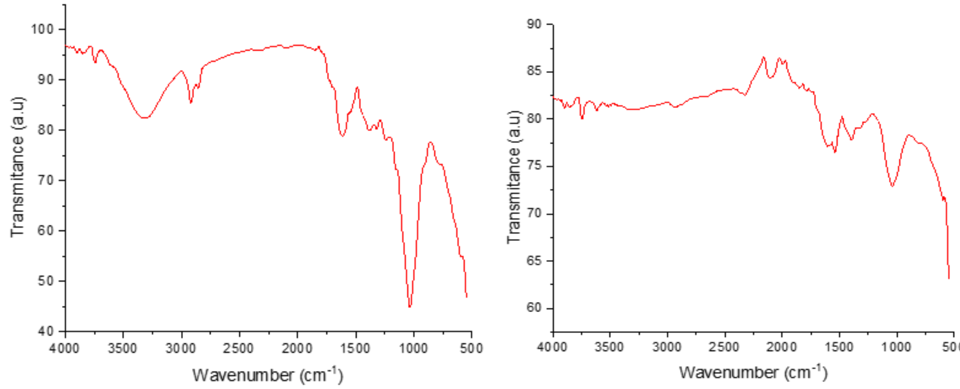


Figure 2A: FTIR spectrum of Hierocloe odorata leaves extract. Figure 2B: FTIR Spectrum of Ag-Cu nanoparticles

The possible biomolecules responsible for reduction, capping and efficient stabilization of the eco-friendly synthesized Ag-Cu bimetallic nanoparticles were investigated using FTIR spectroscopy as shown in Figures (2A and 2B). The former revealed distinct bands for numerous functional groups. IR bands were recorded at 3698, 3317, 2938, 1620 and 1033 cm^{-1}. The bands at 3698 cm^{-1} and 3317 cm^{-1} corresponds to O-H stretching for alcohols and N-H stretching for secondary amines. 2938 cm^{-1} corresponds to C-H stretching for alkanes, the band at 1720 cm^{-1} and 1033 cm^{-1} corresponds to C=O stretching for amides and C-C bending vibration for alkanes. FTIR analysis was also carried out on the refined Ag-Cu NPs to investigate if some biomolecules from the plant extract bonded to the Ag-Cu NPs during the synthesis process. The hydroxyl O-H stretch for alcohols (3693 cm^{-1}), C=N weak stretching for nitriles (2250 cm^{-1}), C=O amides (1620 cm^{-1}) and C-C stretch for alkanes (1033 cm^{-1}) absorption bands revealed peaks in the IR band of Ag-Cu NPs. Aqueous leaves extract of *Hierochloe odorata* is known to be rich in flavonoids and alkaloids, these flavonoid and alkaloid components present in the leaves extract with C=O and C=N functional groups can donate free hydrogen and amino moieties capable of binding to silver and copper surfaces which are responsible for size reduction, capping and prevention of agglomeration of the Ag-Cu bimetallic nanoparticles [31].

XRD Analysis

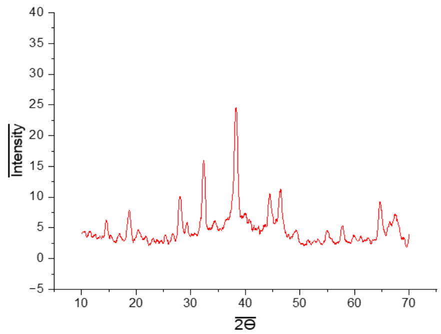


Figure 3: XRD spectrum of Ag-Cu nanoparticles

From the X-ray diffraction patterns of the eco-friendly synthesized Ag-Cu bimetallic nanoparticles as shown in Figure 3, it is clear that the diffraction pattern of the bimetallic nanoparticles were essentially crystalline as shown with the sharp peaks [32]. Estimated diffraction peaks observed at 38.47°, 44.67°, 47.12° and 65.23° corresponds to the (111), (200) and (220) standard face centered cubic structures of silver which confirms the presence of silver oxide in the sample [32, 33]. The XRD pattern also shows peaks of copper oxide at 28.85° and 33.26° brought about by the oxidation of copper particles [34]. The other peaks might be as a result of some bioorganic compounds or proteins present in the plant extract [35]

SEM-EDS Analysis

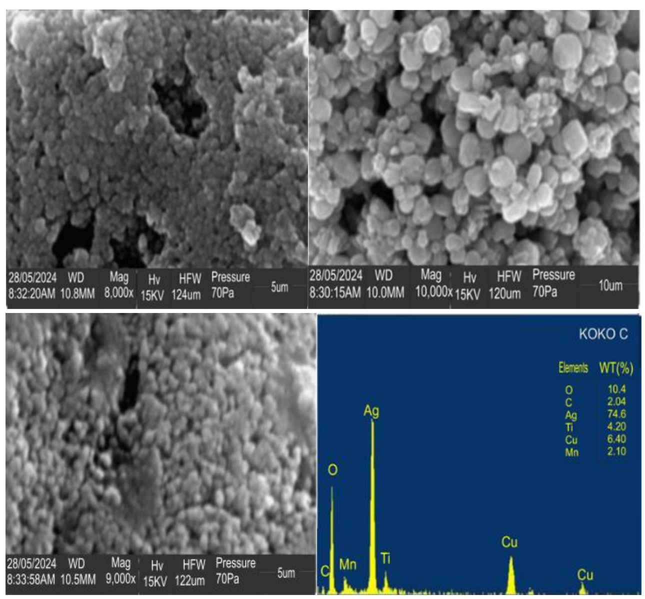


Figure 4: SEM-EDS Micrograps of Ag-Cu nanoparticles

The SEM-EDS analysis was used to investigate the structural, surface morphology and elemental composition of the eco-friendly synthesized Ag-Cu bimetallic nanoparticles. The SEM micrograph (figures 4a, 4b & 4c) revealed that the Ag-Cu nanoparticles are predominantly spherical, having smooth surface and well dispersed with close compact arrangement [36]. EDS analysis was carried out to determine the semiquantitative elemental composition of silver and copper as shown in figure 4d, which revealed a strong signal in the silver region with a percentage weight of

74.6% and 6.4% for Copper. The total metal composition justified the purity of the bimetallic nanoparticles. EDS analysis also revealed weak signals of oxygen, carbon, manganese, sulphur, and titanium, which may have originated from the biomolecules of the plant extract bound to the surface of the Ag-Cu nanoparticle.

Antimicrobial activity

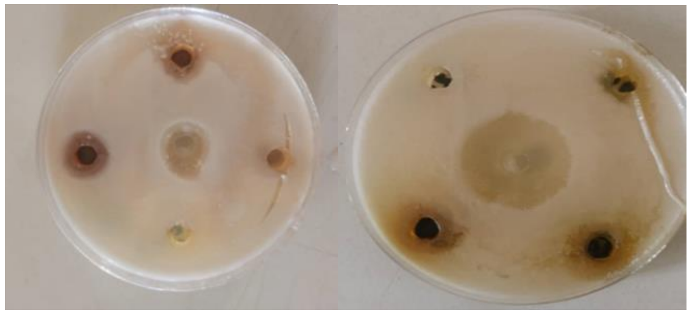


Plate 2: Antimicrobial activity of Ag-Cu NPs against (a) Salmonela typhi (b) Staphylococcus aureus

Antimicrobial activities of the eco-friendly synthesized Ag-CuNPs were evaluated by using standard assay. The nanoparticles showed good inhibition zone against the two strains of bacteria (S. typhi and S. aureus) under study as presented in plates 2a and 2b. The zone of inhibition of Ag-CuNPs obtained against S. typhi and S. aureus are 14 and 16 mm, respectively. It was observed that the zone of inhibition increases with the increase in the concentration of Ag-Cu NPs as shown in table 1 below. MIC test was obtained at 200 mg/ml for both S. typhi and S. aureus. The eco-friendly synthesized Ag-Cu nanoparticles from aqueous leaves extract of *Hierochloe odorata* have been found to be effective against both grams-negative and grams-positive bacterial strains.

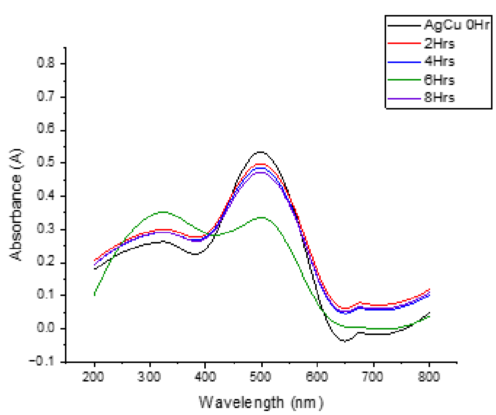
Table 1: Sensitivity test

Organism	400 mg/ml	200 mg/ml	100 mg/ml	50 mg/ml	Control
S. aureus	16	11	6	2	20 mm
S. typhi	14	09	4	-	21 mm

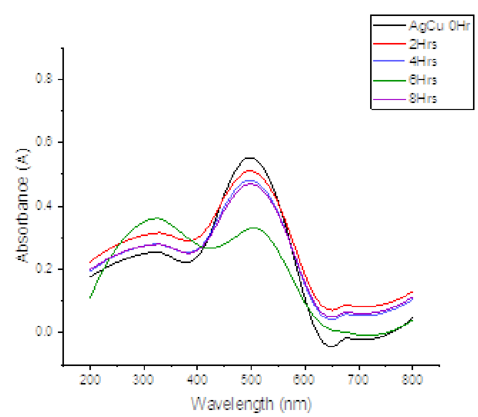
Table 2: Minimum Inhibitory Concentration (MIC) test

Organism	400 mg/ml	200 mg/ml	100 mg/ml	50 mg/ml	Control
S. aureus	+	+	-	-	200 mm
S. typhi	+	+	-	-	200 mm

Congo red degradation



A



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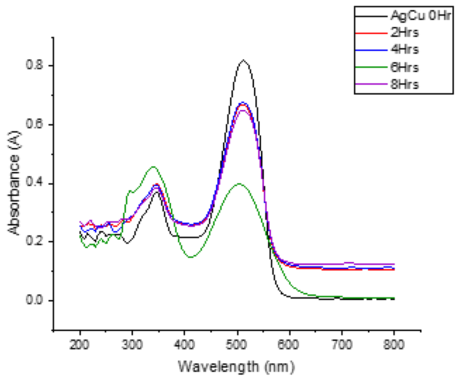

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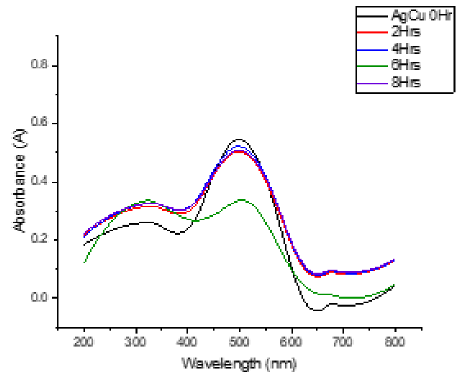

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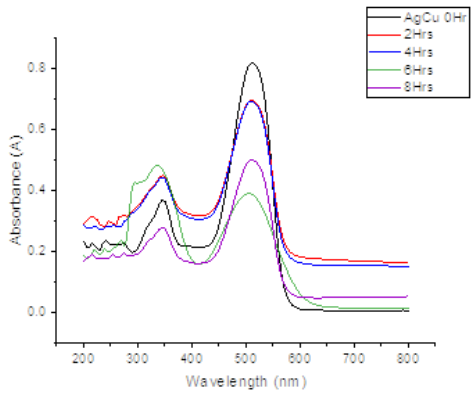

E

Figures 5 A-E: UV-Visible Spectra showing Congo red degradation at various concentration of Ag-Cu nanoparticles and exposure time

Congo red dye is harmful and toxic to a wide variety of organisms including humans and can be carcinogenic and mutagenic. The efficiency of the photocatalytic degradation of Congo red dye by the eco-friendly synthesized Ag-Cu nanoparticles was investigated using UV-vis spectroscopy. (Figures 5A – 5E corresponding to 200, 400, 600, 800 and 1000 ppm of Ag-Cu NPs respectively) shows the degradation of Congo red dye after addition of Ag-Cu NPs of different concentrations, the degradation was observed at uniform time intervals of 2 to 8 hours in each case. Dye absorbance was observe to decrease with time and increase in concentration of nanoparticles with maximum dye degradation of 56% observed at (1000 PPM) after 6 hours exposure time and complete degradation was not observed. Dye degradation process was observed to reduce after 6 hours exposure time, this might be due to the binding of degraded materials to the nanoparticle surface. Guar J. et al., [37] reported photocatalytic degradation of Congo red dye using Carica papaya derived Zinc oxide nanoparticles. Eida S. Al-Farraj and Ehab A. Abddelrahman [38] reported efficient photocatalytic degradation of Congo red dye using facilely synthesized $MgAl_2O_4$ nanoparticles.

4. Conclusion

The eco-friendly method of nanoparticles synthesis is one of the potential methods which has gained lot of attention. It produces novel materials that are cost-effective, facile and eco-friendly and shows applications in the different areas. In this study, aqueous leaves extract of *Hierochloe odorata* was used to for Ag-Cu bimetallic nanoparticle synthesis at room temperature. The plant extract acted as a reducing and capping agent in the nanoparticle synthesis and also provided stability to it. Eco-friendly synthesized Ag-CuNPs showed potential antibacterial activity against

Staphylococcus aureus and *Salmonela typhi*. Photocatalytic degradation using eco-friendly synthesized Ag-CuNPs showed around 56% degradation of Congo red dye. This results shows that aqueous leaves extract of *Hierochloe odorata* can be used in the eco-friendly synthesis of Ag-Cu nanoparticles which can be a viable tool in the degradation of organic dyes and in the treatment of some antibiotic resistant strains of microbes as shown in this study.

Acknowledgement
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