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Green Synthesis, Characterization and Antibacterial Activity of Copper Nanoparticles from the Roots Extract of Piliostigma Reticulata (Fabaceae)

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Abstract: Nanotechnology is a fast-growing area in science which is an interdisciplinary field of both science and technology. In this study, the Roots extracts of Piliostigma reticulata were used for the green synthesis of Copper (Cu) nanoparticles (NPs) from copper Sulphate solution. Green synthesized metallic nanoparticles were characterized by UV– visible spectrophotometer, X-ray diffraction (XRD), Fourier Transform Infra-Red spectrophotometer (FTIR) and Scanning Electron Microscope (SEM). Using the Debye Scherrer formula, the average crystalline size of CuNPs was calculated as 51.0nm respectively. The UV–Vis spectroscopy results showed Surface Plasmon Resonance peaks at 500nm respectively. Fourier transform infrared spectroscopy was measured to identify the possible functional group of the biomolecules in the extract that act as reducing and capping agents of the nanoparticles. SEM analysis describes surface morphology of the synthesized nanoparticles which were roughly spherical in shape. The synthesized Nanoparticles showed antibacterial activity against pathogenic bacteria (*Escherichia coli and Staphylococcus aureus*) and the highest antibacterial activity was found in Cu-NPS compared plant root extract.

Keywords: Metallic nanoparticles; synthesis; characterization; Antibacterial Activity

I. Introduction

Nanotechnology is a fast-growing area in the field of science which is an interdisciplinary field of both science and technology [1] Nanoscience is the study of phenomena at 1-100 nm particle size and Nanomaterials are those which have structured components with at least one dimension less than 100 nm [2]. Metal nanoparticles do not represent a metal-metal chemical bond but are usually defined as isolated particles between 1-100 nm sizes. [3]. This is why Metal nanoparticles (MNPs) have extra ordinarily different properties when compared to their bulk metal which mostly contains a degenerated density of energy states and a large surface-to-volume ratio along with the sizes in the nanometer scale and this is the reason behind they show high chemical activity and specificity as compared to bulk metals [4] The high surface-to-volume ratio along with size effects (quantum effects) gives metal nanoparticles distinctively different properties (chemical, electronic, optical, magnetic and mechanical) from those of bulk metal [5].

A wide range of chemical and physical methods are being used for the synthesis of nanoparticles with great activity but these methods have a few shortcomingsuch as the use of toxic solvents, high energy consumption, hazardous by-products etc. The green or Biological synthesis of NPs has been found to be more advantageous than physiochemical synthesis since biological synthesis is cost-effective, environment friendly and could easily be scaled up for large-scale synthesis and does not use high pressure, temperature and toxic chemicals [**6**]. Green synthesis of nanoparticles using plant extracts is gaining importance over chemical synthesis. Plant extracts with their dual role as surface stabilizing and capping agents act as bio templates for the synthesis of nanoparticles. Better manipulation, crystal growth control and stabilization are other advantages of biological methods [**7**].

The plant Piliostigma reticulate belongs to the Fabaceae family. This plant has one of the most versatile medicinal plants since many years ago, having a wide spectrum of biological activity. All the parts of plants like leaves, stems, fruits and roots habe been used as a medicine for household remedies against various human diseases such as malaria fever, wounds, ulcers, gastric/heart pain, gingivitis anti-inflammatory and antipyretic [**8**].The plant is a cheap and easily available source, this is why we selected the root extract for the synthesis of Cu nanoparticles.

Different researchers have reported that the plant extracts have antioxidant and reducing properties which are key roles responsible for the reduction of metal salt to their respective nanoparticles. The Phytochemicals such as phenols present in plants extracts can act as capping and reducing agents and thus increase the rate of reduction and stabilization of nanoparticles [**9**]. Biosynthesized metal nanoparticles are more stable in nature and their rate of synthesis is faster than that of other methods. The present study describes the green synthesis of Cu nanoparticles and their characterization as well as its application as an antibacterial of some common bacteria.

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II. Materials and Methods

The Copper Nanoparticles (CuNPs) were synthesized using an aqueous extract of Piliostigma reticulata roots extract and the

Copper Sulphate ($CuSO_4 . 5H_2O$) was used as a precursor source for Cu

Sample Collection: The roots of Piliostigma reticulate were collected from Nguru local Government area. The roots were washed thoroughly under tap water to remove the dust, then washed with distilled water twice and dried at room temperature to remove moisture from the roots.

Extraction Method: 25g of dried powder of Piliostigma reticula roots sample in 500mL of distilled water was boiled for 30min at 60C in 1000mL beaker. The extract was cooled to room temperature and filtered with Whatman filter paper no1.

Preparation of $CuSO_4 . 5H_20$ Solution: 0.02M $CuSO_4 . 5H_20$ Solution was prepared by dissolving 5.0g of $CuSO_4 . 5H_20$ in 1000ml volumetric flask and the distilled water was added up to the mark.

Synthesis of copper nanoparticles: 0.02M of $CuSO_4$ solution was added dropwise into the plant extract in a ratio of 1:1 (that is 100ml plant extract and 100ml metal precursor) with constant stirring at 80°C for 60 minutes using magnetic stirrer. Within the first 25 minutes color changes were observed which indicates the reduction process and the formation of nanoparticles. After which it was decanted and dried. The collected nanoparticles at 100°C for 2hours were ground into powdered and kept for further analysis.

Characterisation: Optical properties of metallic nanoparticles were recorded using UV–Vis spectrophotometer. The UV–Vis spectroscopy is the most basic technique used to confirm the formation of nanoparticles. With distilled water as a reference the absorbance spectrum of the sample was in the range of 200–800 nm. X-ray beam diffraction (XRD) of metallic nanoparticles analysis was carried out by using monochromatic Cu radiation (k = 1.5406 A °) and the particle size were calculated using Debye Scherrer equation and SEM analysis were employed in order to describe the morphology of the Cu NPs

Media Preparation: The media preparation depends on the manufacturer's specification. 38g of Mueller Hinton agar was dissolved into 1000ml deionized water and autoclaved at 121°C for 15 minutes,the cooled Mueller Hinton Agar was poured into sterile petri dishes on a level horizontal surface to give uniform depth and Stored the plates at 2-8 °C for analysis.

Broth Dilution Method: For this method, the synthesized nanoparticles were diluted to obtain a stock solution having 2000mg/ml concentration. These synthesized nanoparticles were diluted for primary screening, obtaining 1000mg/ml, 500mg/ml, and 250mg/ml concentrations. The nanoparticles found active against microbes in the primary screening were further diluted in the secondary screening to obtain 200mg/ml, 100mg/ml, 50mg/ml, 25mg/ml, 12.5mg/ml, and 6.250mg/ml concentrations. Each synthesized nanoparticles were diluted using Peptone water as a diluent **[10]**

Antibacterial Assay: The antimicrobial activity studies were carried out by using the agar well method with 6mm diameter Whatmann No.1 filter paper discs to test the antibacterial activity of the green synthesized Cu nanoparticles on gram-negative and gram-positive bacteria(*Escherichia-coli) and* gram positive bacteria (*Staphylococcus aureus)* as well, by following the method according to Saranya e*t al.*, 2017 [11]. 24 hours fresh cultures were prepared and the standardized (McFarland No.0.5) inoculum was used for the antibacterial assay. Each strain was uniformly swabbed on the individual plates. Using micropipettes, 100mg/mL, 200mg/mL, 300mg/mL and 400mg/mL concentrations of nanoparticle solutions were poured into the wells on all plates. After incubation at 37 °C overnight, zones of inhibition were measured.

III.Result and Discussion

The present investigation entitled green synthesis and characterization of copper nanoparticles from the extract of **PILIOSTIGMA RETICULATA** and its bioactivity was conducted. The various outcomes during the investigation have been stated in the form of different tables, figures and chart. From the result obtained during the green synthesis copper nanoparticles, the color change was observed as the first indication of the formation of nanoparticles. The Copper nanoparticles change from black to reddish brown

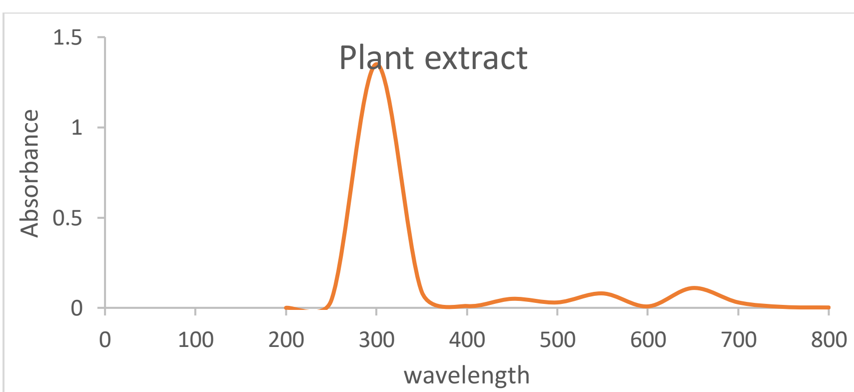

Fig1: UV-Visible spectrum for root Extract

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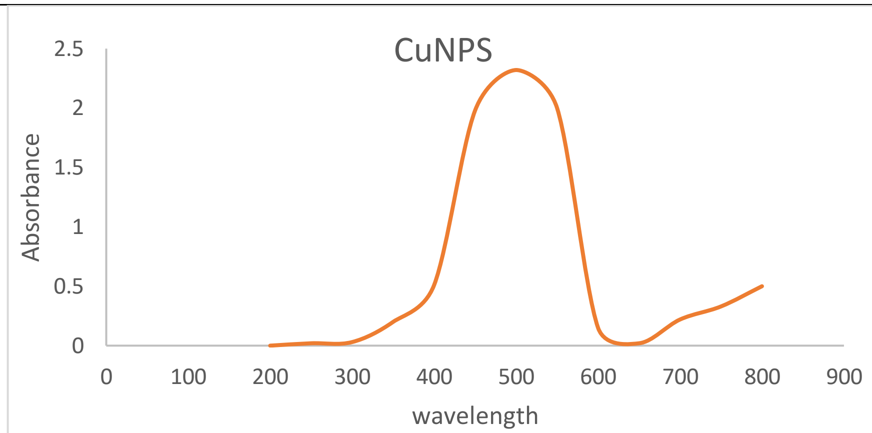

Fig2: UV-Visible spectrum for CuNPS

UV-visible spectroscopy analysis was done to confirm the formation of nanoparticles and observe the extract plasmon vibration and excitation of Copper nanoparticles and the Plant extracts. The wavelength will be varied at regular wavelengths of 200nm, 300nm, 400nm, 500nm, 600nm, 700nm and 800nm respectively. The colour reaction arises from the excitation of surface Plasmon vibration in the metal nanoparticles [12].The phytochemical present in the extract not only reduced the metal ions but also stabilized the metal nanoparticles by preventing them from being oxidized after synthesis [13]. The Beer-Lambert law is applied where a beam of monochromatic light is passed through a solution of an absorbing materials and the intensity of radiation monitored. The rate decrease of intensity of radiation with thickness of the absorbing solutions is proportional to the incident radiation and the concentration of the solution.

III. Fourier Transform Infrared Spectroscopy (FTIR)

In this study, Fourier transform infrared spectroscopy analysis (FTIR) was utilized todiscern the surface composition of the synthesized nanoparticles (NPS), with a particular focus on identifying functional groups that may contributory role in the formation of these nanoparticles(NPs). Thus, the technique provides information on the interaction between the phytochemicals available in the plant extracts and metal ions responsiblefor the production and capping of the nanoparticles. Though, the nanoparticles possessed absorption peakssimilar to those of the extracts, the differences in intensities suggest the importance of functional groups in the reduction and capping processes. The FTIR spectra showseveral peaks which indicate the presence of important functional groups of O-H. N-H, C-H, –C=C, and C-Cl stretching for halogen compounds as shown in the spectrum. The FTIR spectra of the CuNPs in Fig4 below shows several peaks at 3862 cm^{-1}, 3758 cm^{-1}, 3432 cm^{-1}, 2387 cm^{-1}, 1951 cm^{-1}, 1669 cm^{-1}, 1575 cm^{-1}, 1471 cm^{-1}, 1343 cm^{-1}, 1162 cm^{-1} and 803 cm^{-1}The peak at 3862 cm^{-1} represent O-H stretching of phenolic groups. The absorption band at 3432cm^{-1} indicate O-H stretching due to water, peak at 1669 cm^{-1} is for –C=O of carbonyl, peaks at , 1575 cm^{-1} and 1471 cm^{-1} is due to the C=C ring of aromatics the absorbance band at 1343 cm^{-1} is for C-H symmetry of -CH_2, the peak at 803 cm^{-1} is for to C-Cl stretching for halogen compounds.This was confirmed in terms of the individual band intensities observed in the extracts and their corresponding nanoparticles (CuNPs)

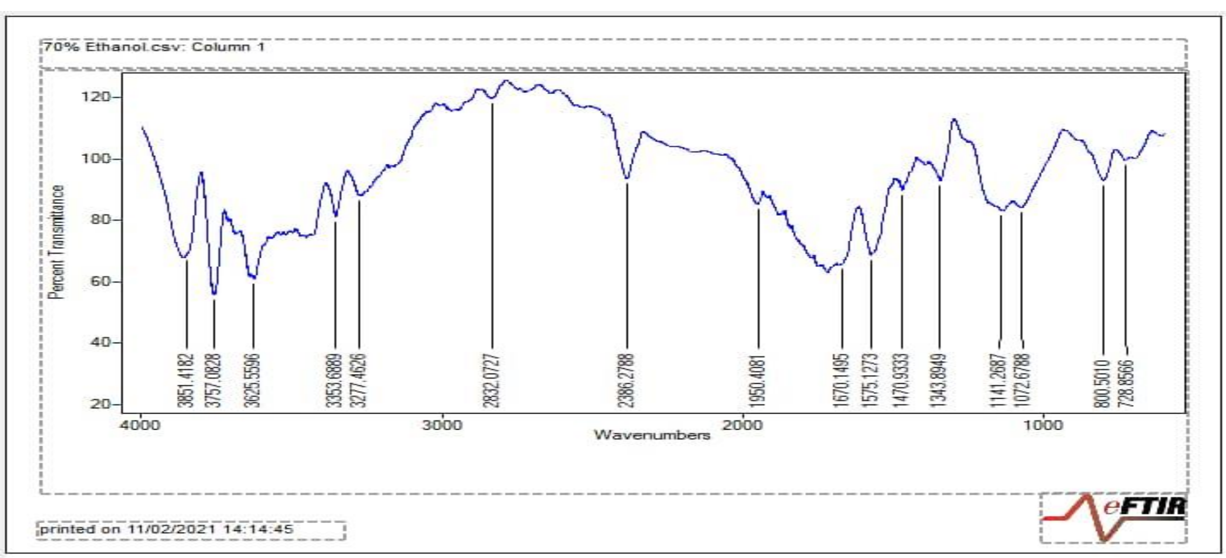

Fig3: FTIR spectrum of the Extract

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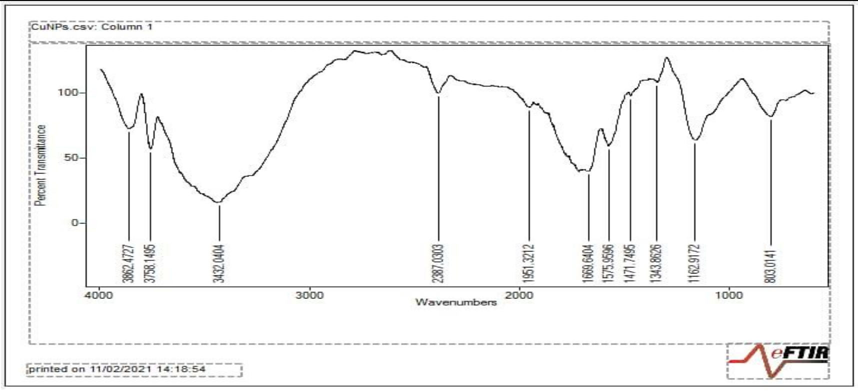

Fig4: FTIR spectrum of the CuNPs

IV. Sem (Scanning Electron Microscopy)

The SEM results for Copper nanoparticles (CuNPs) elucidate the surface morphology of CuNPs. SEM analysis underscores remarkable capability of plant extract to facilitate the synthesis of nanoparticles characterized by a predominantly spherical shape and uniformdistribution. SEM images showed that most of the NPs are predominately spherical in shape having smooth surfaces and are well dispersed with close compact arrangement.

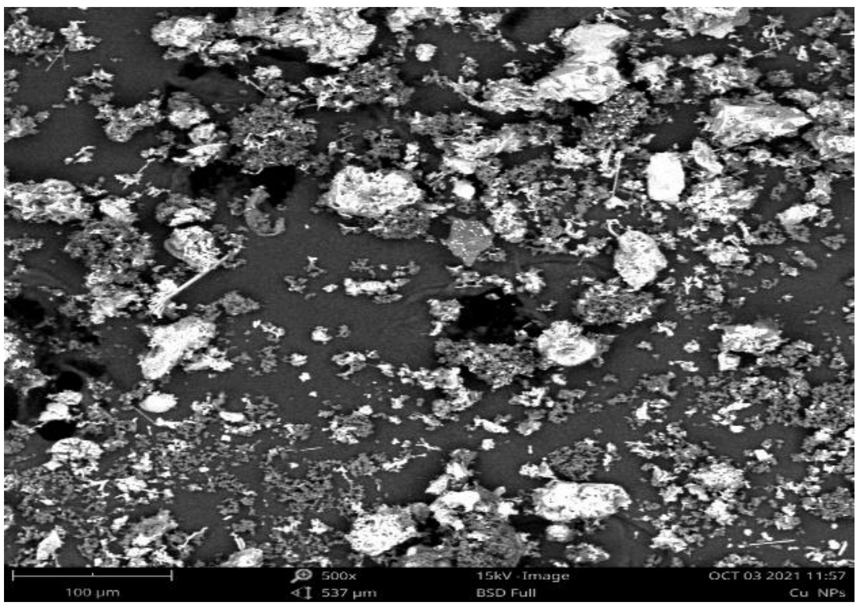

Fig5: Scanning electron microscopic image of Cu NP

XRD (X-Ray Diffraction) Analysis

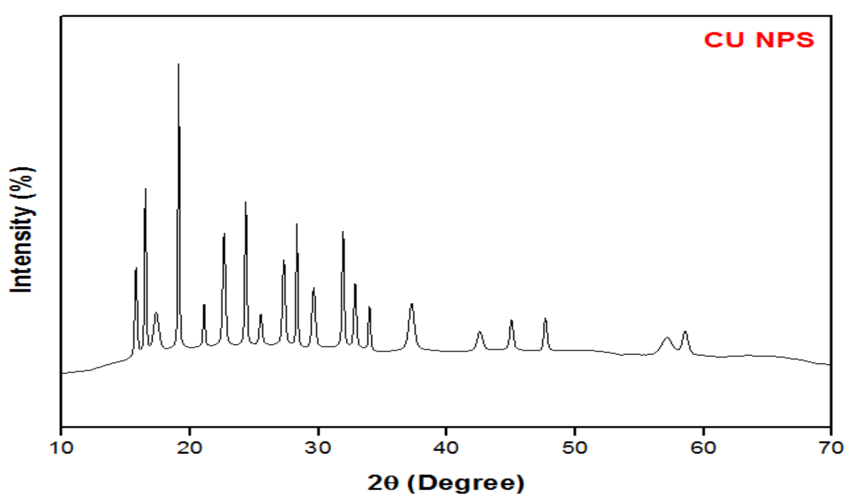

Fig6: XRD analysis of Cu NPs

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Powder X-ray diffraction (XRD) analysis was applied to examine the surface crystallinity of the biosynthesized nanoparticles and determine their crystalline size. The synthesized Cu Nanoparticles underwent XRD analyses across a two theta (2θ) range spanning from 0 to 70^0 respectively. The XRD analysis of the copper nanoparticles with the braggs angle of 0-70° observed along with the weak peaks, it was found that, four prominent peaks are observed at 2θ =17.36°, 66.95°, 51.61° and 68.09° which corresponds to the plane(110), (111), (200) and (220) respectively which matches with the Cu JCPDS, File No. 04-0836. It shows Face Centered Cubic [FCC] structure and the average crystalline size 51.00nm according to the Scherer equation calculated. The result corresponds to the literature reported by Fatemeh *et al.*, 2017 [**14**], which shows FCC (face centered cubic) structure for the green synthesized silver nanoparticles using *tapidiumdraba* weed extract with respect to the plane of (110), (111), (200) and (220) and the diffraction peaks are indexed based on JCPDS file number 64-0783.

Table1. Particle size calculation using Debye Scherer Equation for Cu Nanoparticles:

S/N	2Ɵ	FWHM	Λ	D = 0.9λ /BCOSƟ
1.0	17.36	0.4728	0.1541	16.99nm
2.0	19.11	0.1181	0.1541	66.95nm
3.0	24.32	0.1574	0.1541	51.61nm
4.0	28.30	0.1181	0.1541	68.09nm
Average Size = 50.91				

Antibacterial activity of green synthesized nanoparticles

With ever increasing concerns about the use of antibiotics, alternatives to antibiotics are being looked upon. Metallic nanoparticles are being reported to have good antibacterial activity due to their large surface area-to-volume ratio. The antibacterial activities of CuNPs and Plant extract against the pathogenic organisms are shown below. It is quite interesting to note that all bacterial species tested in this study showed good sensitivity towards the green synthesized nanoparticles. Zone of inhibition was observed to be more in gram-negative bacteria when compared to gram- positive bacteria which might be due to the differences in bacterial pathogen's membrane structures. The antibacterial activity test copper nanoparticles (CuNPS) also showed that *Escherichiacoli* shows an increase in bacterial growth inhibition with an increase in concentration of the green synthesized drug (nanoparticles). The maximum zone of inhibition was recorded at 400 mg/ml with 11.5 mm followed by 300 with 8.5mm, 200mg/ml with 8mm and 100mg/ml revealed 4.0 mm of zone of inhibition. For the *Staph. Aureus* shows high sensitivity to high concentrations of nanoparticles at 400mg/ml with 10mm. *Staphylococcus aureus* shows an increasing order in bacterial growth inhibition with an increase in the concentration of the CuNPS (synthesized drug).

Table 2: Showing the results obtained from the antibacterial activity test for CuNPs

Zone of Inhibition in Millimeters (mm)						
Test organism	100mg/ml	200mg/ml	300mg/ml	400mg/ml	Control(300mg/ml)	
E. Coli	4	8	8.5	11.5	20	
S.Aureus	4	4	8	10	23	
Control = Augmentin E. Coli = Escherichia Coli, S.Aureus = Staphyloccocus Aureus, mg/ml = Milligram per mil						

Table3: Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of synthesized nanoparticles and aqueous roots extract

Bacterial strain	Antimicrobial Agents	MIC(mg/ml)	MBC(mg/ml)
E.Coli	CuNPS	50	100
E.Coli	Plant extract	100	--
E.Coli	Augmentin	100	100
S.Aureus	CuNPS	50	100
S.Aureus	Plant extract	100	--
S.Aureus	Augmentin	50	200

The Table3 aboveshows the antibacterial activity of CuNPs in comparison with Plant extract. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) were observed for CuNPs and Plant extract. The results reveal that all

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antimicrobial agents (Cu NPs and extract) show Minimum Inhibitory Concentration (MIC) on all bacterial strains. The Minimum Bactericidal Concentration (MBC) was only observed in CuNPS while Plant extract was not.

V. Conclusion

A Green synthesis method of nanoparticles has advantages overphysiochemical methods like it is free from toxic reagent, low cost, low energy, ecofriendly and simple process. In this study Plant extracts from *Piliostigma reticulata* were used for the green synthesis of Copper (Cu) nanoparticles (NPs) from copper Sulphate solution respectively. FTIR analysis showsdifferent functional group that are responsible for natural reducing agents, SEM images showed that NPs having smooth surface and are spherical in shape, the crystalline sizes of the NPs was determined by XRDand UV-Vis techniques confirmed the formation of Cu nanoparticles which are in nano range and are stable. Green synthesized nanoparticles in the present study show good antibacterial activity against the human pathogens (*Escherichia coli* and *Staphylococcus aureus)*

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Conflicts of Interest: The authors declare that there is no conflict of interest.

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