

Green synthesis and characterization of CuO nanoparticles using *Angelonia angustifolia* plant extract for biological application

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Abstract

Biological synthesis of Cu NPs free from toxic chemical components, using plant extract of *Angelonia angustifolia* has been proposed in the present investigation. To confirm its direction and crystal arrangement Copper NPs were characterized by, X-ray diffraction (XRD) & UV spectrophotometer. The morphology and particle size are investigated using Electron Microscopy (SEM and HR-TEM). The probable bio-reducing agents within the plant extract are established by using FTIR analysis. The investigational study (XRD, SEM and HR-TEM) recommended that the biological synthesis method by using plant extract has capable effect for the synthesis of spherical shape Cu NPs average size of 0.21 to 6.18 nm. The antibacterial activity of Cu NPs has also been investigated. According to the present study, the green synthesis is without any toxic chemical, Cu metal NPs with preferred size, shape, morphology and the required properties by using plant extract.

1. INTRODUCTION

The present cohort is all about nanotechnology and nanoparticles. Nanoparticles have varied applications in life sciences such as drug expansion, protein revealing and gene deliverance and antimicrobial action [1]. Last Decades metal nanoparticles are profoundly used in cosmetics, medicines and food preservatives. Generally, nanoparticles of metal and metal oxides be able to be synthesized using a diversity of physical and chemical methods such as chemical reduction, photochemical reduction, electrochemical reduction, heat evaporation, etc. [2–5] which are not environment friendly. However, physical method is very costly, it required sophisticated instrument [6]. On the other hand, in chemical process toxic chemicals like lead oxide and sodium citrate are involved, this method not only expensive but also involve toxic & hazardous chemicals, this may increase adverse effect may pose potential environment and biological risks [7]. However, these methods are strongly depended on rigorous reaction conditions, such as, aggressive agents, harmful solvent system to environment and ecology, higher temperature and pressure and so on [8]. On the other hand, plant-based biological synthesis of NPs is a non-hazardous, simple and safe and sound process is substitute to chemical and physical method. NPs synthesis using plant extract can be beneficial over other biological processes because No chemical reagent or template are required in this method and it eliminates the highly structured process of maintaining cell cultures and can also be rightfully scaled up for large-scale synthesis of NPs [9]. It is a base up advance for synthesizing Cu NPs where either reduction or oxidation reaction is occurred [10]. In this study, we synthesized Cu NPs using plant extract of *Angelonia angustifolia*. It is a wild herbaceous plant and rich source of several secondary metabolites [11]. Plant based extract and microorganisms for the synthesis of Cu NPs offer numerous benefits as they are compatible for biological application with zero chemical toxicity on the application and environment [12]. *Angelonia angustifolia* plant offer synergistic effect to enhance the antimicrobial properties of the synthesized Cu NPs. Plant parts or entire plants have been used for the green synthesis of Cu NPs, the extracts of a range of parts of plant species that comprise *Azadirachta indica* leaf [13], *Thymus vulgaris* L. [14], *Ginkgo biloba* Linn [15], *Punica granatum* [16], *Citrus medica* Linn [17], *Zingiber officinale* [18], *Asparagus adscendens* [19], *Terminalia catappa* [20], *Eclipta prostrata* [21]. We have deliberate outcome of

particle size and its allocation. Cu NPs were characterized by using Fourier transform infrared spectroscopy (FTIR) analysis was used to categorize the biomolecule liable for reducing Cu NPs ion and stabilizing Cu NPs. The optical properties were monitored using UV-Vis spectroscopy. Surface morphology, particle size and its allotment were confirmed using scanning electron microscopy (SEM) and transverse electron microscopy (TEM). The X-ray diffraction analysis confirmed the creation of crystalline Cu NPs. The antibacterial activities of Cu NPs have been investigated by means of number of bacteria.

2. MATERIAL & METHODS

For the synthesis of Copper nanoparticles *Angelonia angustifolia* whole plant used (Fig. 1). Plant was collected from Nawegaon/bandh national park in Dist. Gondia, Maharashtra, India [11].

A. Preparation of extract from plant material:

The collected plant washed thoroughly with tap water. The procedure was carried out two or three times in order to remove all dust and undesired particles. Cut them into small pieces and allow them to shed dry for some weeks. After complete removal of moisture whole plant crushed in mortar and pestle and dry powder is made. Take 20 gm powder of dry plant powder of *Angelonia angustifolia* in beaker, add 200 ml distilled water into it. Then boil at 70-80⁰Cf or 2 hrs. in soxhlet. After passing the extract through a basic filter paper and Whatman filter paper number 1, the final product was refrigerated for later use.

B. Synthesis of Cu NPs:

For Cu NPs synthesis, take 50 ml of *Angelonia angustifolia* plant extract in 500 ml beaker. Then prepare a solution of metal oxide is (Cu(NO₃)₂.6H₂O) Copper Nitrate Trihydrate (3 gm of copper nitrate trihydrate in 100 ml distilled water). Add this metal solution in burette and adjust its drops. Add metal solution drop by drop into plant extract in beaker with continues stirring and keep its temperature at 40-50 ⁰C up to complete addition of metal solution (it take near about 2 hrs.). After complete addition, increases its temperature up to 80 ⁰C for half an hr. After again increase its temperature up to 100 ⁰C to remove excess of water. Keep 100 ⁰C up to the paste of solution will appear. Collect that paste into crucible for calcination. Calcination done at 400 ⁰C for 2 hrs. After calcination blakish or black coloured powder is formed which indicate presence of Cu NPs. For further characterization, the collected nanoparticles of copper stored in air tight container and preserved under dark to avoid photolytic reaction occur under sunlight.

C. Characterization of Nanoparticles:

Through the use of characterization techniques, we are able to accurately and consistently interpret the measured results by understanding the unique features of the nanocrystals under study. The characterization of green synthesized nanoparticles was done by using UV-VIS spectrophotometer, X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR), Scanning electron microscopy energy

dispersive X-ray spectroscopy (SEM & EDAX) and High-resolution transmission electron microscopy (HR-TEM).

3. RESULTS AND DISCUSSION

1) *UV-Vis Spectrophotometer analysis*

Utilizing the UV-Vis absorption spectra, the optical characteristics of the biologically produced Cu NPs were examined. [22]. The size and form of the particles, the distance between them, the material surrounding them, and their nature all affect optical absorption. [23].

Au, Ag and Cu NPs all have characteristic colors related with their particle size, for Cu NPs, observation of UV-Vis spectra can be useful method in characterizing metal particles [24]. The UV-Vis spectrophotometer was used to investigate the bio-synthesised Cu NPs, and the results revealed characteristic surface plasmon resonance (SPR) spectra with absorbance in the 300–1100 nm region. The resulting optical plot is displayed in (Fig.2). The peaks at 560, 550, 540, 430 and 400 nm with the absorption 0.9555, 0.9568, 0.9586, 0.966 and 0.9558 AU respectively. The maximum absorbance can be found in between 400 nm to 650 nm at about 540 nm is 0.9586 AU. The precise location of the SPR band may change based on the capping agents, shape and size of each particular particle [25]. In the current experiment, the reaction mixture revealed a single SPR band confirming the spherical shape of Cu NPs, which was further confirmed by TEM pictures in accordance with Mie's theory. [26].

2) *FTIR Spectroscopy analysis*

FTIR analysis is used to determine potential biomolecules in the *Angeloniaangustifolia* and functional groups surrounding or capping on the surface of Cu NPs. FTIR analysis was used to identify the natural chemicals that were selectively attached to the metal oxide nanoparticle surface. [22]. The FTIR analysis is measured in between the 4000 to 500 cm^{-1} region. (Fig.3) shows the absorption band at 3165, 1472, 1384, 1117, 865 and 526 cm^{-1} respectively. 1472 cm^{-1} shows the presence of alkanes with bending vibrations of $-\text{CH}_2$ [27]. Bands at 1395 cm^{-1} that come from hydroxyl groups and a medium band at 1384 cm^{-1} that shows the presence of amine groups. Amide I and II b and s originate from vibrations of carboxyl stretch and N-H deformation in the amide bonds of the proteins that comprise it. [28]. A peak at 865 cm^{-1} represent the aromatic H out of plane bending [29]. The bands in the 600–400 cm^{-1} range may be caused by metal oxygen (Cu-O). [30]. The band at 526 cm^{-1} confirmed the presence of Cu NPs. This indicate functional group plays a major role in the synthesis of Cu NPs as they provided reducing group which help in the synthesis of NPs.

3) *XRD Analysis*

XRD reveals crystal size, regularity in an atomic arrangement of sample and structural characterization of Cu NPs. The Cu NPs show high crystalline nature which corresponds the diffraction angle [31]. The purity and creation of the nanoparticles have been verified by X-ray diffraction (XRD), which was used to

assess the peak intensity, location, and width of the particles. [32]. The biosynthesized Cu NPs for 2θ values in the range of 10° - 80° (Fig.4) shows XRD pattern of Cu NPs prepared using *Angelonia angustifolia* plant extract. The intense diffraction peaks of Cu NPs are clearly visible at 28.402° , 32.623° , 38.873° , 48.794° , 58.444° and 61.629° . Which correspond to crystal planes are (110), (-111), (200), (-112), (-202) and (020) respectively. These crystal planes and 2θ values were in very close agreement match with the standard JCPDS data file no.00-04-0254 of standard Cu NPs [33]. shown in (Fig.4). The XRD pattern revealed that the synthesized CuO NPs were polycrystalline in nature and depicted to be monoclinic structure phase of CuO structure [34-37].

4) HR-TEM

To find out the size and morphology of the biosynthesized Cu NPs, TEM examination was used.

According to obtained TEM image analysis, the estimated Cu nanoparticle size ranges from 0.21 to 6.18 nm (Fig. 5). It is evident that crystalline nanoparticles with a specific particle size and minimal coagulation are produced from the plant extract of *Angelonia angustifolia*. Mostly spherical and near spherical shape of Cu NPs was observed. The majority of the nanoparticles had rough edges and a round shape. TEM of biosynthesized Cu NPs shows hexagonal crystalline nature. The pattern of the chosen area electron diffraction (SAED) has been recorded and is presented in Fig.6. The hexagonal wurtzite crystallite structure's known lattice planes are represented by the rings visible in the SAED pattern.[38].

5) SEM & EDAX Analysis

Scanning electron microscopy (SEM) provided the morphology and size details of Cu NPs. The result of structural studies revealed that samples had crystallites in the nano size range [39,40]. As shown in (Fig.7) obtained SEM images of Cu NPs is having narrow space between two molecules with mixed morphology [41]. The particles are largely irregular in shape, it is due to the aggregation of particles or due to protein which are bound on surface of NPs. Variations in shape and size of synthesized NPs is common by biological systems [42]. EDAX analysis is used for elemental mapping of biosynthesized Cu NPs. After synthesized Cu NPs analyzed by using EDAX spectrum which identifies the two characteristic signals of copper and oxygen each. The analysis revealed the presence of oxygen and copper (Weight percentage-19.08% and Atomic percentage-46.06%) and (Weight percentage-71.72% and Atomic percentage-43.61%) respectively as shown in (Fig.8), confirming the formation and high purity nature of synthesized Cu Nanoparticles. In EDAX spectra, the Cu atom showed a significant signal while the O atom showed a minor signal. The very pure CuO NPs that made up the reaction product were indicated by the results [22].

6) Microbiological Results

The results for antibacterial activity of Cu NPs are shown in (Fig.9). The solution of Cu NPs (1mg/ml) was vortexed and used for antibacterial activity. The biosynthesized Cu NPs were tested for antibacterial

activity by testing multidrug resistant organisms such as gram-negative *E. coli*, *P. aeruginosa*, *K. pneumoniae* and gram positive *S. aureus*, *E. faecalis* were screened by well diffusion assay as with lawn culture method and well having load of 25 µl (0.0025 mg), 50µl (0.05 mg) and 100 µl (0.1 mg) concentration of nanoparticles respectively and positive control antibiotic sensitivity test against various antibiotics. For Cu NPs *E. coli* showcased no inhibition in 25µl , 50 µl and 100µl which was resistant. *S. aureus* showcased 25 µl (no inhibition), similarly, in 50µl and 100µl of zone of inhibition which were resistant. *P. aeruginosa* recorded no zone of inhibition in case of 25µl , 50 µl and 100 µl which showcased resistant inhibition. *K. pneumoniae* was no inhibition in case of 25 µl and 50 µl which was resistant in case of 100 µl with 16 nm of zone of inhibition which was intermediate. *E. faecalis* showcased no inhibition (resistant) in case of 25µl , 50µl and 100µl respectively.

CONCLUSION

From the above results we can able to conclude that, *Angeloniaangustifolia* extract can synthesis Cu NPs in an easy, less toxic and effective manner. The characterization of copper nanoparticle was done by FTIR, HR-TEM, XRD Analysis, UV-Vis spectroscopy and SEM-EDS. Cu NPs produced are stable and comparable average size 0.21 to 6.18 nm. Mostly NPs are spherical in shape. The biosynthesized NPs were confirmed by XRD and HR-TEM which shows that the particle size nanometer with hexagonal crystalline structure.

Declarations

Acknowledgments

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Conflict of Interest:

All authors declare that they have no competing interests.

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Figures



Figure 1

Photographs of *Angelonia angustifolia* plant

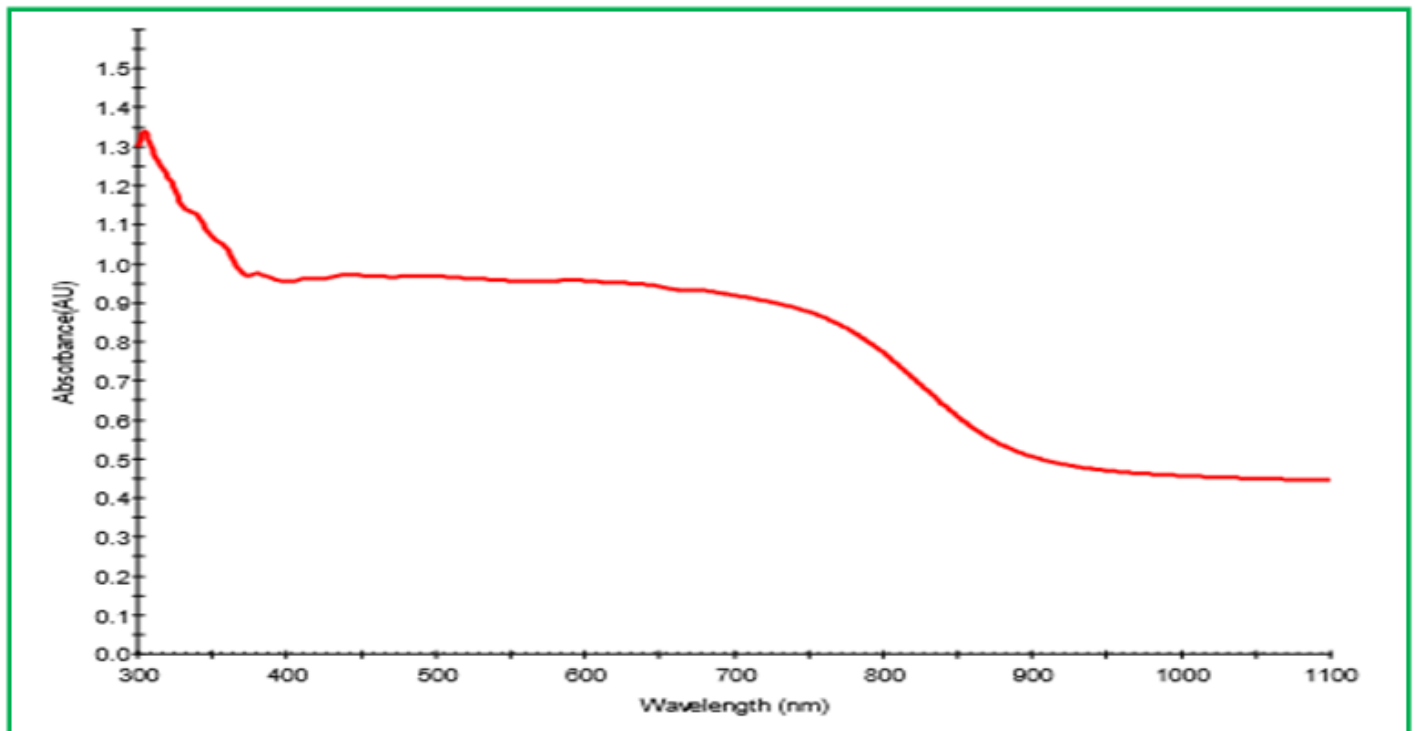


Figure 2

U-Vis spectroscopy analysis of Cu NPs

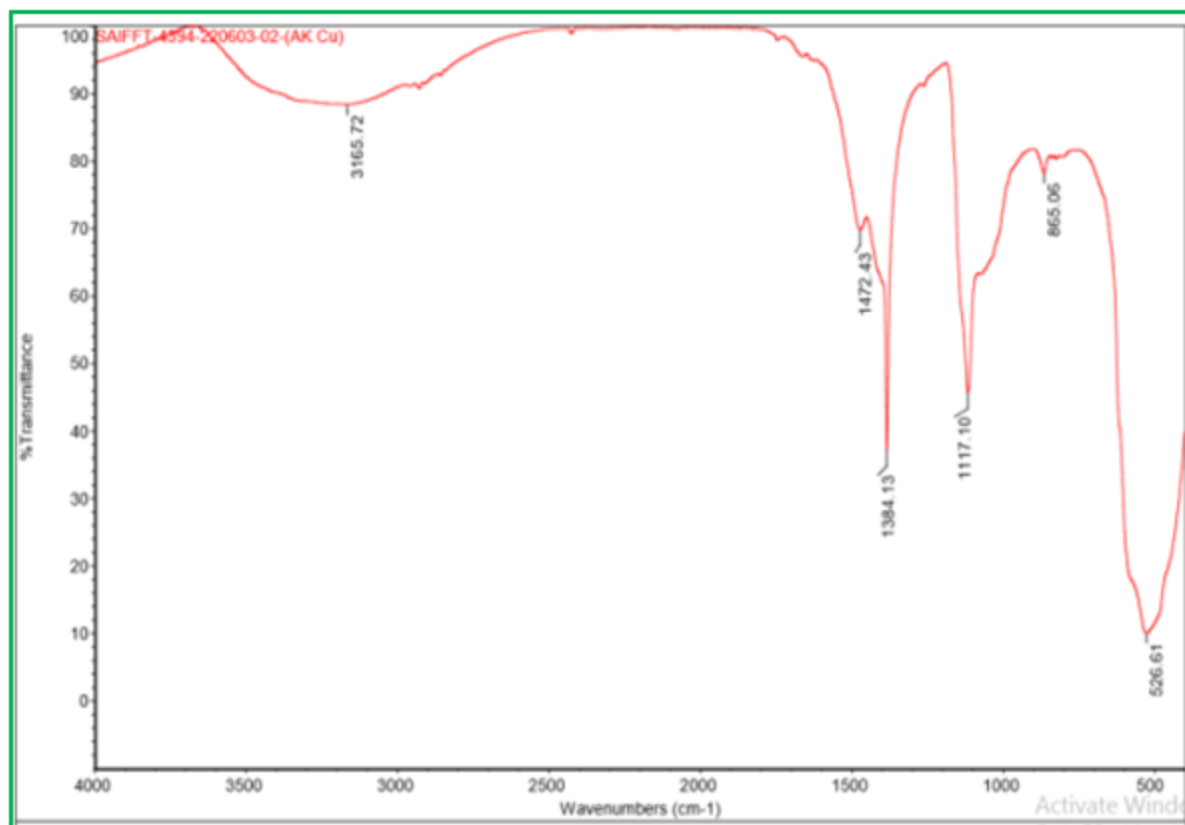


Figure 3

FTIR analysis of Cu NPs

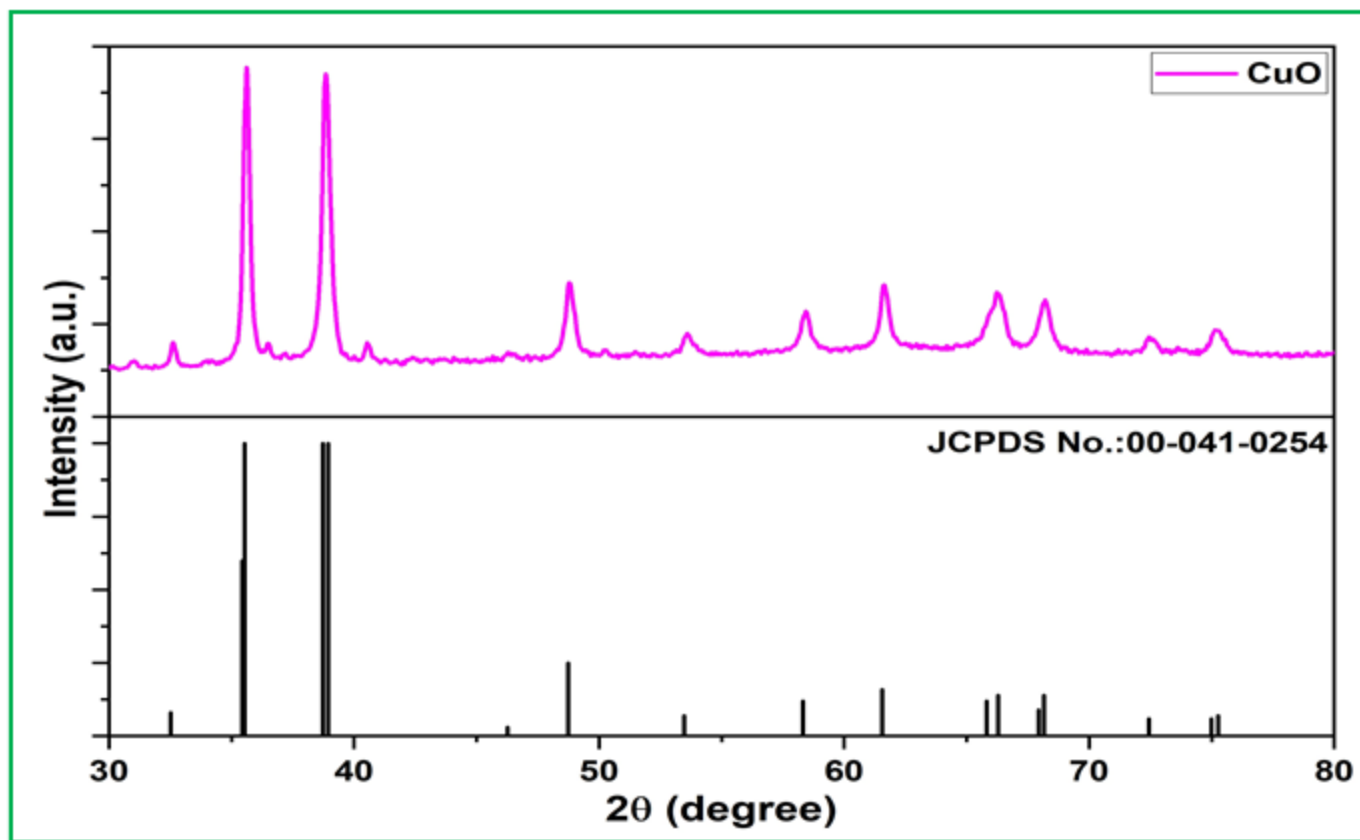


Figure 4

XRD patterns for biosynthesized Cu NPs using *Angelonia angustifolia* plant

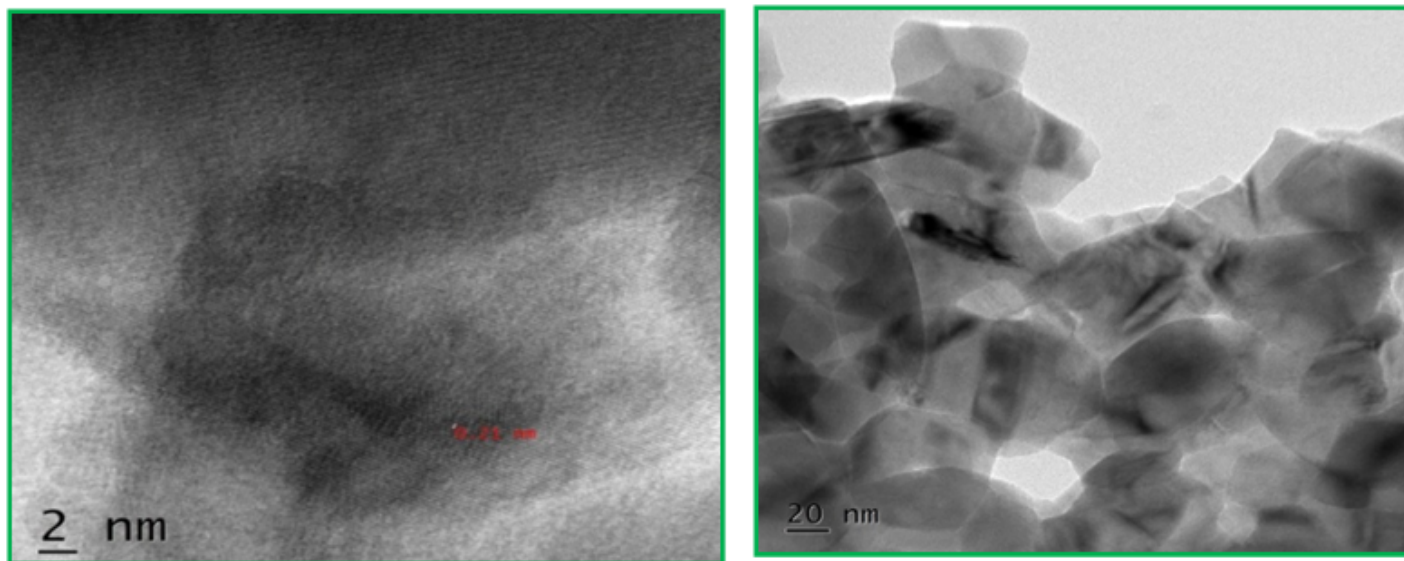


Figure 5

TEM images of synthesized Cu NPs (a) magnified image at 2nm scale (b) showing size range from 0.21 to 6.18 nm

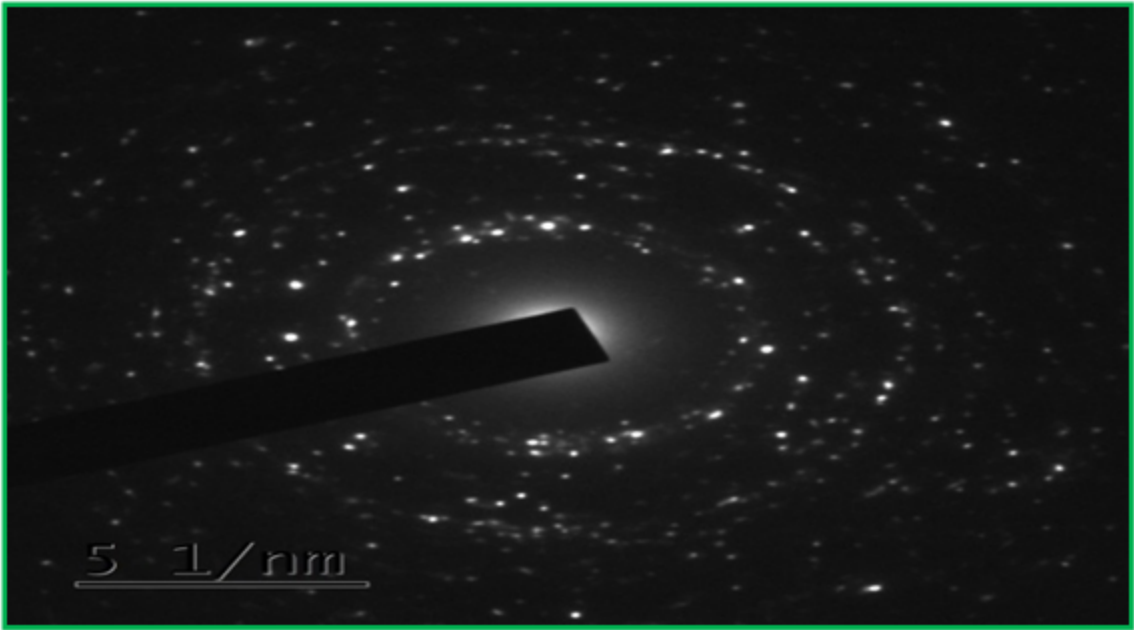


Figure 6
SAED pattern (electron diffraction) of synthesized Cu NPs

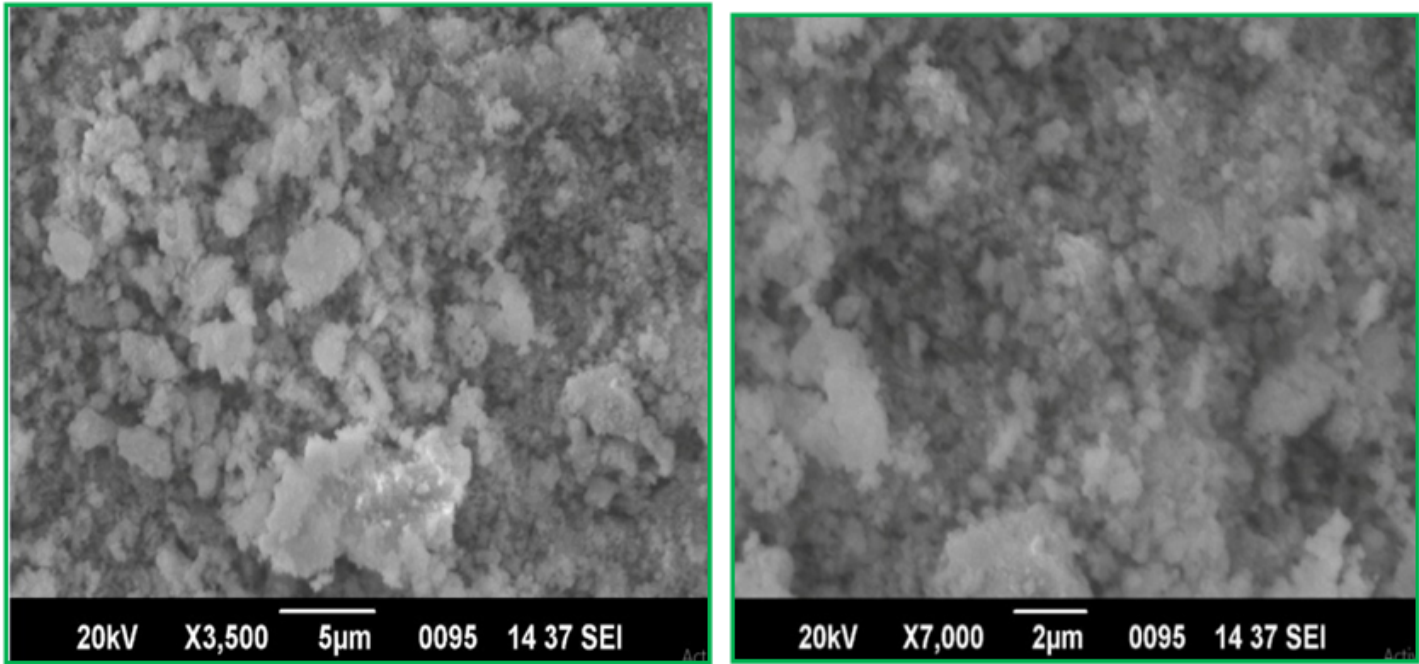


Figure 7
SEM images of synthesized Cu NPs in magnification (a) 2μm (b) 5μm

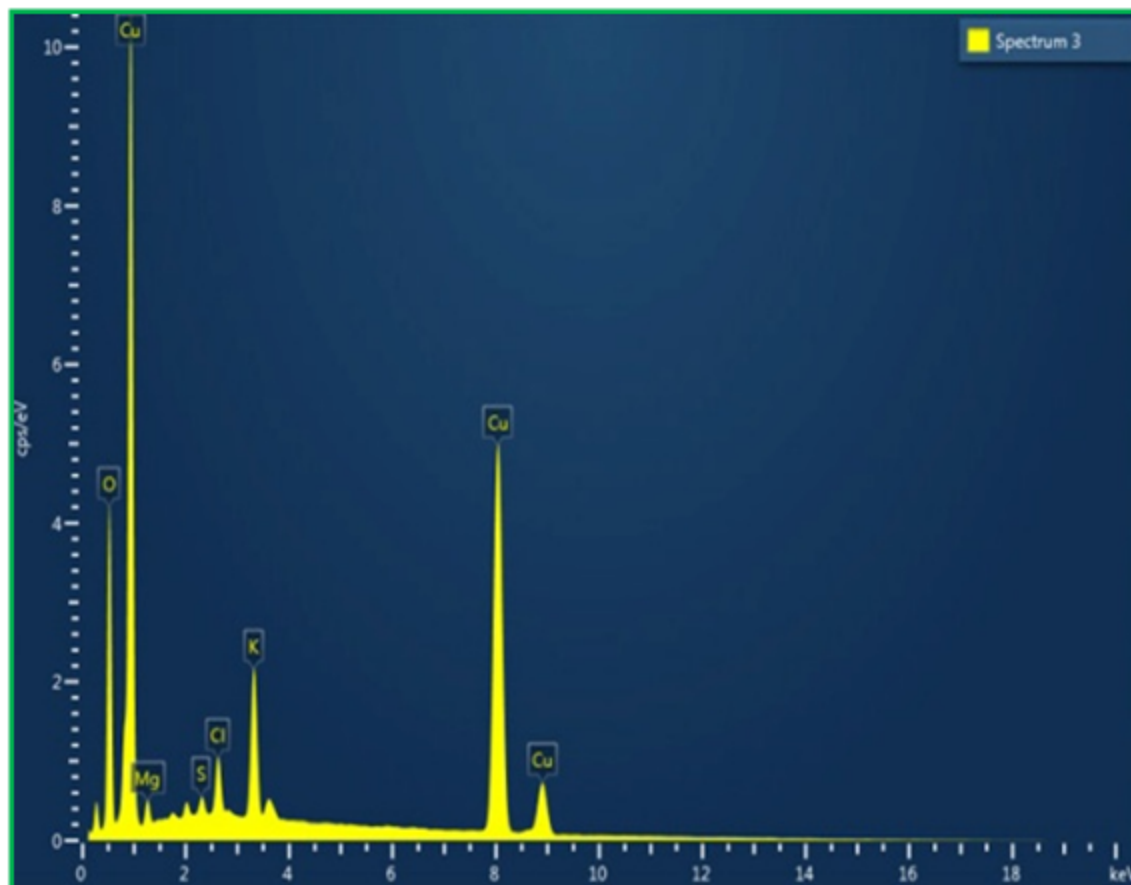


Figure 8

EDAX synthesized spectra of Cu NPs

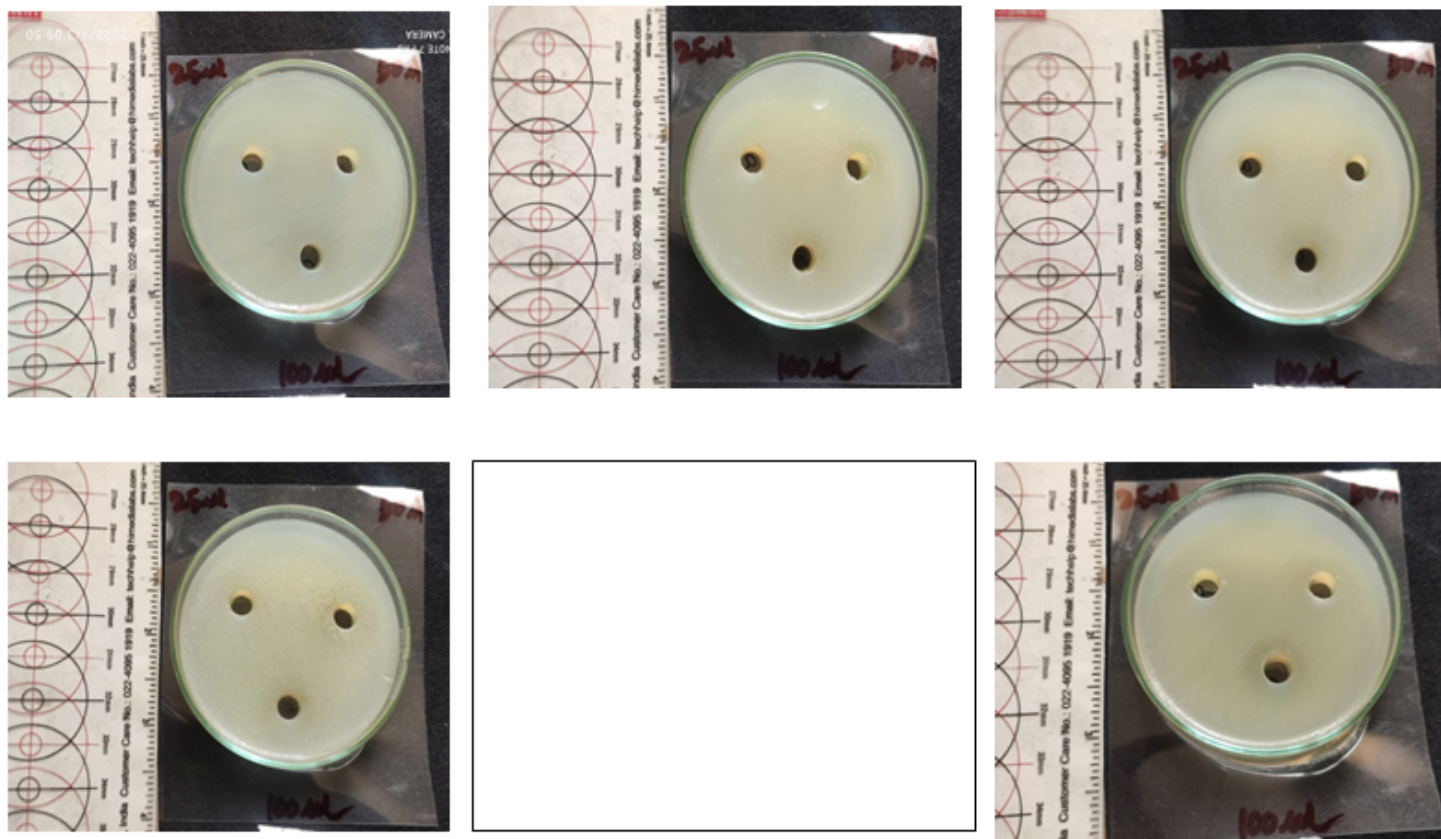


Figure 9

Antibacterial activity of Cu Nps against 1. *E. coli* 2. *S. aureus* 3. *P. aeruginosa* 4. *K. pneumonia* 5. *E. faecalis*