# Biosynthesis and characterization of copper nanoparticles with andrographis paniculata for antimicrobial applications

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This study presents a synthesis of copper nanoparticles in which an intermediate aqueous extract of the leaves of Andrographis paniculata. The synthesis copper nanoparticles (CuNPs) was supervised by UV-visible spectroscopy at room temperature. FTIR analysis prepared nanoparticles exhibited the attendance of comparable peaks established in the leaves extract spectra. XRD analysis inveterate the crystalline structure of synthetic copper nanoparticles. SEM's images presented that greatest balls were a spherical, like a shaped one. Subsequently, CuNPs was further analyzed for anti-bacterial effects from the Gram positive and Gram negative was obviously detected.

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## 1. Introduction

Nanotechnology is a fast-growing area that is used for a variety of materials, and is used various industrial applications. The advancement and main principles of nanotechnologies and characterize innovativeness and also modern times of discovery and highlights in these areas. Nanotechnologies have been proposed as potential ecofriendly alternatives to chemical and physical methods. The Biosynthetic procedures have been examined as an alternative to chemical and physical properties. Biosynthesis of nanoparticles is an emerging recognition more and more attention has been professional due to the applications of nanotechnologies and biotechnology, given the growing need to develop environmentally friendly technologies in materials synthesis [1-4]. Amongst inorganic nanoparticles, metal oxide nanoparticles are commonly considered as antibacterial effects. Cuppers nanoparticles have been synthesized using biological bases and have been potential applications from plant extracts are playing an energetic character due to the size and morphology [5-6].

More information for metal oxides has easy synthesis methods that can be controlled to modify the size and shape of nanoparticles and are likely to environmental (or) pollution toxic decreases in nanoparticles used. Many nanoparticles, such as silver nanoparticles and gold, have recently been synthesized from a number of biological foundations sources such as bacteria, fungi, algae and plants. The development of biologically methods motivated biosynthesis research processes of nanoparticles will become an important branch of nanotechnology and these productions can be achieved by different methods and various applications in industries [7-9].

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It is very significant for Cu metal oxides; the n-type of semiconductor is rich in environment, balanced, fewer harmless and is charity in the production of a multifunction air filtration membrane. In general, copper exists in both amorphous and crystalline forms; there have been infiltrated uses such as solar cells, drug delivery system, photocathodes, and anti-microbial presentations**.** The effective biological methods used in the production of the CuNPs are different, for example leaf, flower buds, fruit, stem, root and flower extracts. In general, it is mentioned that material-based plants promise an interpretation for the synthesis of copper nanoparticles because this process is very simple to scale [10-13].Andrographis paniculata (AP)Wales (Acanthaceae family) is one of the greatest common therapeutic plants conventionally used in the executive of some diseases such as cancer, diabetes, high blood pressure, ulcer, itching, bronchitis, skin diseases, bloating, , flu, dysentery, dyspepsia and malaria have been in the world for centuries [14] (Fig 1). It has several photochemical components with high-class and attention-grabbing pharmacological activities. This work designates the current research work into Andrographis paniculata in relation to pharmacological use, phytochemistry, pharmacological action, and therapeutic use to fill opening future research capabilities. Diterpenes, flavonoids, xanthtones, noriridoides and other various compounds were isolated from the plant. It has been found that the extract and the pure plant compounds are used as antimicrobial, anti-protozoan, anti-inflammatory, anti-oxidant, immuno-stimulants, anti-diabetes, anti-angiogenic, hepatic nerve protection, hepatic enzyme modulation, insecticide and toxic effects [15-17].

The prevalence of antibiotic resistance pathogens has commanded to a continuous exploration for innovative alternatives [18]. Anopheles stephensi carries viral pathogens to humans, which reasons a yellow fever and a fever in malaria and the human population is currently being risk for this motivation. Effective processes are being occupied to combat mosquitoes, but there are still difficult challenges, including increased confrontation to creatures to mosquito. Larviciding is used in several route control programs and this research work is intended to explore the profitable alternatives to active control of the route of mosquito. This research for applied and profuse of the plant extract as mosquito larvae and the procedure from the plant extract is favorable for various reasons of effortlessness of use and it is cost-effectiveness in applications in pharmaceuticals.

*Fig 1. Andrographis Paniculata (AP)*

## 2. Materials and methods

### 2.1. Preparation of leaves extract and synthesis of CuNPs

Andrographis paniculata leaves have been carefully washed in tap water to eliminate dust particles and clean briefly in deionized water. The aqueous solution has been equipped by collecting 10 g of washed and excellently complete leaves in 250 ml. The Erlenmeyer flask with 100 ml of the deionized water and the mixture were boiled at 60°C for 15 minutes. This extract was filtered over no.1 whatmann filter paper and used for supplementary experiments.

### 2.2. Green synthesis of copper nanoparticles

The synthesis of copper nanoparticles was obtained by addition 5 ml of the plant extract to 95 ml of copper sulphate (HiMedia Mumbai, India). Aqueous solution of copper sulphate (0.02 mol) is prepared in round bottom flask. About 0.4 g of NaOH is added to above heated solution till pH reaches to 6-7, and then 20 mL of the *J. curcas* leaf extract was added to the mixture and constantly stirred for another 24 hours at room temperature. The large amount of yellowish brown precipitation was formed, indicating the formation of the CuNPs. It is centrifuged and washed 3-4 times with deionized water. The obtained precipitate was dried in oven for 12 hrs.

### 3.3. Green fabrication of CuNPs by visual observation

In this study, Andrographis paniculata leaf extract was used as a reactant agent to nucleate nanoparticles in the reaction mixture. The optical production of nanoparticles of metal is definitely established due to the transformation of the color of the reaction mixture. Therefore, the fabrication of CuNPs production of the cuvette in the current test was determined by reducing the copper ions (Cu2+) using the Andrographis leaf extract and copper sulphate represented the transformation of color from blue to dark green. The dark green color of the reaction mixture indicated the fabrication of CuNPs. The transformation of the color in the aqueous solution was due to occurrence of surface plasma reforming (SPR) in metallic NPs. This effect includes the combined oscillatory electrons in the NPs metal conduction band where the frequency of the incident photon corresponds to the frequency of the NPS metal. This interesting phenomenon occurs principally when particle sizes are reached at the level of nanoparticles.


*Fig. 2. Green fabrication of CuNPs using the Andrographis leaf extract and copper sulphate.*

## 3. Results and discussion

### 3.1. X-ray diffraction analysis

The crystal grid and the structure of the biosynthetic CuNPs have been determined by X-ray powder diffraction analysis (XRD). The X-ray diffraction have shown well-disconnected four peaks of diffraction peak at 2θ angles approximation 38.04°, 44.22°, 64.40° and 77.37°, which are in line with crystal planes of cubic structures (FCC) of copper nanoparticles 111, 200, 220 and 311, respectively is shown in Fig 3. Similar reports were also achieved by Zahrah Alhalili, wherever well-strong and shaped diffraction peaks of nanoparticles at 38.28° (111), 44.33° (2000), 64.33°(220) and 77.53° (311) were also enclosed in the reflection of Bragg of copper cubic structures. The remaining insignificant peaks are the consideration of the crystal-organic particles on the surface of the CuNPs. The XRD formula achieved in line with previous plant-based synthesis reports [20]**.** The CuNPs sizes have been dogged by approximating the complete width to half the maximum (FWHM) of the greatest visible peaks of the XRD formula using the Debye-Scherrer's equation and mean crystal size is 34 nm. Nonappearance peaks of impurities in the diffractogram which demonstrated the formation of pure crystalline copper nanoparticles.

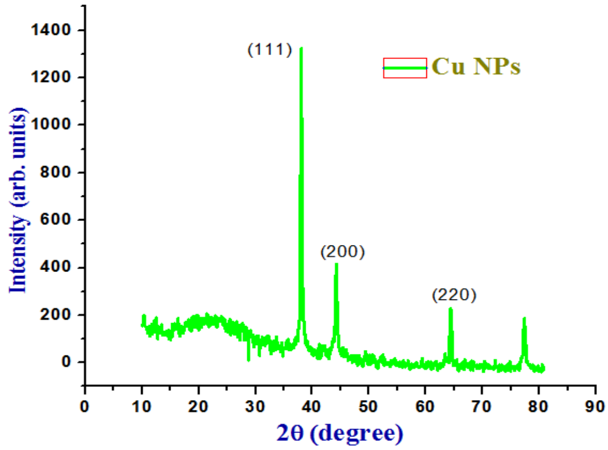


*Fig. 3. X-ray diffraction spectrum of synthesized CuNPs using leaves extracts of Andrographis paniculata.*

### 3.2. UV-visible absorption analysis

UV spectrum of synthesized copper nanoparticles from the leaves of Andrographis paniculata the extracts are presented in Figure 4. The appropriate production of CuNPs from the bio-reduction of copper oxide and copper ions should be determined. This is observed visually by changing the color of the solution mixture from light blue to dark green after 30 minutes. The change in color of the solution can be explained by a double-sided electromagnetic field caused by coordinated oscillations of the free electrical conductivity units which indicate the presence of a surface Plasmon resonance [20]. Absorption of the CuNPs is provisional on particle size, medium and chemical surroundings environment. The reduction of silver ion improvement observed by UV-vis spectroscopy analysis in the wavelength is ranged from 200 to 600 nm. The maximum absorption spectrum of nanoparticles was approximately 246 and 282 nm.

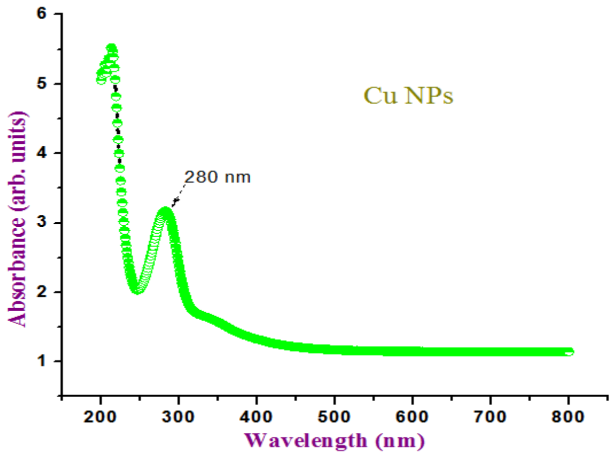


*Fig. 4. UV visible spectrum of synthesized Cu NPs with leaves extracts.*

### 3.3. FTIR analysis data

Biosynthetic copper nanoparticles are usually stabilized by phytochemical compounds through molecular interactions with metal surfaces. The nature of the interaction of molecules can be investigated through the FTIR analysis, and different closing measures are suggested based on the position of peaks of functional groups. This study is charity to analyze the interaction between copper sulphate and biomolecules existing in the Andrographis paniculata leaf extract in this respect as revealed in Fig. 5, several characteristic peaks at 3425, 2924, 1722, 1628, 1528cm$^{-1}$ have been obtained, and 1383, 1263, 1123, 618 and 448cm$^{-1}$, completely representative peaks have been achieved in bio-synthesized copper nanoparticles. In addition, all peaks corresponding to the molecules that can be convoluted in the synthesis and stabilized of the copper nanoparticles. The peaks in 3425cm$^{-1}$ indicated to the presence of the amide groups from flavonoids, triterpenoid and polyphenols. Thus, the interaction between the metal ions and the group of amides in leaf extract of Andrographis paniculata convoluted in the synthesis and stabilization (capping) of copper nanoparticles. Peaks at a distance of 2924 cm$^{-1}$ can be attributed to the groups of phenols -OH, -NH amines and the existence of stretching alkanes - CH. The peaks appeared on 1628 cm$^{-1}$ due to the two-carbon (carbon-carbon) double bond. A sharp peak at a distance of 1383 cm$^{-1}$ matches to the stretch vibration of CN band aromatic amines. The remaining bands of 618 cm$^{-1}$ exhibited similarity to the groups of alkanes which are found in the plant extract, may have an effect on the synthesis of nanoparticles.

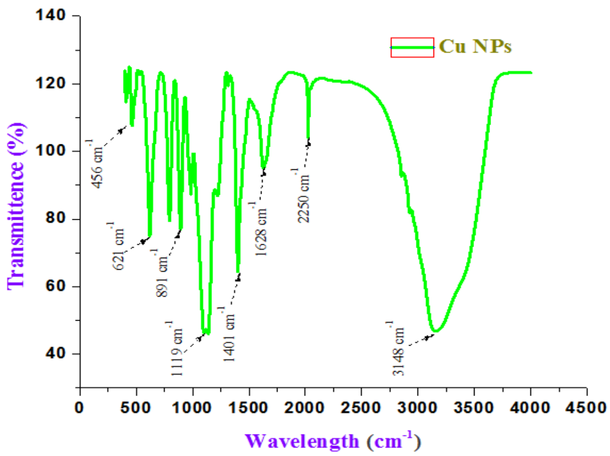


*Fig. 5. FTIR spectrum of synthesized CuNPs with leaves extracts of Andrographis paniculata.*

### 3.4. Scanning electron microscope (SEM) data analysis

Surface morphology was involved to visualize the size and shape of copper nanoparticles synthesized by Andrographis paniculata of the extract leaves. The characteristics of SEM synthetic nanoparticles are presented in Fig 6. Particles have been established to be spherical in shape, well dispersed and homogeneous (5 µm and 1 µm). These particles were prepared by Cu ions for a zero-value atom by reducing the coatings of various biological molecules having hydroxyl groups of surfaces, which resulted in an increase in agglomeration. The SEM illustration displayed a bit of an identical shaped nanoparticles manufactured with a diameter range of 500 nm. It specifies the end of competition of the nanoparticles synthesis process.

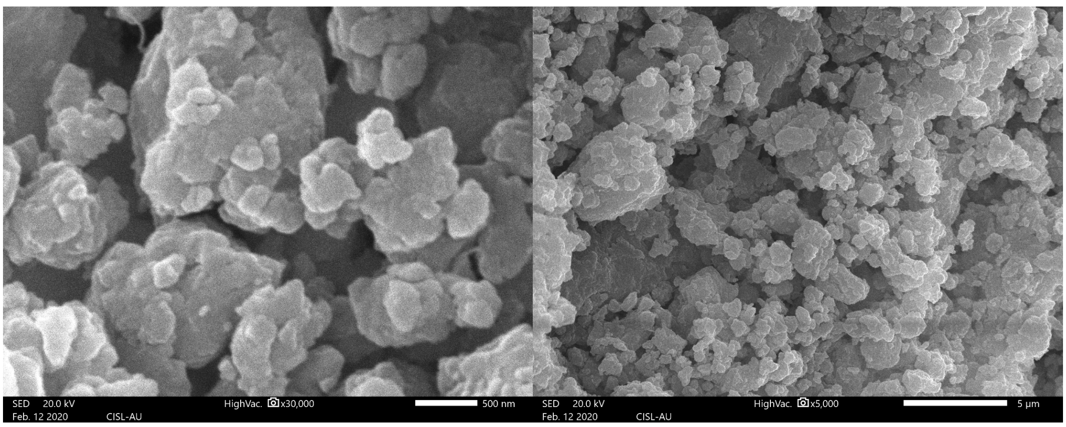


*Fig. 6. Scanning electron microscopy images of synthesized Cu NPs with leaves extracts.*

### 3.5. Antibacterial activities

Numerous analytical mechanisms are used to evaluate the antimicrobial activity of the CuNPs. Allowing to the standard, disk diffusion is the most sensitive process. The anti-microbial activity of a petal extract containing silver nanoparticles against Gram positive (Staphylococcus aureus) and negative Gram negative (Escherichia coli) is revealed in the Fig 7 as the standard mean values for zone braking. CuNPs has a strong inhibitory activity against Gram positive in bacterial species and to Gram negative bacteria Escherichia coli. The maximum inhibition of zone 28 mm has been detected for Staphylococcus aureus. And CuNP showed a higher bactericidal activity in Staphylococcus aureus compared to Gram negative Escherichia coli. However, the appropriate procedure for the achievement of antibacterial copper has not been elucidated. Where's, there is some possible way to find out the interference of the proton gradient by the phospholipid mixture with the bacterial membrane proton pump.

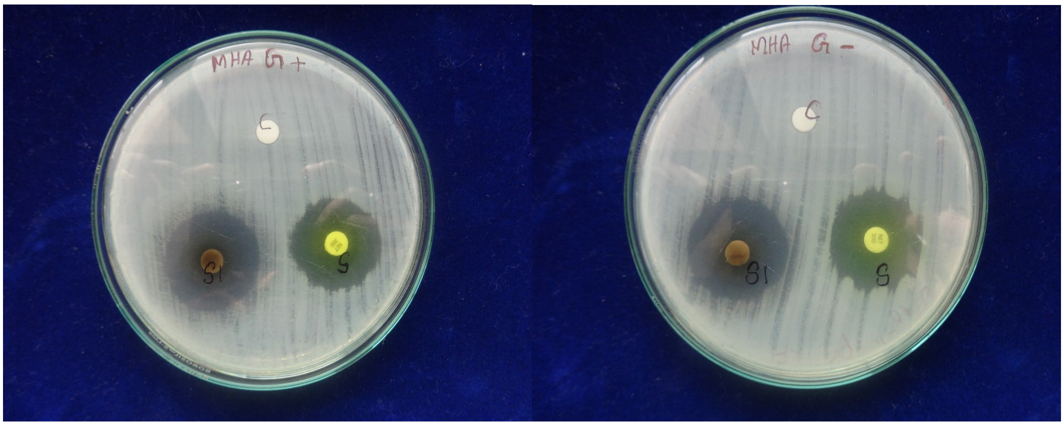


*Fig. 7. Antibacterial activity of CuNPs on Gram Positive and Gram Negative stains.*

### 3.6. Biological activity of Larvicidal

The nanoparticles metal biosynthesis is now recognized as a developing area for research and development in the field of Nano-sciences. Biosynthetic NPs biomaterials are beneficial and have advantages attendant to chemical and physical methods become an economical and environmentally friendly method as an ecofriendly and nontoxic to the environment. A number of plants for the green synthesis of copper nanoparticles (CuNPs) have been examined in recent times. The combination of nanoparticles and bio-activated components of plant extracts has been revealed to increase productivity. The larvicic potential of the synthesis of deciphers from Andrographis paniculata was studied against various species of mosquitoes. The improved prospective of extracts from the plant has been observed when used as copper nanoparticles, which are suggested as an inexpensive, highly effective, environmentally friendly and promising option in the fight against Zika, malaria and filariasis vectors.

The current work examines the potential of common weeds, CuNPs as mosquito control agent as a control measure for mosquitoes. In our study, the initial phytochemical analysis of CuNP indications the presence of Andrographis paniculata extracts as the main component that affects the larval mortality. The impermanence was detected at CuNPs against mosquitoes, a change of rank was observed, while in our studies low-concentration mortality was observed using CuNPs aqueous leaf extract. Synthetic CuNPs are a good, fast and environmentally ecofriendly source for mosquito control.


*Fig 8. The nanoparticles biosynthesis of Larvicidal.*

### 3.7. Antibacterial activity assay

The anti-microbial activity was investigated following adaptation of the technique eventually described by Bauer et al. (1966). The prepared Müller Hiton has been sterilized in an autoclaved system at a pressure of 15 Ibs for twenty minutes and then has cooled. The medium has been accelerated on sterile panels and allowed to solidify. The plates with the carrier are inoculated with a suitable microbiological suspension with a sterile swab. Individual solvents extract equipped discs separately placed on every Petriplates, and regulator and reference substances (nitrofurattoin 300 µg) placed for Bacteria disc. The plates were incubated for one day at 37°C and remained formerly for measured and exposed in mm around the paper.

## 4. Conclusions

The green biosynthesis of copper nanoparticles (CuNPs) exhausting a leaf extract from *Andrographis paniculata has* been recognized in our existing research work. The principles of green biochemistry for the synthesis of metal nanoparticles (NPs) are Non-toxic and eco-friendly has been charity for the synthesis of nanoparticles through a green approach and is pronounced scientific attentiveness due to its extensive application. The results of the current study investigated that Andrographis paniculata extracts are able to fast, ecofriendly, economically and renewable CuNPs synthesis.

The synthetic nanoparticles of copper were crystal-clear in nature and the appearance focused on a cubic (FCC), trim the dispersed shape of the sponge and an average size of 34 nm. Photochemistry, i.e. flavonoids, proteins and phenolic compounds is present in the Andrographis paniculata leaf extract, answerable for the appropriate reduction and stabilization of copper nanoparticles application. Nanoparticles of copper indication a greater antimicrobial activity for gram positive (Staphylococcus aureus) compared to Gram-negative bacteria (Escherichia coli). Advanced technology for effective control of mosquitoes vectors takes time. This study emphasizes a cost-effective and environmentally friendly approach to the synthesis of nanoparticles of CuO using an extract from the leaves of Andrographis paniculata. Larviditic activity against malaria, chikungruya and yellow fever transmit Anopheles stephesi species were studied using CuO nanoparticles and the outcomes have been confirmed in the work and may be applied in future work because the Cu nanoparticles attracted great attention researchers due to the uses as biological properties and potential antimicrobial activity against infectious organisms *as Staphylococcus aureus* and *Escherichia Coli.*

The work attractive application of nanoscience and agrees for the improvement of antimicrobial activity response rates in addition to a significant reduction of the systemic activity of CuPNs associated with chemotherapy applications for anti-bacteria activity for future work .

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