

GREEN SYNTHESIS OF COPPER NANOPARTICLE FROM *PASSIFLORA FOETIDA* LEAF EXTRACT AND ITS ANTIBACTERIAL ACTIVITY

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ABSTRACT

Objective: Development of green nanotechnology is generating interest of researchers toward eco-friendly biosynthesis of nanoparticles (NPs). In this study, biosynthesis of stable copper (Cu) NPs was done using *Passiflora foetida* leaf extract.

Materials and Methods: First, we prepared leaf extract of *Passiflora foetida* in deionized water. This extract added to 20 mMol of Cupric sulfate solution, and we observed the change in color of the solution from colorless to colored solution; this indicates that there is a formation of CuNPs.

Results: These biosynthesized CuNPs were characterized with the help of ultraviolet visible spectroscopy, X-ray diffraction, and Fourier transform infrared spectroscopy. The spectroscopic analysis of synthesized CuNPs showed the maximum absorbance at 350 nm indicating the presence of biosynthesized CuNPs in the reaction mixture.

Conclusion: It was observed that the *P. foetida* leaf extract can reduce Cu ions into CuNPs within 8-10 minutes of reaction time. Thus, this method can be used for rapid and eco-friendly biosynthesis of stable CuNPs.

Keywords: Antimicrobial activity, Biosynthesis, Copper nanoparticles, *Passiflora foetida*.

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INTRODUCTION

In recent years, nanotechnology has attracted many researchers from various fields such as biotechnology, physics, chemistry, material sciences, engineering, and medicine. Nanoparticles (NPs) are synthesized by physical and chemical methods; these are suffering from drawbacks such as hazardous reaction condition, longer time, expensive reagent, tedious process to isolate NPs [1,2]. Hence, there is scope to develop new methods for the synthesis of NPs which should be required less drastic reaction condition, inexpensive reagent, and eco-friendly. In recent years, copper (Cu) NP have attracted much attention of researchers because of its application in wound dressings and biocidal properties [3,4] potential industrial use such as gas sensors, catalytic process, high-temperature superconductors, and solar cells [5-7]. In literature, the CuNPs are synthesized from vapor deposition [8], electrochemical reduction [9], radiolysis reduction [10], thermal decomposition [11], chemical reduction of Cu metal salt [12], and room temperature synthesis using starch and hydrazine hydrate [13]. In recent, green synthesis of CuNPs was achieved using microorganisms [14], plant extract [15]. *Passiflora foetida* is commonly known as passion fruit. It is an exotic fast-growing perennial vine. The genus *Passiflora* belongs to *Passifloraceae* family that includes the passion fruit. Medicinal plants have played a significant role in a variety of ancient traditional systems of medicine. They are rich sources of bioactive compounds and thus serve as an important raw material for drug production and have become a target for the search of new drugs [16]. The plants are the invaluable, incredible, and traditional sources for the curability of various diseases in the form of medicines. Leaves of the plant utilized as folk medicine for treatment of antianxiety, stress, and insomnia. In addition, the plant has been used for its properties such as sedative, antiproliferative, antibacterial, antianxiety, leishmanicidal, antispasmodic, emetic, dressing for wounds, and antiulcer. The constituent of *P. foetida* is alkaloids, phenols, glycosides, flavonoids, cyanogenic compounds, passifloricins, polypeptides, and alpha-pyrone [17].

MATERIALS AND METHODS

Materials

All the reagents used in this experiment were obtained from Sigma-Aldrich Chemicals, India. Double-distilled water was utilized for this process. Filtration was established using Whatman No. 1 filter papers. Glasswares used for the complete reactions were washed well, rinsed with double-distilled water and dried in hot air oven.

Collection of plant leaf

P. foetida leaf was collected from Villupuram district. The collected leaf was tightly packed with a polyethene bag and then transfer to the laboratory. Then, it was washed with distilled water twice and kept under room temperature for 2 weeks in the dark condition. Then, it was made into powder using blender.

Preparation of leaf extracts

Plant leaf extract of *P. foetida* was prepared by first washing the leaves surface with running tap water followed by distilled water. The leaves were then dried in oven and crushed in powder form. To prepare the plant broth solution, 5 g powder of *P. foetida* leaves was taken in a 250 ml beaker with 100 ml of distilled water. Stirred vigorously and boiled the solution for 20 minutes at 80°C. The extract was filtered through Whatman No. 1 filter paper. The filtrate thus obtained was stored in refrigerator for further experiments.

Synthesis of CuNPs using *P. foetida* leaf extracts

About 40 ml of *P. foetida* aqueous leaf extract was added to 20 ml of 20 mM Cu sulfate solution in a 250 ml flask under vigorous stirring. After 15 minutes, the green color of the solution was turned into reddish brown on keeping the reaction mixture in an oven at 80°C for 4 hrs. The appearance of reddish brown color indicated the complete reduction of CuSO₄ solution and formation of CuNPs. The CuNPs thus obtained were purified by repeated centrifugation method at 9000 rpm for 30 minutes followed by redispersion of the pellet in distilled water. The formation of CuNPs was strongly inferred by visual observation

followed by ultraviolet-visible (UV-Vis) spectrum, Fourier transform infrared (FTIR), scanning electron microscope (SEM), X-ray diffraction (XRD), and energy dispersive analysis X-ray (EDAX) studies.

Characterization of silver (Ag) NPs

The synthesized CuNPs were characterized through UV-Vis spectrophotometer HITACHI U2300. The reduction of CuNPs was monitored by UV-spectrophotometer range of absorbance from 250 to 480 nm. Synthesized AgNPs were characterized by FTIR to identify the biomolecules that were responsible for the reduction of AgNPs Jusco 5300 model with the wavelength range from 400 to 4000 cm^{-1} were used. SEM analysis was used to characterize surface morphology, using Supra Zeiss with a resolution of 1 nm at 30 kV with 20 mm Oxford EDS detector. XRD studies were carried out to study the crystal structure of the synthesized AgNPs, the dried samples were coated on XRD grid, and the spectra were recorded using PANalytical X-pert X-ray generator operated at a voltage of 40 kV and a current of 30 Ma.

Antimicrobial activity

Antibacterial activity of CuNPs synthesized from *P. foetida* aqueous leaf extract was determined by Agar well diffusion method. Muller and Hinton agar (MHA) medium (Hi-Media Pvt. Ltd., Mumbai) was used as a medium for this study [18]. The medium and other required glass wares were sterilized through autoclave process at 121°C for 15 minutes. After some time, MHA medium is poured into the petri plate. Following the solidification of medium, the inoculums were spread on the solid plates with sterile swab moistened with the bacterial suspension. The disc was placed in the wells made on MHA plates, and 20 μl of sample (Concentration: 100, 75, 50 and 25 $\mu\text{g}/\text{ml}$) were placed in the disc. The plates were incubated at 37°C for 24 hrs. Then, the antimicrobial activity was determined by measuring the diameter of zone of inhibition.

RESULTS AND DISCUSSION

UV-analysis

UV-Vis spectroscopy measures the extinction (scatter + absorption) of light passing through a sample. NPs have unique optical properties that are sensitive to the size, shape, concentration, agglomeration state, and refractive index near the NP surface, which makes UV-Vis a valuable tool for identifying, characterizing, and studying nanomaterials. The synthesized CuNPs were characterized through UV/Vis spectrophotometer Lambda 35. The biosynthesized CuNP was monitored by UV-spectrophotometer range of absorbance from 250 to 800 nm. UV-Vis absorption spectrum shows peaks characteristics of the surface plasmon resonance of CuNPs [19]. The spectroscopic analysis of synthesized CuNPs showed the maximum absorbance at 350 nm indicating the presence of biosynthesized CuNPs in the reaction mixture. These experimental investigations were found to be in good agreement with the results already presented in the literature by Ashajyothi *et al.*, 2014 (Fig. 1) [20].

FTIR analysis

FTIR measurements were done for both the aqueous fresh *P. foetida* leaf extract and the synthesized dried CuNPs to recognize the possible phytoconstituents responsible for the bioreduction, capping and efficient stabilization of the synthesized CuNPs. The FTIR spectra of the leaf extract and the synthesized CuNPs are shown in Fig. 2a and b. The spectrum was recorded in the wavelength region between 400 and 4000 cm^{-1} .

The spectrum of aqueous fresh leaf extract (Fig. 2a) shows the peaks at wave numbers 3474, 674 cm^{-1} and 1644, 2361 cm^{-1} . The peak at 1644 cm^{-1} was due to the presence of C=O stretching. The peak at 3474 cm^{-1} showed broad O-H stretching of phenolic compound. The N-H stretching of primary amine was obtained from the peak at 674 cm^{-1} . The alkyne stretching frequency was obtained at 2361 cm^{-1} .

FTIR measurement of CuNPs showed the absorption peaks at 3412, 1625, 623, 2361 cm^{-1} in Fig. 2b. Peak at 1625 corresponds to C=O stretching of amides and 3412 corresponds to O-H stretching of

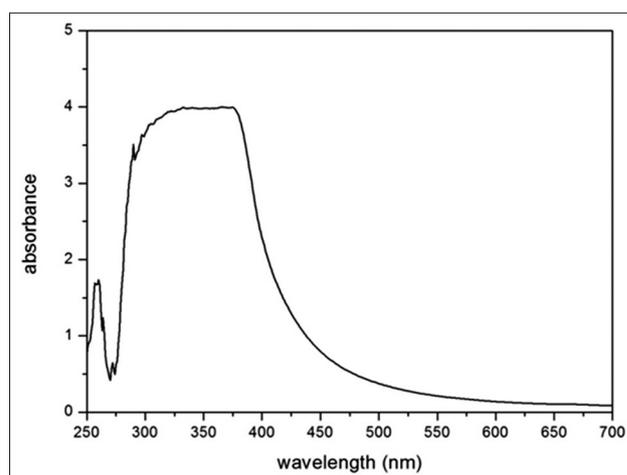


Fig. 1: Ultraviolet-visible spectrum of copper nanoparticles

phenolic compound. The other peaks obtained at 623 and 2361 cm^{-1} were due to N-H stretching of primary amine and alkyne. On the basis of the peaks obtained for CuNPs, it is concluded that biosynthesized CuNPs might be surrounded by any one of these bioactive molecules such as polyphenols, alkaloids, and terpenoids which are in compliance with the already established facts in the literature [21].

The FTIR spectrum of the CuNPs indicates the lowering of peak intensity for O-H stretch of phenolic compounds, confirming the reduction of CuNPs, which have been possibly proceeded via these groups. This indicates that water soluble compound such as polyphenols and terpenoids are present in extract. Therefore, it can be inferred that the biomolecules present in leaf extract, namely, flavonoids, alkaloids, and terpenoids might be responsible for the reduction of Cu ions to CuNPs due to their remarkable reducing capacity. Ulubelen *et al.* studied the species of *P. foetida* and reported the presence of C-glycosyl flavonoids such as chrysoeriol, apigenin, isovitexin, vitexin, 2''-xylosylvitexin, luteolin-7-B-D-glucoside, and kaempferol in enormous amount, which further supports our analysis for concluding flavonoids as one of the major reducing and capping agent (Fig. 2).

SEM analysis

The morphology of the biosynthesized dried CuNPs was examined by S-3400N SEM. The obtained SEM image shows that the product is mainly made of particle-like Cu nanoclusters with size ranges from 150 to 200 nm. However, with high magnification, further observation reveals that these Cu nanoclusters are assembled by smaller NPs, which exhibit good uniformity, and the average diameter is about 24.54 nm (Fig. 3) [22].

XRD analysis

The crystal structure and size of the NPs are verified by XRD analysis. Peaks observed at 2θ values of 42.47, 51.73 and 73.42 correspond to (111), (200) and (220) planes of zero-valent CuNPs. These three peaks are quite consistent with those of the standard JCPDS Card No. 04-0836 for the standard spectrum of the pure face centered cubic (FCC) CuNPs (Fig. 4).

Besides the zero-valent CuNPs peaks, several other diffraction peaks appeared at 36.43, 61.58, 73.42 and 77.19 corresponding to (111), (220), (311) and (222) planes of cuprite NPs, respectively, indicate the formation of cubic Cu (I) oxide nanocrystals [23]. XRD peaks observed for cuprite NPs are matched well with the standard powder diffraction card of body centered cubic cuprite (JCPDS No. 05-667) [24]. The mean size of the crystalline zero-valent Cu and Cu oxide NPs calculated from the major diffractions peaks using the Scherrer formula is about 24.54 and 21.19 nm, respectively.

Scherrer formula, $D = K\lambda/\beta \cos\theta$

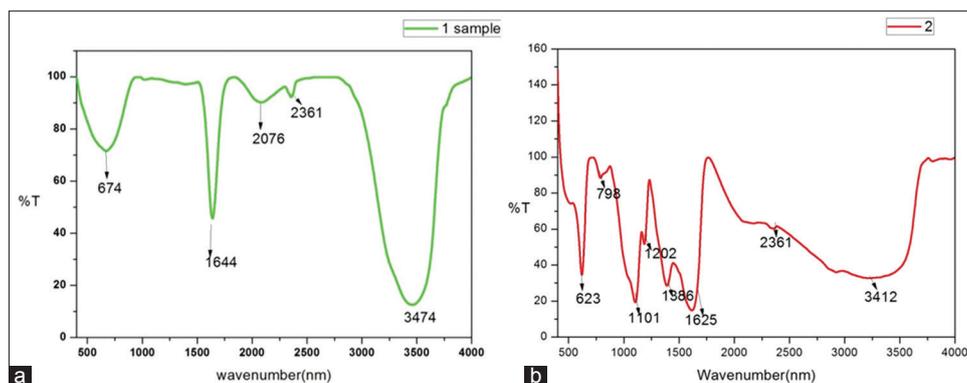


Fig. 2: (a) Fourier transform infrared (FTIR) spectrum of leaf extract, (b) FTIR spectrum of copper nanoparticles

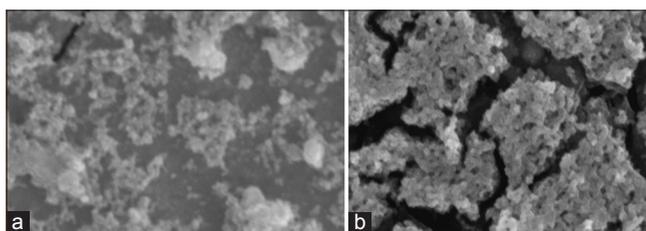


Fig. 3: (a) Scanning electron microscope image of biosynthesized copper nanoparticles, (b) high magnification

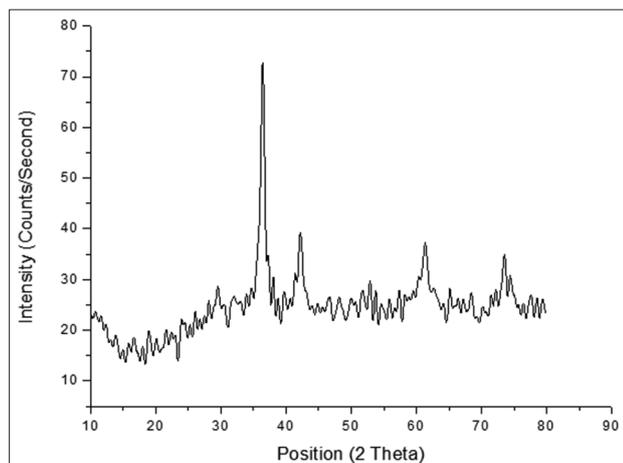


Fig. 4: X-ray diffraction pattern of biosynthesized copper nanoparticles at 2θ

Where, D is the average size of crystallite, K is the Scherrer constant with a value from 0.9 to 1, λ is the wavelength of the X-ray source (0.1541 nm) used in XRD, β is the full width at half maximum of the diffraction peak, and θ is the Bragg's angle.

EDAX analysis

Energy-dispersive X-ray report confirms the elemental composition for the synthesized CuNPs.

This analysis also gave information about the weight percentage of CuNPs, depicting 49.95 weight % of Cu and 50.05 weight % of oxygen. As the analysis was done 1 month after the powder preparation, an aerial oxidation might have taken place which led the formation of Cu oxide (Fig. 5) [25].

Dynamic light scattering

The average size of the particles, size distribution, and polydispersity index (PDI) of the CuNPs was determined by particle size analyzer.

Table 1: Antimicrobial activity of biosynthesized CuNPs against pathogens at different concentrations

Organisms	Zone of inhibition (mm)			
	Concentration ($\mu\text{g/ml}$)			
	100	75	50	25
<i>E. coli</i>	15	13	10	6
<i>S. typhimurium</i>	14	12	9	6
<i>A. aceti</i>	10	7	5	

E. coli: *Escherichia coli*, *S. typhimurium*: *Salmonella typhimurium*, *A. aceti*: *Acetobacter aceti*, CuNPs: Copper nanoparticles

Size distribution graph shows the average particle diameter is 318 nm and PDI is 0.200. The average particle size and PDI revealed that the produced CuNPs are monodispersed (Fig. 6) [26].

Antimicrobial activity of CuNPs

Antimicrobial activity of biosynthesized CuNPs was studied against various pathogenic bacteria using agar well diffusion method. The test organisms used were *Escherichia coli*, *Salmonella typhimurium*, and *Acetobacter aceti*. Biosynthesized CuNPs showed clear zone of inhibition as indicated in Table 1. Maximum zone of inhibition (15 mm) was observed with *E. coli*, next was *S. typhimurium* with 14 mm zone of inhibition and *A. aceti* showed least zone of inhibition of 10 mm at 1000 $\mu\text{g/ml}$ of CuNPs (Plate 1).

From the above result, it can be concluded that the zone of inhibition increases with increase in concentration of NPs. At the lower concentration of 250 $\mu\text{g/ml}$, *A. aceti* was found to be resistant and at higher concentration showed the antimicrobial activity against *Acetobacter*. The occurrence of an inhibition zone clearly indicates the antimicrobial potential of CuNPs, disrupting the membrane. The reason could be that the smaller size of the CuNPs along with bound phytoconstituents, viz., flavonoids (chrysoeriol and apigenin), polyphenols and other biomolecules get tightly adsorbed on the surface of the bacterial cells. The presence of these bound phytoconstituents might be responsible for further enhancing the antimicrobial potential of biosynthesized CuNPs, leading to the disruption of bacterial membrane, thereby causing the leakage of intracellular components, thus killing the bacterial cells. Another proposed mechanism involves the association of Cu with oxygen and its reaction with sulfhydryl (-S-H) groups on the cell wall to form R-S-S-R bonds, thereby blocking respiration and causing cell death [27].

CONCLUSION

CuNPs were synthesized using aqueous leaf extract of *P. foetida* through green synthesis route. The leaf extract of *P. foetida* was found efficient for the synthesis of CuNPs. This method has merits over other reported methods such as easy availability of starting materials, inexpensive

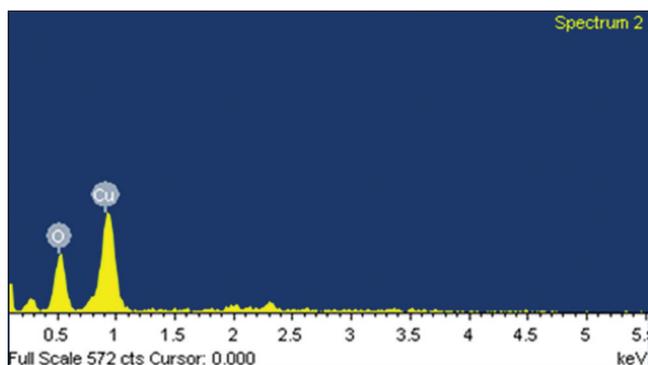


Fig. 5: Energy dispersive analysis X-ray analysis of copper nanoparticles

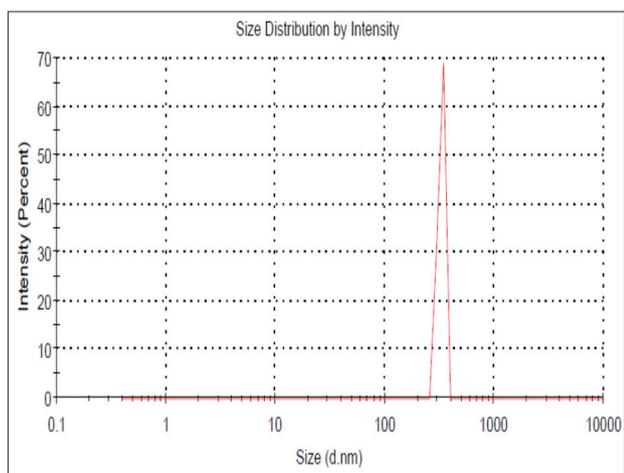


Fig. 6: Particle size distribution



Plate 1: Diameter of zones of inhibition of (a) *Escherichia coli*, (b) *Salmonella typhimurium*, and (c) *Acetobacter aceti*

process, ease of conduction at laboratory, simple reaction conditions, avoidance of use of expensive, hazardous and toxic reagents and pollution free. The synthesized CuNPs were characterized and assessed by UV-Vis spectrometer, FTIR spectroscopy, powdered XRD, SEM with EDAX (SEM with EDAX) and dynamic light Scattering. UV surface plasmon peak reveals the formation of CuNPs in the reaction mixture, which is further confirmed by EDAX analysis. FTIR interpretation gives an insight about the probable bioactive molecules which are acting as reducing and capping agents during the biosynthesis of CuNPs. XRD

analysis affirms the crystalline nature (FCC) and mean size (24.54 nm) of CuNPs, which is in accordance with SEM result. XRD and EDAX result indicate the presence of Cu oxide NPs along with Cu zero valent NPs. The phenolic groups, for instance, chrysoeriol and kaempferol, presents in fresh aqueous *P. foetida* leaf extract exhibit excellent antioxidant activity and these phenols can react with a free radical to form the phenoxyl radicals. Therefore, the use of natural anti-oxidant for the synthesis of CuNPs seems to be an effective root for the green synthesis. The biologically synthesized CuNPs shows excellent antibacterial activity in the reaction mixture.

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